THE EURASIA PROCEEDINGS OF HEALTH, ENVIRONMENT AND LIFE SCIENCES

ISRES Publishing

VOLUME 13 ICGEHES CONFERENCE
ISSN: 2791-8033
ISBN: 978-625-6959-48-4
ICGEHES 2024: 4th International Conference on General Health Sciences (ICGeHeS)
May 02 - 05, 2024
Alanya, Turkey
Edited by: Mehmet Ozaslan (Chair), Gaziantep University, Turkey

ICGEHES 2024

4th International Conference on General Health Sciences (ICGeHeS)

Proceedings Book

Editor

Mehmet Ozaslan
Gaziantep University, Turkey

ISBN: 978-625-6959-48-4

Published by the ISRES Publishing

Address: Askan Mah. Akinbey Sok. No: 5-A/Konya/Turkey

Web: www.isres.org

Contact: isrespublishing@gmail.com

Dates: May 02 - 05, 2024

Location: Alanya, Turkey

https://www.2024.icgehes.net

About Editor

Prof Dr. Mehmet Ozaslan
Department of Biology, Gaziantep University, Turkey
Website: mehmetozaslan.com
E-mail: ozaslanmd@gantep.edu.tr

Language Editor

Lecturer Ceren Dogan
School of Foreign Languages, Necmettin Erbakan University, Turkey
Email: cerendogan@erbakan.edu.tr

CONFERENCE PRESIDENTS

Prof. Dr. Mehmet Özaslan - ISRES Turkey & Gaziantep University, Turkey
Prof. Dr. Merita Rumano - University of Tirana, Albania

SCIENTIFIC BOARD

Ahmet Ozaslan - Gazi University, Turkey
Alexander Oleynik - Si Dnipropetrovsk Medical Academy, Ukraine
Aruna M Jarju - King Graduate School-Monroe College, United States
Betül Apaydın Yıldırım - Atatürk University, Turkey
Cahit Bagci - Sakarya University, Turkey
David Wortley, International Society of Digital Medicine, United Kingdom
Djamil Krouf - University of Oran, Algeria
Elena Lukianova - Rudn-University, Russia
Eva Trnova - Masaryk University, Czech Republic
Gergely Pápay - Szent István University, Hungary
Gulfaraz Khan - United Arab Emirates University, U.A.E
Harun Ulger - Erciyes University, Turkey
Ildikó Járdi - Szent István University, Hungary
Iwona Bodys-Cupak, Jagiellonian University Medical College, Poland
Jaleela Alawi - University of Bahrain, Bahrain
Karim Hajhashemi - James Cook University, Australia
Károly Penksza - Szent István University, Hungary
Khandakar A. S. M. Saadat- Gaziantep University, Turkey
Kristina Kilova - Medical University Plovdiv, Bulgaria
Marinela Asenova Kozhuharova - Shterev Medical Center Reproductive Health, Bulgaria
Mariusz Jaworski - Medical University of Warsaw, Poland
Merita Rumano - University of Tirana, Albania
Mousa Attom- American University of Sharjah, U.A.E
Muhammad Safdar - Cholistan University, Pakistan
Mustafa Saber Al-Attar-Selahaddin University, Iraq
Nigora Muminova - Center for Professional Development of Medical Personal, Uzbekistan
Oltinoy Yakubova - Andijan State Medicine Institute, Uzbekistan
Pınar Gül - Ataturk University, Turkey
Rıdwan Lawal, Lagos university, Nigeria
Rifat Morina - University of Prizren Ukshin Hoti, Kosovo
Sai Yan - Minzu University of China, China
Teuta Ramadani Rasimi, University of Tetova, Republic of Macedonia
Vladimir Protsenko - Rudn-University, Russia
Volodymyr Sulyma - Si Dnipropetrovsk Medical Academy, Ukraine
Yulduz Rasul-Zade - Tashkent Pediatric Medical Institute, Uzbekistan
Wenxia (Joyl Wu, Eastern Virginia Medical School, U.S.A.
Zhaklina Trajkovska-Anchevska - PHI University Clinic for Hematology-Republic of Macedonia

ORGANIZING COMMITTEE

Aynur Aliyeva - Institute of Dendrology of Anas, Azerbaijan
Dominika Falvai - Szent Istvan University., Hungary
Elman Iskender - Central Botanical Garden of Anas, Azerbaijan
Ishtar Imad - Rasheed University College, Iraq

Editorial Policies

ISRES Publishing follows the steps below in the procedings book publishing process.
In the first stage, the papers sent to the conferences organized by ISRES are subject to editorial oversight. In the second stage, the papers that pass the first step are reviewed by at least two international field experts in the conference committee in terms of suitability for the content and subject area. In the third stage, it is reviewed by at least one member of the organizing committee for the suitability of references. In the fourth step, the language editor reviews the language for clarity.

Review Process

Abstracts and full-text reports uploaded to the conference system undergo a review procedure. Authors will be notified of the application results in three weeks. Submitted abstracts will be evaluated on the basis of abstracts/proposals. The conference system allows you to submit the full text if your abstract is accepted. Please upload the abstract of your article to the conference system and wait for the results of the evaluation. If your abstract is accepted, you can upload your full text. Your full text will then be sent to at least two reviewers for review. **The conference has a double-blind peer-review process.** Any paper submitted for the conference is reviewed by at least two international reviewers with expertise in the relevant subject area. Based on the reviewers' comments, papers are accepted, rejected or accepted with revision. If the comments are not addressed well in the improved paper, then the paper is sent back to the authors to make further revisions. The accepted papers are formatted by the conference for publication in the proceedings.

Aims & Scope

Compared to other fields, developments and innovations in the fields of medical and health sciences are very fast. In this century, where the human population is rapidly increasing and technology is developing rapidly, health problems are constantly changing and new solutions are constantly being brought to these problems. With the Covid 19 epidemic, it has emerged that a health problem affects all humanity and all areas of life. For this reason, this conference focused on the changes and innovations in the field of Medical and Health Sciences.

The aim of the conference is to bring together researchers and administrators from different countries, and to discuss theoretical and practical issues of Medical and Health Sciences. At the same time, it is aimed to enable the conference participants to share the changes and developments in the field of Medical and Health Sciences with their colleagues.

Articles: 1-17

CONTENTS

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 1-8

ICGeHeS 2024: International Conference on General Health Sciences

Evaluation of XRCC2 Expression in Breast Cancer

Naser Gilani
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract: Breast cancer (BC) is a leading cause of mortality among women worldwide. The X-ray repair cross-complementing group 2 (XRCC2) gene is implicated in DNA repair processes, and its role in BC remains controversial. This study aimed to analyze XRCC2 mRNA expression in BC tissues compared to normal breast tissues to elucidate its potential role in BC pathogenesis. An observational analytical study with a case-control design was conducted at Zheen International Hospital, Erbil, Iraq, from 2021 to 2024. The study included 44 adult women diagnosed with BC, and XRCC2 mRNA levels were measured using real-time quantitative reverse transcription PCR (qRT-PCR). RNA was extracted, converted to cDNA, and analyzed by qRT-PCR. The XRCC2 mRNA expression levels were normalized using GAPDH and statistically analyzed using the $2^{-\Delta Ct}$ method. The study found a significant upregulation of XRCC2 expression in BC tissues compared with normal controls ($p<0.05$), especially in patients aged 40-55 years and those > 56 years ($p=0.0392$ and $p=0.0191$, respectively), and in higher BC grades II and III ($p=0.0013$ and $p=0.0051$, respectively). Invasive ductal carcinoma exhibited a notable increase in XRCC2 expression ($p=0.0006$). In conclusion, the increased XRCC2 mRNA expression in BC tissues suggests a possible oncogenic role of XRCC2 in BC development. The correlation between age and cancer grade indicates its potential as a marker for BC progression.

Keywords: Breast cancer, Gene expression, qRT-PCR, XRCC2

Introduction

Breast cancer (BC) is one of the most common (with 2.26 million cases in 2020) and the second leading cause of death (with 685,000 deaths in 2020) from cancer in women (Wilkinson & Gathani, 2022). It is a complex and heterogeneous disease, with various genetic and environmental factors contributing to its development and progression (Abiola et al., 2024). One of the key aspects of BC research is the study of DNA repair genes, as they play a crucial role in maintaining genome integrity and preventing the accumulation of mutations that can lead to cancer (Moon et al., 2023).

Among these genetic elements, the X-ray repair cross-complementing group 2 (XRCC2) gene has emerged as a candidate of interest due to its involvement in DNA repair mechanisms. The XRCC2 gene is integral to the homologous recombination (HR) repair pathway, a critical system for maintaining genomic stability and repairing DNA double-strand breaks, which, if left unrepaired, can lead to tumorigenesis (Andreassen and Hanenberg, 2019, Prime et al., 2024).

Recent studies have suggested that alterations in the expression levels of XRCC2 may be associated with the development of certain cancer types, including BC (Shi et al., 2022, Liu et al., 2023, Yu & Wang, 2023). The gene's product, XRCC2 protein, is a part of the RecA/Rad51-related protein family, is known for facilitating the exchange of strands between homologous DNA molecules (a key step in the repair process) (Liu et al., 2023). In

BC, the fidelity of DNA repair mechanisms is particularly crucial, as genetic mutations driving the disease are often a result of DNA repair errors (Alhmoud et al., 2020).

Recent advances in molecular techniques have presented new opportunities to dissect the complexities of cancer biology. mRNA expression analysis, in particular, has become a cornerstone in studying gene expression alterations in various cancers, including BC (Malone et al., 2020, Velaga & Toi, 2022). By quantifying mRNA levels, researchers can infer the activity of genes of interest and elucidate their potential involvement in tumorigenesis and progression. This approach is instrumental in validating biomarkers for cancer diagnosis and prognosis, as well as in identifying new therapeutic targets (Perron et al., 2018).

The necessity to delve into the molecular landscape of BC is underscored by the heterogeneous nature of the disease, which impedes the efficacy of a one-size-fits-all approach to treatment. The present study is predicated on the hypothesis that XRCC2 expression levels may serve as a diagnostic and prognostic marker in BC and could offer insights into the disease's molecular phenotype. Therefore, the present study aims to provide a detailed analysis of XRCC2 mRNA expression in BC tissues, employing real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) to achieve high sensitivity and specificity.

Method

Study Design and Setting

In this observational analytical research study employing a case-control design, the focus was on investigating the XRCC2 gene's correlation with BC. Conducted between 2021 and 2024, this study involved the meticulous collection of specimens from Zheen International Hospital in Erbil, Iraq.

Participants

The study included adult women 18 years and older who had been diagnosed with BC through histological confirmation and had given informed consent. Patients with previous malignancies, undergoing chemotherapy or radiation therapy prior to sample collection, lacking complete medical records, or declining to participate were excluded from the study. All subjects provided informed permission before sample collection, and the Local Ethics Committee accepted the study procedure (Approval number: 05.01.2020\17). A total of 88 samples, comprising 44 normal and cancerous tissue samples from the breast, were analyzed using the prevailing sampling technique.

Table 1. The key reagents and conditions

Step	Component	Volume for reaction	Volume for negative control	Incubation conditions
One	Total RNA	1-6 µl	-	-
	Primer d(T)23 VN	2 µl	-	-
	Nuclease-free H2O	Variable	-	-
Two	Denaturation	-	-	5 min at 70°C
Three	M-MuLV Reaction Mix	10 µl	10 µl	-
	M-MuLV Enzyme Mix	2 µl	-	-
	Nuclease-free H2O	-	Variable	-
Four	Incubation	-	-	1 hr at 42°C
	(If random primer used)	-	-	5 min at 25°C
Five	Inactivation & Dilution	-	-	80°C, then dilute

RNA Extraction and Complementary DNA Synthesis

The extraction and synthesis of RNA into cDNA from breast tissue can be summarized in a streamlined process with attention to specific conditions and reagent volumes. Initially, RNA was extracted using a Thermofisher

kit, and its concentration was measured with a NanoDrop device. The conversion of RNA to cDNA involved the Ipsogen RT Kit and thermal cycling with the Master-cycler pro-PCR System. To prevent contamination, the workspace was cleaned with 70% ethanol, and filter tips were used throughout the process. A no-reverse transcriptase control was included to check for DNA contamination. For cDNA synthesis, the procedure began by mixing RNA with a primer d(T)23 VN in RNase-free conditions, followed by a denaturation step to improve yield, particularly for long mRNAs and CG-rich regions. This was achieved by heating the RNA-primer mixture at 70°C for 5 minutes, then quickly cooling it on ice. Next, reaction mixes and enzymes were added to initiate synthesis, with specific conditions for the negative control. Incubation at 42°C for an hour allowed for cDNA synthesis, with a preliminary step at 25°C for 5 minutes when using a random primer mix. The reaction was terminated by heat inactivation at 80°C. The final product was diluted (30 µl with H2O) and stored for PCR, ensuring the cDNA did not exceed 10% of the PCR reaction volume (Table 1).

Primer Design

In the study, the design of primers was a critical step for amplifying the XRCC2/Exp mRNA sequences. This task was accomplished using an online primer design tool (http://workbench.sdsc.edu). The primers were meticulously crafted to span the entire coding sequence of the gene of interest, incorporating one or two exon-exon junctions within the design. The inclusion of these junctions was strategic to ensure the amplification was specific to the RNA transcript, avoiding the unintended amplification of any possible genomic DNA contaminants. Details regarding the primer sequences, their specific annealing temperatures, and the expected sizes of the PCR products were systematically cataloged in Table 2.

Table 2. Primer sequences.

Gene name		Primer sequence	Optimal annealing temperature	PCR product Size
XRCC2	F R	TGTTTGCTGATGAAGATTCAC TCGTGCTGTTAGGTGATAAAGC	59.2 °C	255 bp
GAPDH	F R	GGTCCACCACCCTGTTGCTGT AGACCACAGTCGATGCCATCAC	59,4 °C	456 bp

Real-time PCR

To optimize the PCR conditions for specific cDNA primers, a gradient PCR experiment was carried out using an ABI Vertti PCR System. The most effective annealing temperature was determined by evaluating the yield on a 2% agarose gel, which was identified to be 59.2°C for the XRCC2 primers. The PCR mixture, with a total volume of 25µL, included 15µL of dH2O, 2.75µL of 10X PCR buffer with ammonium sulfate, 2µL of 25 mM MgCl2, 1.5µL of 2 mM dNTP mix, 1µL each of 20 mM forward and reverse primers, 0.125µL of 5 U/mL Taq DNA polymerase, and 1.5µL of cDNA template. Table 3 shows the PCR gradient reaction conditions.

Table 3. Conditions of gradient PCR reaction.

No	Step	Temperature	Time
1	Pre denaturation at	94°C	7 minutes
2	Denaturation at	94°C	40 seconds
3	Primer annealing	55°C - 60°C	40 seconds
4	Extension	72°C(40 cycles)	40 seconds
5	Final extension	72°C for	5 minutes
6	Hold	4°C	0

After the PCR, the products were evaluated via 2% agarose gel electrophoresis stained with ethidium bromide, subjected to a 100-volt run for 60 minutes, and observed under UV light. Real-time PCR analysis was carried out on a RotorGene 5 plex system utilizing RT² SYBR Green ROX FAST Mastermix. The reaction mixture for evaluating XRCC2 expression was prepared with 10.5µL of the master mix, 1µL each of forward and reverse primers at 10 µM, 15.5µL of RNAse/DNAse free water, and 2.5µL of cDNA (50 ng), resulting in a total reaction volume of 30µL. The Real-Time PCR procedure involved an enzyme activation at 94°C for 10 min, succeeded by 40 cycles of denaturation at 94°C for 10 sec, primer annealing at the optimized 59.2°C for 40 sec, and extension at 72°C for 40 sec.

Normalization and Statistical Analysis

Normalization of gene expression data was conducted by employing GAPDH as an internal control gene. The relative quantification of XRCC2 mRNA levels was computed through the 2−ΔCt method, and statistical analysis was carried out utilizing SPSS software (version 22.0). The statistical significance of disparities in XRCC2 expression between tumor and normal tissue was assessed using Student's t-test, with a significance level set at 0.05.

Results and Discussion

Table 4 shows the basic data and statistical significance according to age, cancer grade, and cancer types of the patients. In terms of age distribution, most of the participants were 20 (45.45%) between 40- 55 years old. For the Grades variable, most of the participants 18 (40.9%) had grade II cancer. Additionally, in the cancer types, Metaplastic carcinoma, matrix producing type was observed in 12 participants (27.27%), invasive ductal carcinoma in 22 participants (50.0%), and carcinoma Medullary-like in 10 participants (22.73%).

The increased expression of XRCC2 exhibited notable significance within the age groups of 40-55 years and those aged above 56 ($P<0.05$). Similarly, a statistically significant elevation in XRCC2 expression was observed among patients diagnosed with BC grades II and III ($P<0.05$). Conversely, this augmentation did not reach statistical significance in individuals with cancer grade I ($P>0.05$). Moreover, a significant rise in XRCC2 expression was identified in cases of invasive ductal carcinoma ($P<0.05$) (Table 4).

Table 4. Statistical significance according to age, cancer grade and cancer types.

Variables	NO. (%)	XRCC2 Expression (p-value)	Mean of differences	SD of differences	SEM of differences
			Age		
<40 years	13 (29.54)	0.8149	0.050	0.7832	0.2093
40- 55 Years	20 (45.45)	0.0392	0.0280	0.5653	0.1264
> 56 years	11 (20.45)	0.0191	0.3200	0.3553	0.1123
Cancer Grade					
I	9 (20.45)	0.8273	-0.06667	0.8874	0.2958
II	18 (40.9)	0.0013	0.3722	0.4099	0.09661
III	17 (38.65)	0.0051	0.2412	0.5789	0.1404
Brest cancer type					
Invasive ductal carcinoma	22 (50.0)	0.0006	0.3682	0.4247	0.09055
Carcinoma Medullary like	10 (22.73)	0.8088	0.0500	0.6346	0.2007
Matrix producing metaplastic	12 (27.27)	0.6647	0.1083	0.8426	0.2432

SEM: Standard error of means

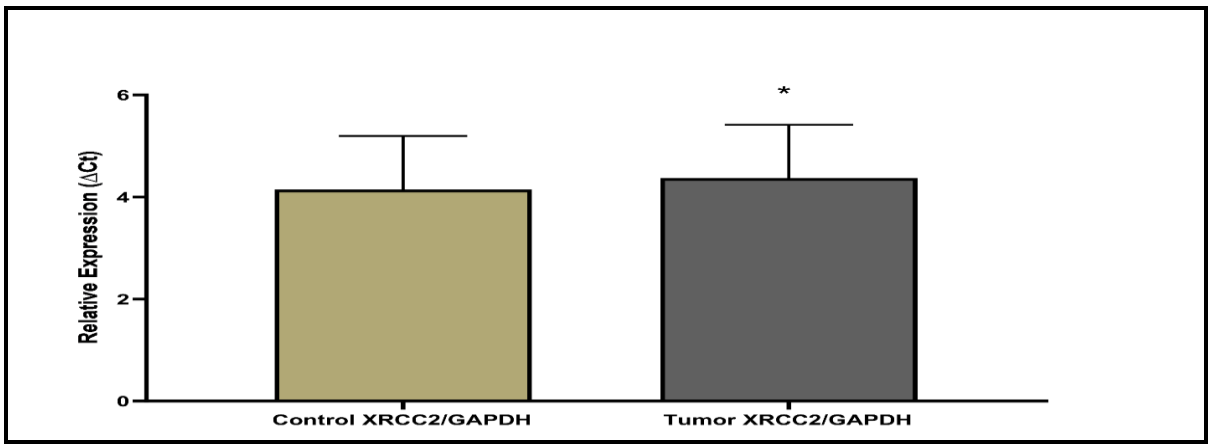


Figure 2. The level of expression of mRNA

Figure 2 illustrates the statistical results of the level of expression of mRNA in both normal controls and tumors. The results reveal that the expression of the XRCC2 gene is significantly increased (upregulated) in tumor samples compared with normal tissues ($p<0.01$). In addition to the statistical data, Figure 3 presents the amplification curve of XRCC2 in real-time PCR. Also, Figure 4 shows the melting curve of XRCC2 in real-time PCR.

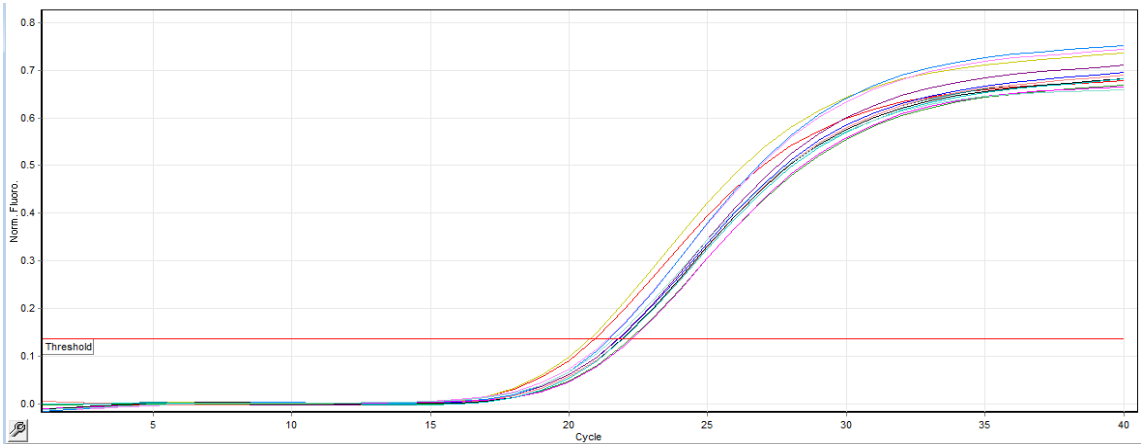


Figure 3. Amplification curve of XRCC2 in real-time PCR.

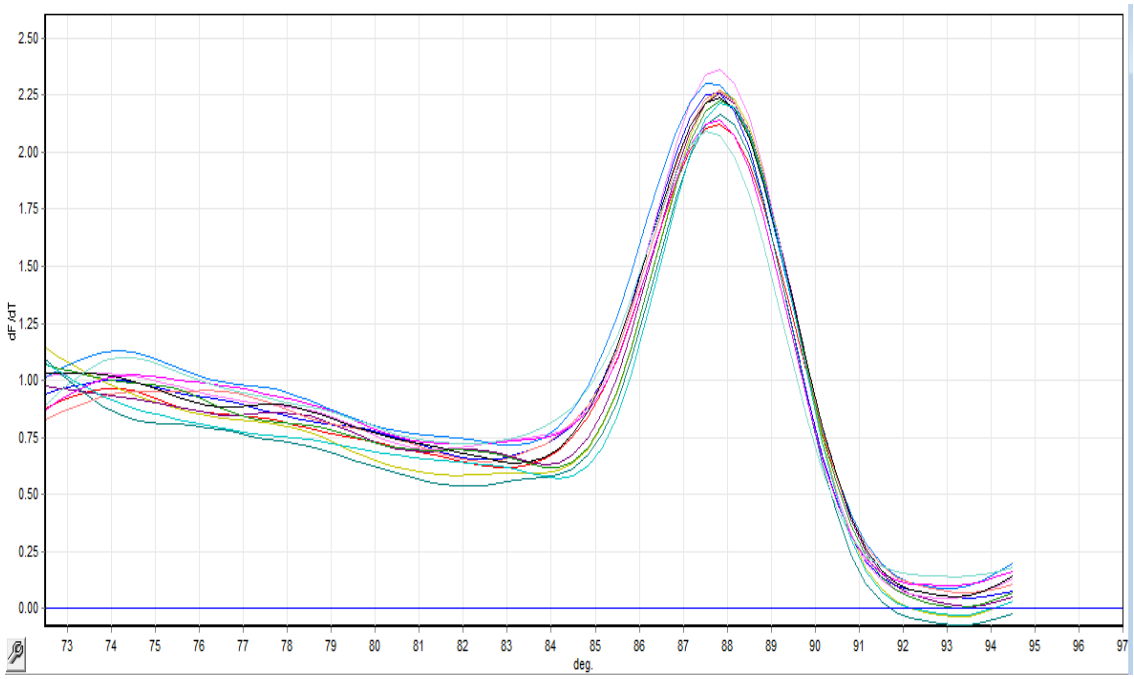


Figure 4. Melting curve of XRCC2 in real-time PCR

Breast cancer stands as the prevalent form of cancer in women and a primary contributor to female mortality rates (Siegel et al., 2024). Studies have shown that XRCC2 gene polymorphisms can impact BC susceptibility (Wei-Yu et al., 2011). Additionally, meta-analyses have highlighted the significance of genetic variability in DNA repair genes like XRCC2 in BC risk (Yu & Wang, 2023).

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) is a highly sensitive and specific method for measuring gene expression levels, including the expression of XRCC2 in BC (Castelló et al., 2002, Ho-Pun-Cheung et al., 2009). The gene expression change of XRCC2 was done by the RT-qPCR method and the data was analyzed by the ΔCT method. The results showed that the expression level of this gene in tumor samples upregulated significantly compared to the adjacent healthy tissue. Considering the regenerative function associated with this particular gene (Andreassen and Hanenberg, 2019), it is anticipated that the level of its expression would diminish throughout the progression of tumorigenesis, exhibiting a tumor-

suppressive characteristic; However, findings from this investigation indicate an upregulation of this gene in cases of BC, suggesting an oncogenic attribute.

The results showed that XRCC2 mRNA expression was significantly upregulated in BC tissues compared to normal controls, suggesting that XRCC2 plays a role in the development of BC. In line with the results of the present study, Shi et al. (2023) support the role of XRCC2 in BC, but what draws attention is that, in their study, they point out that the expression of XRCC2 mRNA decreased; It is contrary to the results of the present study (Shi et al., 2022). Bashir et al.'s (2014) study also discovered that XRCC2 mRNA expression is decreased in BC (Bashir et al., 2014). This difference may be caused by the grade and type of BC and the number of participants and the methods used. Therefore, more studies are needed to investigate this difference. In contrast, other studies also confirm the findings of the present study and state that XRCC is overexpressed in glioblastoma (Liu et al., 2021), colorectal (Xu et al., 2014), stomach cancer (Gok et al., 2014), and BC (Mohamed et al., 2021).

Statistical studies showed that the expression of this gene has a significant relationship with the age of the patients so it showed a higher level of expression in patients 40 years old and above. The studies conducted by Qureshi et al. (2015) and Kluźniak et al. (2019) also confirm this finding, because most people who had mutations in the XRCC2 gene were over 40 years old (Qureshi et al., 2015, Kluźniak et al., 2019).

In line with the present study, the study by Chen et al. (2018), and Zhang et al. (2012) showed that XRCC2 is highly expressed in human tumor cell lines and tissues (Zheng et al., 2012, Chen et al., 2018). Additionally, the results of the present study showed that the increase in cancer grade is related to the higher expression of XRCC2. A study undertaken by Zhang et al. (2017) demonstrated a notable elevation in XRCC2 expression in colorectal cancer (CRC) tissues compared to normal tissues. This heightened XRCC2 expression was linked with more advanced T staging, M staging, TNM staging, Duke's staging, and increased liver and lymph node metastases. The study suggests that XRCC2 expression could serve as an independent prognostic marker for patients with CRC (Zhang et al., 2017). As mentioned, despite accepting the carcinogenicity of XRCC2, some studies suggest that it is premature to consider XRCC2 as a BC-predisposing gene (Kluźniak et al., 2019). Therefore, more studies on the carcinogenesis of XRCC2 in BC are needed.

Conclusion

In conclusion, in the present study, the expression level of XRCC2 was notably higher in tumor samples than in adjacent healthy tissues, suggesting an oncogenic role for XRCC2 in BC development. Discrepancies in the literature regarding the role of XRCC2 in BC highlight the complexity of its function, as some studies report a decrease in its expression. However, conflicting data and variability in study designs necessitate further research to fully elucidate the role of XRCC2 in BC carcinogenesis.

Recommendations

Further investigations are recommended to elucidate the inconsistent findings regarding XRCC2 expression in BC across different studies, with particular attention to cancer grade and type. Larger, more diverse cohorts should be included in subsequent research to validate the findings and account for variability in gene expression due to cancer grade, type, and patient demographics. Additionally, functional studies are warranted to understand the mechanistic role of XRCC2 in tumor development and progression.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

References

Abiola, S. A., Ben-Chioma, A. E., Fidelis, B. G., Aloy, S. C. & Elekima, I. (2024). Epigenetic modulation in breast cancer: From mechanisms to therapeutic ınterventions. *International Research Journal of Oncology, 7(*1**),** 1-13. 1047336.

Alhmoud, J. F., Woolley, J. F., Al Moustafa, A. E. & Malki, M. I. (2020). DNA Damage/repair management in cancers. *Cancers (Basel), 12*(4**),** 1050.

Andreassen, P. R. & Hanenberg, H. (2019). XRCC2 (X-ray repair cross complementing 2). *Atlas Genet Cytogenet Oncol Haematol,* 23(1**),** 1-7.

Bashir, N., Sana, S., Mahjabeen, I. & Kayani, M. A. (2014). Association of reduced XRCC2 expression with lymph node metastasis in breast cancer tissues. *Familial Cancer, 13*(4**),** 611-617.

Castelló, R., Estellés, A., Vázquez, C., Falcó, C., España, F., Almenar, S. M., Fuster, C. & Aznar, J. (2002). Quantitative real-time reverse transcription-PCR assay for urokinase plasminogen activator, plasminogen activator inhibitor type 1, and tissue metalloproteinase inhibitor type 1 gene expressions in primary breast cancer. *Clin Chem, 48*(8**),** 1288-95.

Chen, Y., Li, Z., Xu, Z., Tang, H., Guo, W., Sun, X., Zhang, W., Zhang, J., Wan, X., Jiang, Y. & Mao, Z. (2018). Use of the XRCC2 promoter for in vivo cancer diagnosis and therapy. *Cell Death & Disease, 9*(4**),** 420.

Gok, I., Baday, M., Cetinkunar, S., Kilic, K. & Bilgin, B. C. (2014). Polymorphisms in DNA repair genes XRCC2 and XRCC3 risk of gastric cancer in Turkey. *Bosn J Basic Med Sci, 14*(4), 214-8.

Ho-Pun-Cheung, A., Bascoul-Mollevi, C., Assenat, E., Boissière-Michot, F., Bibeau, F., Cellier, D., Ychou, M. & Lopez-Crapez, E. (2009). Reverse transcription-quantitative polymerase chain reaction: description of a RIN-based algorithm for accurate data normalization. *BMC Mol Biol, 10,* 31.

Kluźniak, W., Wokołorczyk, D., Rusak, B., Huzarski, T., Gronwald, J., Stempa, K., Rudnicka, H., Kashyap, A., Dębniak, T., Jakubowska, A., Lener, M., Szwiec, M., Tomiczek-Szwiec, J., Jarkiewicz-Tretyn, J., Cechowska, M., Domagała, P., Szymiczek, A., Bagherzadeh, M., Lubiński, J., Narod, S. A., Akbari, M. R., Cybulski, C., Bębenek, M., Godlewski, D., Gozdecka-Grodecka, S., Goździ, S., Haus, O., Janiszewska, H., Jasiówka, M., Kilar, E., Kordek, R., Kozak-Klonowska, B., Książkiewicz, G., Mackiewicz, A., Marczak, E., Mituś, J., Morawiec, Z., Niepsuj, S., Sibilski, R., Siołek, M., Sir, J., Surdyka, D., Synowiec, A., Szczylik, C., Uciński, R., Waśko, B., Wiśniowski, R., Byrski, T., Górski, B. & The Polish Hereditary Breast Cancer, C. (2019). Inherited variants in XRCC2 and the risk of breast cancer. *Breast Cancer Research and Treatment, 178*(3**),** 657-663.

Liu, Q., Peng, Q., Zhang, B. & Tan, Y. (2023). X-ray cross-complementing family: The bridge linking DNA damage repair and cancer. *J Transl Med, 21*(1), 602.

Liu, Z., Zhang, W., Cheng, X., Wang, H., Bian, L., Wang, J., Han, Z., Wang, Y., Lian, X., Liu, B., Ren, Z., Zhang, B., Jiang, Z., Lin, Z. & Gao, Y. (2021). Overexpressed XRCC2 as an independent risk factor for poor prognosis in glioma patients. *Mol Med, 27*(1), 52.

Malone, E. R., Oliva, M., Sabatini, P. J. B., Stockley, T. L. & Siu, L. L. (2020). Molecular profiling for precision cancer therapies. *Genome Medicine, 12*(1), 8.

Mohamed, R. I., Bargal, S. A., Mekawy, A. S., El-Shiekh, I., Tuncbag, N., Ahmed, A. S., Badr, E. & Elserafy, M. (2021). The overexpression of DNA repair genes in invasive ductal and lobular breast carcinomas: Insights on individual variations and precision medicine. *Plos One, 16*(3), e0247837.

Moon, J., Kitty, I., Renata, K., Qin, S., Zhao, F. & Kim, W. (2023). DNA Damage and ıts role in cancer therapeutics. *International Journal of Molecular Sciences, 24*(5**),** 4741.

Perron, G., Jandaghi, P., Solanki, S., Safisamghabadi, M., Storoz, C., Karimzadeh, M., Papadakis, A. I., Arseneault, M., Scelo, G., Banks, R. E., Tost, J., Lathrop, M., Tanguay, S., Brazma, A., Huang, S., Brimo, F., Najafabadi, H. S. & Riazalhosseini, Y. (2018). A general framework for ınterrogation of mRNA stability programs ıdentifies RNA-binding proteins that govern cancer transcriptomes. *Cell Reports, 23*(6), 1639-1650.

Prime, S. S., Darski, P., Hunter, K. D., Cirillo, N. & Parkinson, E. K. (2024). A review of the repair of DNA double strand breaks in the development of oral cancer. *International Journal of Molecular Sciences, 25*(7**),** 4092.

Shi, Y., Shen, M., Xu, M., Tao, M., Chen, K. & Zhu, Q. (2022). Comprehensive analysis of the expression and prognosis for RAD51 family in human breast cancer. *Int J Gen Med,* 15, 4925-4936.

Siegel, R. L., Giaquinto, A. N. & Jemal, A. (2024). Cancer statistics, 2024. *CA: A Cancer Journal for Clinicians, 74*(1**),** 12-49.

Velaga, R. & Toi, M. (2022). Advances in molecular medicine for breast cancer practice: a narrative review. *Annals of Breast Surgery, 7,* 38-49.

Qureshi, Z., Mahjabeen, I., Baig, R. & Kayani, M. (2015). Correlation between selected XRCC2, XRCC3 and RAD51 gene polymorphisms and primary breast cancer in women in Pakistan. *Asian Pacific Journal of Cancer Prevention, 15*(23), 10225-10229.

Wei-Yu, L., Nicola, J. C., Lisa, A. C.-A., Kristina, A. B., Sabapathy, B., Malcolm, W. R. R., John, L. H., Carmel, A., Graham, G. G., Melissa, C. S., Roger, L. M., Jose, I. a.-P., Primitiva, M.-R., Javier, B., Magdalena, G., Natalia, D., Tjoung-Won, P.-S., Thilo, D., Montserrat, G.-C., Jonine, F., Mark, S., Jolanta, L., Douglas, F. E., Alison, M. D., Preetha, R., Alice, J. S., Michele, M. D., Martha, S. L., Paul, D. P., Marjanka, K. S., & Angela, C. (2011). A role for XRCC2 gene polymorphisms in breast cancer risk and survival. *Journal of Medical Genetics, 4*8(7), 477.

Wilkinson, L. & Gathani, T. (2022). Understanding breast cancer as a global health concern. *Br J Radiol, 95*(1130)**,** 20211033.

Xu, K., Song, X., Chen, Z., Qin, C., He, Y. & Zhan, W. (2014). XRCC2 promotes colorectal cancer cell growth, regulates cell cycle progression, and apoptosis. *Medicine (Baltimore), 93*(28), e294.

Yu, J. & Wang, C. G. (2023a). Relationship between polymorphisms in homologous recombination repair genes RAD51 G172T、XRCC2 & XRCC3 and risk of breast cancer: A meta-analysis. *Front Oncol,* 13(1)**,** 1047336.

Zhang, Y.-Z., An, J. H., Liu, Y. X., Wu, X. C., Han, S. S., Ren, X. Q. & Qin, C.-J. (2017). XRCC2-deficient cells are highly sensitive to 5-Fluorouracil in colorectal cancer. *Cellular Physiology and Biochemistry, 43*(3), 1207-1219.

Zheng, Z., Ng, W. L., Zhang, X., Olson, J. J., Hao, C., Curran, W. J. & Wang, Y. (2012). RNAi-mediated targeting of noncoding and coding sequences in DNA repair gene messages efficiently radiosensitizes human tumor cells. *Cancer Res, 72*(5), 1221-8.

Author Information

Naser Gilani
Gaziantep University
Gaziantep, Türkiye

Mehmet Ozaslan
Gaziantep University, Department of Biology. 27310
Gaziantep - Turkiye
Contact e-mail: *ozaslanmd@yahoo.com*

To cite this article:

Gilani, N. & Ozaslan, M. (2024). Evaluation of XRCC2 expression in breast cancer. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 1-8.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 9-19

ICGeHeS 2024: International Conference on General Health Sciences

Mathematical Models for Tuberculosis Disease Transmission in Southeast Asia: A Systematic Literature Review

Zulfaidil Zulfaidil
Institut Teknologi Bandung

Haryanto Haryanto
Universitas Pendidikan Indonesia

Sri Redjeki Pudjaprasetya
Institut Teknologi Bandung

Warsoma Djohan
Institut Teknologi Bandung

Abstract: Research on tuberculosis (TB) continues to be a primary focus, given its status as a serious threat to global health, including in the Southeast Asia region. Enhancing understanding of the TB transmission model is imperative, with the main objectives of this study being (1) identifying developed models, (2) describing the study methodologies employed, and (3) identifying proposed interventions within these models. By applying for a Systematic Literature Review following the 2020 PRISMA guidelines, we successfully collected 872 articles from the Scopus database, specifically focusing on those studying TB spread through compartmental mathematical models. However, only 21 articles met the eligibility criteria for further analysis utilizing the meta-synthesis analysis method. Each article was then thoroughly analyzed to identify its characteristics and research context. Various interventions proposed in each model were evaluated, identified, and summarized to understand the potential for model development in future research. The entire content of the articles discusses the role of mathematics in analyzing TB models and transmission studies, with various interventions explained in detail. The results of the analysis indicate that the mathematical modelling of TB transmission can be enhanced by developing models with direct and indirect interventions for the human population. Various approaches in tuberculosis transmission dynamics, including compartmental models and spatial modelling techniques, are highlighted in this research. Evaluating the effectiveness of interventions and control measures implemented in the models also serves as a focal point to assess their impact on TB spread. This review contributes to synthesizing existing knowledge, identifying research gaps, and highlighting opportunities for future advancements in mathematical modelling for TB control strategies in the Southeast Asia region.

Keywords: Tuberculosis disease, Mathematical models, Systematic literature review

Introduction

Tuberculosis (TB) remains a significant global health challenge, posing hurdles to healthcare systems worldwide. This infectious disease is caused by Mycobacterium tuberculosis, primarily affecting the lungs but potentially impacting other body parts and spreading to others through respiratory droplets in the air (Campo & Kawamura, 2017). *Mycobacterium tuberculosis* plays a crucial role in granuloma formation and maintenance, ensuring disease dissemination and preventing its progression to severe illness (Russell, 2007). Its transmission involves numerous factors, including respiratory illness, bacilli release, environmental survival, and host defenses, with gaps in knowledge and limited animal models (Turner et al., 2017). Despite significant progress

in prevention and treatment, TB continues to afflict millions each year, especially in resource-limited settings where factors such as poverty, population density, and lack of healthcare access contribute to its transmission and persistence. Understanding TB transmission is vital for developing effective control strategies and reducing its burden on global public health systems. Hence, it is crucial to explore various aspects of TB transmission, including its routes, risk factors, and the importance of early detection and intervention in controlling its spread.

In Southeast Asia, TB prevalence remains high, with thousands of new cases reported annually. The WHO Southeast Asia Region accounted for 45% of new TB cases and 50% of global TB deaths in 2021, significantly supporting global TB efforts (Bhatia et al., 2023). Moreover, poor TB detection in Southeast Asia, where a third of cases go undetected or untreated outside national health programs, could hinder the region's goal to reduce TB prevalence and deaths by half from 1990 levels by 2015 (Padma, 2010). Thus, a deep understanding of TB transmission in Southeast Asia is crucial for designing effective intervention strategies. Efforts to raise public awareness about the importance of early detection, easier access to healthcare services, and promoting healthy lifestyles are critical steps in addressing the TB burden in this region. Therefore, it is important to delve into greater detail about the factors influencing TB transmission in Southeast Asia and the strategies that can be adopted to reduce its impact on public health.

Mathematical modelling is a valuable tool in understanding, analyzing, and forecasting real-world phenomena in various fields, including epidemiology and infectious disease control, aiding in constructing and utilising epidemiological models (Garnett, 2002). Mathematical models represent a system, process, or relationship using mathematical concepts and equations. By translating complex phenomena into mathematical language, we can gain insights, make informed decisions, and solve problems efficiently. In this exploration of mathematical modelling, we will delve into the various applications and methodologies behind its creation. From differential equations describing celestial motion to optimization models guiding business strategies, mathematical modelling bridges theoretical understanding and practical solutions.

As previously established, the complex transmission of Tuberculosis (TB) poses a barrier to effective control and prevention strategies. In the effort to understand and address this public health threat, mathematical modelling emerges as a powerful tool. By summarizing the intricate interactions among biological, social, and environmental factors, mathematical models offer invaluable insights into TB transmission patterns and the impact of interventions. In the realm of mathematical modelling in the context of TB, we explore various approaches, assumptions, and outcomes that shape our understanding of disease spread. Through the lens of mathematical abstraction, we unravel the dynamics of TB transmission within populations, providing insight into key factors such as transmission routes, latent periods, and host susceptibility.

In the complex landscape of public health, the battle against tuberculosis (TB) in Southeast Asia poses a serious challenge. With diverse demographics, socioeconomic factors, and varying healthcare infrastructures, this region has become a complex environment in which to understand and address the spread of this infectious disease (Pramono, 2021). In this endeavor, mathematical modeling emerges as a vital ally, offering a systematic approach to uncovering the complex dynamics of TB transmission. TB transmission models exhibit considerable discrepancies regarding the progression of the disease to active TB, with 40% of outcomes being more than double or less than half of empirical estimates (Menzies et al., 2018). This systematic literature review embarks on a journey through the broad realm of mathematical models used to analyze TB transmission dynamics in Southeast Asia. Through careful analysis and synthesis of existing research, this study aims to provide a comprehensive overview of the methodologies, findings, and insights gained from mathematical modelling efforts in this region. By delving into various mathematical models, ranging from compartmental models to agent-based simulations, this review seeks to uncover fundamental patterns of TB transmission dynamics across different settings, populations, and epidemiological contexts in Southeast Asia. Through the lens of mathematical abstraction, we strive to understand the complex interactions among factors influencing TB spread, including demographic characteristics, healthcare access, treatment adherence, and social determinants of health.

Furthermore, this review seeks to evaluate the strengths, limitations, and gaps in existing mathematical modelling approaches, providing insights into areas ready for further investigation and refinement. By synthesizing various studies, we hope to draw conclusions that can form the basis for developing and implementing TB control and eradication interventions in Southeast Asia. Essentially, this systematic literature review is a collective effort to harness the power of mathematical modelling as a guide in the fight against TB in Southeast Asia. By uncovering the intricate web of TB transmission dynamics, we aim to pave the way for evidence-based strategies that have the potential to reduce the TB burden and protect the health and well-being of communities in this region. Research on mathematical modelling for tuberculosis (TB) has been extensive.

Therefore, we aim to enhance understanding of TB transmission models by identifying developed models, explaining the research methodologies used, and identifying proposed interventions in these models. Hopefully, this review contributes to synthesizing existing knowledge, identifying research gaps, and highlighting opportunities for future advancements in mathematical modelling for TB control strategies in the Southeast Asia region.

Method

This section outlines the research methodology, the PRISMA method. The PRISMA method, also known as Preferred Reporting Items for Systematic Reviews and Meta-Analyses, is a manuscript methodology that must undergo a selection process. Using this strategy, article elements included in the database are identified through analysis (Moher et al., 2009; Stovold et al., 2014). It addresses the basic research questions used to find, select, and evaluate related research to understand the research problem.

Table 1. Eligibility criteria

No.	Inclusion criteria	Exclusion criteria
1.	Article published between 2013 and 2023.	The articles are not in English.
2.	Journal articles only	The articles are not open-access
3.	Published as final peer review	The articles are not in Southeast Asia

Articles were found using the keywords "Mathematical Model" AND "Tuberculosis" in the Scopus database. A maximum of 872 results were available for selection, with publication years restricted between 2013 and 2023. We summarized this field's latest advancements using references encompassing current research. We identified 21 articles relevant to these terms through this technique for general understanding. Figure 1 illustrates the selection process.

As depicted in Figure 1, the article under analysis underwent a series of stages before publication, including identification, screening, and selection. The initial stage involved limiting the years and using predetermined keywords to retrieve 872 articles from the Scopus database. In the second round, 872 items were examined, with 843 disqualified according to the specified inclusion and exclusion criteria (see Table 1). During this screening process, the primary focus was on articles conducted or affiliated with the Southeast Asian region.

The remaining articles passed through thorough analysis in the third step. In the final phase, an analysis was conducted to address the research question. This analysis was carried out on 29 publications selected for systematic review. Before conducting the analysis, the abstracts of each article were carefully reviewed to identify relevant themes or subthemes. Subsequently, each article was investigated further to gather more in-depth data aligned with the research objectives. Thus, 21 articles were selected for further examination within the framework of this study. These stages demonstrate meticulous and systematic efforts to ensure that the selected articles meet the established criteria and are relevant to the research question.

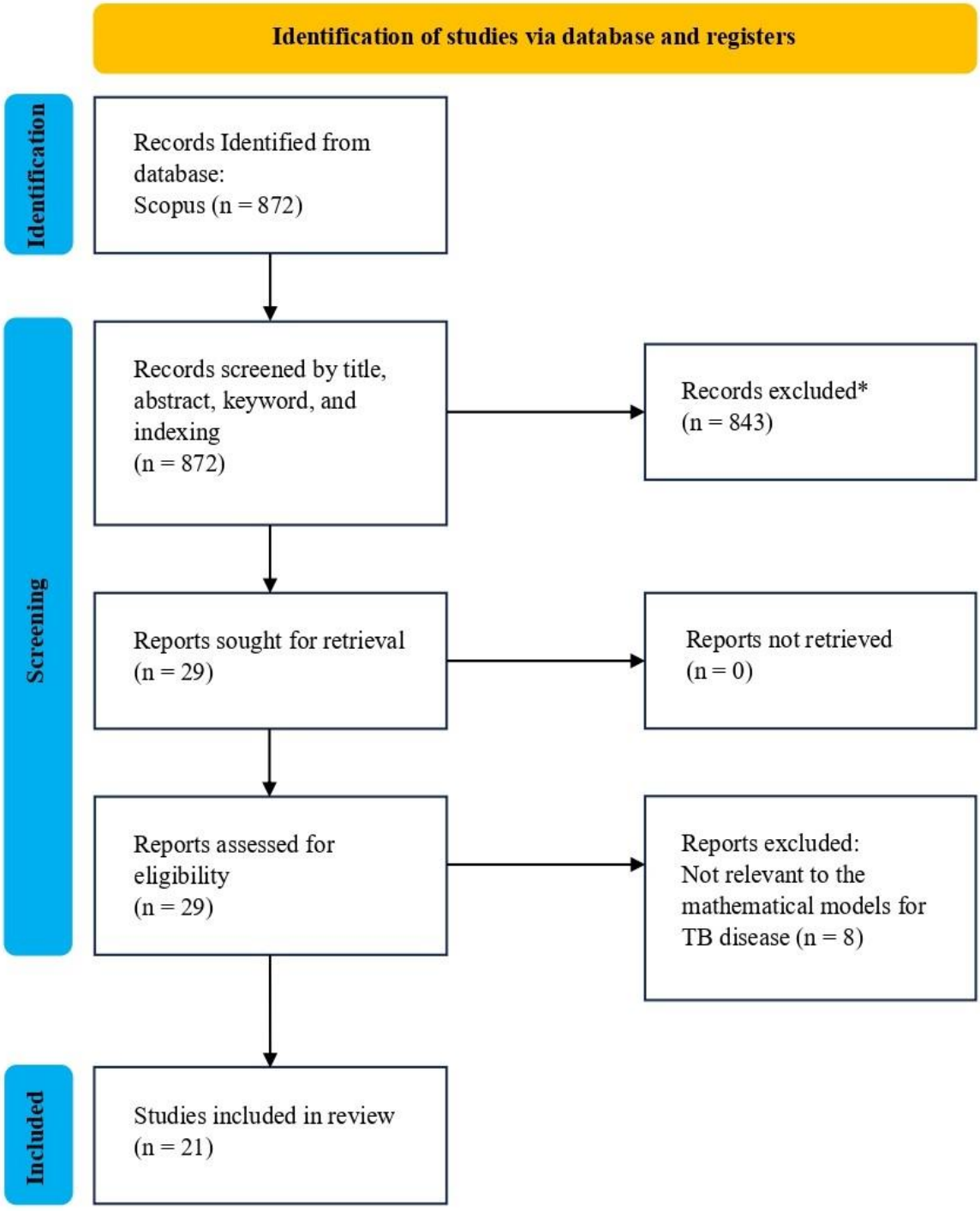
Results and Discussion

Identifying Models

The first mathematical model to simulate the spread of tuberculosis was introduced by (Waaler et al., 1962). They divided the population into three classes based on the epidemiological characteristics of the disease. This model provided a mathematical representation of the TB spreading process and laid the foundation for an initial understanding of the disease dynamics. Subsequently, the model by (Revelle et al., 1967) was further developed by (Revelle et al., 1967) using a system of nonlinear ordinary differential equations.

The resulting model introduced the concept that the spread of tuberculosis depends on the proportion of its prevalence, providing a more accurate depiction of how the number of infected individuals in the population influences TB spread. Over time, this model continued to evolve and was utilized to discuss strategies for controlling the spread of tuberculosis. This included research on the effectiveness of various interventions such as anti-TB immunization programs, sanitation programs, and treatment. By employing these models, researchers could predict the impact of various control strategies and identify the most effective approaches to reducing TB transmission in communities. Essentially, these models provide an important mathematical framework for understanding and addressing the spread of TB and guiding the development of more effective policies and

interventions to control this disease. We explored all identified papers, including authors, compartment notation, and whether they were analyzed mathematically (see Table 2). Table 3 explains the notation of each compartment. The aim is to introduce the entire article and obtain some basic information for further exploration



*Note:

1. The publication does not comprise the final article (n = 218).
2. The publication does not include the journal source type (n = 5).
3. The publication is not in English (n = 12).
4. The publication is not open access (n = 198).
5. The publication does not originate from Southeast Asia (n = 410).

Figure 1. The flowchart of the selection process.

Table 2. Identification of compartments on articles

Name, Year	Cited	Analyzed Mathematically	Compartment	Interventions
Ahmadin & Fatmawati, (2014)	14	Yes	$S_1, I_S, I_r, R_1, S_2, I_2, R_2$	Treatment
Vinh et al., (2018)	2	No	$U, L, ExPTBn, PTBn,$ $U_h, L_h, ExPTBn_h, PT_i$	-
Aldila et al., (2019)	9	Yes	$x_1, x_2, x_3, y_1, y_2, y_3, y_4$	Medical mask intervention, Quarantine on cattle
Gomes et al., (2019)	9	No	U_i, P_i, I_i, L_i	-
Fatmawati et al., (2020)	43	Yes	$S_C, L_C, I_C, S_A, L_A, I_A$	-
Kabunga et al., (2020)	27	Yes	$S, L_e, L_f, I, R_1, R_2, T, K$	Prevention, Screening, Treatment
Kim et al., (2020)	17	No	S, E, I, L	Educational health campaigns, Treatment, Quarantine, Isolation
Han et al., (2021)	4	No	DS and MDR	Treatment
Kasbawati et al., (2021)	1	Yes	S, E, I, I_h, R	Treatment
Rahman et al., (2021)	49	Yes	$\mathbb{S}, \mathbb{E}, \mathbb{I}, \mathbb{T}, \mathbb{R}$	Treatment
Sulayman et al., (2021)	21	Yes	S, V, E, I, R, E	Vaccination
Weerasuriya et al., (2021)	15	No	-	Vaccination, Therapy
Biswas et al., (2022)	1	Yes	$S, L_T, I_H, H_S, E_{TH}, E_{HT_i}$	Vaccination, Treatment
Inayaturohmat et al., (2022)	7	Yes	$S, I_C, I_T, I_{TC}, Q, T, R$	Isolation, Treatment
Nuraini et al., (2022)	3	Yes	$S, Q_1, E_{co}, I_{co}, R_{co}, Q_2,$	Quarantine
Qu et al., (2022)	9	Yes	S, E, I, T, R	-
Singh et al., (2023)	5	Yes	S, E_H, E_C, I, R	-
Chukwu et al., (2023)	3	Yes	S, E, I, R	-
Muhafzan et al., (2023)	1	Yes	S, I, T, R	Treatment
Tamhaji & Hamdan, (2023)	-	Yes	B, S, E, I, R	Vaccination
Vo et al., (2023)	-	No	SROI	The economic evaluation of healthcare interventions

Table 2 provides details of the identities of 21 new relevant articles. From the data, it can be inferred that the work by (Rahman et al., 2021) is the most frequently cited, with 49 citations. Meanwhile, the studies conducted by (Tamhaji & Hamdan, 2023; Vo et al., 2023) have never been cited in the recorded literature. The article by (Vinh et al., 2018) developed a mathematical model depicting Tuberculosis (TB) transmission by dividing the population into two groups, namely G1 (non-hyper-susceptible) and G2 (hyper-susceptible). In the model they presented, it was assumed that the transition of individuals from G1 to G2 occurs uniformly across TB-affected regions. As a result, the resulting model has 14 compartments depicting transmission dynamics, which appear complex even though G1 and G2 essentially have similar characteristics. Additionally, (Nuraini et al., 2022) has developed a mathematical model explaining the impact of COVID-19 quarantine on TB and Diabetes Mellitus cases. This article also involves the formation of 12 compartments in its model, indicating high complexity in its resolution.

Table 3. Notations description

Notations	Description	Additional
$\mathbb{S}, S, S_1, S_2$	Susceptible population/individuals	-
S_C	Susceptible children	-
S_A	Susceptible adults	-
x_1	Susceptible cattle	-
y_1	Susceptible human who has direct contact with cattle	-
z_1	The susceptible human who has no direct contact with cattle	-
$\mathbb{E}, E$	Exposed population/individuals, high-risk latent	-
E_H	Exposed Tuberculosis	-
E_C, E_{co}	Exposed COVID-19/coronavirus	-
E_{TH}	infected with HIV (pre-AIDS) exposed to TB	-
E_{HT}	AIDS individuals exposed to TB	-
E_{T0}	Exposed to TB only	-
x_2	Exposed cattle	-
y_2	Exposed human who has direct contact with cattle	-
z_2	Exposed human who has no direct contact with cattle	-
$\mathbb{I}, I, I_2$	Infectious population/individuals	-
I_C, I_{co}	Infectious children, Infectious with COVID-19/coronavirus	-
I_A	Infectious adults	-
I_T, I_{tb}	Infectious with tuberculosis	-
I_{TC}	Individuals coinfected with COVID-19 and tuberculosis	-
I_s	infected sensitive	-
I_r	resistant to the class of anti-TB drugs	-
I_H	Infectious with HIV only	-
I_h	Infectious and hospitalized	-
I_i	Active tuberculosis disease	-
x_3	Infectious cattle	-
y_3	Infectious human who has direct contact with cattle	-
z_3	Infectious human who has no direct contact with cattle	-
$\mathbb{T}, T$	Treatment, Transferred	-
$\mathbb{R}, R, R_1, R_2$	Recovered population/individuals	-
R_h	Recovered hyper-susceptible	-
R_T	Recovered with temporal immunity	-
R_{co}	Recovered coronavirus	-
R_{tb}	Recovered tuberculosis	-
y_4	Recovered human who has direct contact with cattle	-
z_4	Recovered human who has no direct contact with cattle	-
L_C	Latent TB children	-
L_A	Latent TB adults	-
L_e	Latent early	-
L_f	Latent late	-
L	Low-risk latent	-
L_i	Latent infection	-
L_h	Latent hyper-susceptible	-
L_T	Latent TB with no HIV	-
L_{tb}	Latent tuberculosis	-

K	People who stop treatment	-
V	Vaccinated	-
B	BCG vaccinated	-
DS	Drug-susceptible	*DS* refers to cases of TB that are susceptible to standard treatment with commonly used anti-TB drugs.
MDR	Multidrug-resistant	*MDR* refers to cases of TB that are resistant to standard treatment with the most used anti-TB drugs.
SROI	the Social Return on Investment	SROI is a method for measuring values that are not traditionally reflected in financial statements, including social, economic, and environmental factors.
Q	Isolated with COVID-19 infection	-
Q_1	Quarantined susceptible	-
Q_2	Quarantined infected coronavirus	-
S_T	Symptomatic TB	Symptomatic TB refers to the stage of (TB) infection where an individual exhibits noticeable symptoms of the disease.
H_S	HIV infected displaying AIDS symptoms	-
H_{DT}	AIDS individuals dually infected with TB	-
D_{tb}	Diagnosed tuberculosis	-
D_{dm}	Diabetes without complications	-
C_{dm}	Diabetes with complications	-
U_i	Uninfected	-
U_h	Uninfected hyper-susceptible	-
P_i	Primary infection	Primary infection is a subpopulation that is infected for the first.
$ExPTBn$	Active extra-pulmonary TB new cases	-
$PTBn$	Active pulmonary TB new cases	-
$ExPTBr$	Active extra-pulmonary TB relapsed cases	-
$PTBr$	Active pulmonary TB relapsed cases	-
$ExPTBn_h$	Active extra-pulmonary TB new cases of hyper-susceptible	-
$PTBn_h$	active pulmonary TB new cases hyper-susceptible	-
$ExPTBr_h$	Active extra-pulmonary TB relapsed of cases of hyper-susceptible	-
$PTBr_h$	Active pulmonary TB relapsed cases of hyper-susceptible	-

Out of the 21 included articles, five of them (Chukwu et al., 2023; Fatmawati et al., 2020; Muhafzan et al., 2023; Qu et al., 2022; Rahman et al., 2021) incorporate their analyses using fractional models. This fractional model approach offers a robust framework for investigating TB transmission dynamics, predicting disease outcomes, optimizing intervention strategies, understanding complex transmission dynamics, and evaluating public health policies. The integration of fractional models with TB analysis in this context is expected to significantly contribute to designing more effective intervention strategies and estimating the impact of these interventions more accurately. On the other hand, three other articles (Inayaturohmat et al., 2022; Nuraini et al., 2022; Singh et al., 2023) evaluate the impact of the COVID-19 pandemic on TB cases. Reviewing this impact is important to understand how TB is managed during the pandemic, considering shifting priorities and limited healthcare resources. Analysis of how to address TB amidst the COVID-19 pandemic is crucial in formulating appropriate strategies to mitigate the dual impact of both diseases.

The development of these models is highly dependent on the assumptions used and the complexity of the problem under investigation. Therefore, more complex models may emerge in the future tailored to more complicated or specific issues. Advances in understanding disease dynamics, better data availability, and the development of mathematical and computational modeling techniques may enable the development of more sophisticated models.

In the context of infectious diseases such as tuberculosis, where factors such as variability in individual immunity, changes in social behavior, and population interactions play crucial roles in disease spread, more complex models may be required to account for all these variables accurately. Thus, ongoing research will continue to drive the development of more advanced models tailored to the complexity of the problems being studied, with the hope that these models will provide deeper insights and more effective solutions in addressing complex public health issues.

Identifying Research Methodology

The mathematical analysis in the mentioned articles represents a crucial approach to understanding the dynamics of diseases and the effectiveness of health interventions. In 15 out of the 21 papers, the authors employ various mathematical theories to analyze the epidemiological models under study. The aim of this analysis is to gain a deeper understanding of the positivity of solutions (i.e., whether the model solutions state the existence and uniqueness of solutions), solution constraints (such as whether the model solutions are bounded within a certain range), disease-free equilibrium points (i.e., situations where there are no disease cases), disease equilibrium points (i.e., situations where the disease persists), as well as the local and global stability of these equilibrium points (Aldila et al., 2019; Kabunga et al., 2020; Sulayman et al., 2021; Tamhaji & Hamdan, 2023). Additionally, several additional analyses have been conducted, such as bifurcation analysis to understand qualitative changes in system behavior when parameters change (Kabunga et al., 2020; Singh et al., 2023), and the utilization of continuous-time Markov chain models to account for uncertainty and stochasticity in the system ((Kasbawati et al., 2021)). Optimal control theory is utilized in several articles to analyze conditions where optimal control can be achieved by considering the associated control costs (Ahmadin & Fatmawati, 2014; Biswas et al., 2022; Singh et al., 2023).

While not all articles apply analytical approaches, some rely on primary data to simulate population dynamics (Han et al., 2021; Kim et al., 2020; Vinh et al., 2018), while others only use secondary data. Economic analyses are also outlined in several articles, including the economic evaluation of tuberculosis treatment models by community health workers and the importance of considering risk inequality in TB policy development (Ahmadin & Fatmawati, 2014; Quang Vo et al., 2023).Furthermore, the epidemiological, cost-effectiveness, and budgetary impacts of new vaccines for multidrug-resistant tuberculosis are also investigated (Weerasuriya et al., 2021).

Overall, the mathematical analysis and modeling in these articles provide valuable insights into understanding the dynamics of specific diseases and the effectiveness of different health interventions. They combine various mathematical methods and analytical approaches to generate a better understanding of the factors influencing disease spread and the effects of various health policies. Thus, these articles make significant contributions to the fields of epidemiology and public health, providing a foundation for the development of more effective interventions in disease control.

Identifying Interventions

Mathematical models for understanding and controlling the spread of tuberculosis (TB) have become a major focus in public health research. By considering the various interventions available, researchers are attempting to develop effective strategies to address this problem. Treatment is one of the most used interventions in TB control (Ahmadin & Fatmawati, 2014; Biswas et al., 2022; Han et al., 2021; Inayaturohmat et al., 2022; Kabunga et al., 2020; Kasbawati et al., 2021; Kim et al., 2020; Muhafzan et al., 2023; Rahman et al., 2021). Research has consistently confirmed that treatment is the best approach to immediately stop the spread of the disease. The focus of these interventions is to provide treatment to the infected population to stop the immediate spread of the disease. However, although treatment is an important step, it cannot completely prevent the spread of the disease. Therefore, preventive measures are very important in reducing the risk of infection. One preventive measure that has been widely studied is the use of medical masks to prevent transmission of TB through air and breathing (Aldila et al., 2019).

The safety of the human population is a major concern in studying the TB phenomenon. This is why some researchers have begun focusing on development models considering direct preventive measures against human populations. Vaccination, for example, has been shown to be effective in reducing the risk of infection in human populations (Biswas et al., 2022; Sulayman et al., 2021; Tamhaji & Hamdan, 2023; Weerasuriya et al., 2021). However, it is important to remember that although vaccination can be effective, no measure has yet been able

to eliminate this disease. Therefore, researchers also considering other preventive measures such as educational campaigns (Kim et al., 2020), quarantine (Aldila et al., 2019; Kim et al., 2020; Nuraini et al., 2022), and isolation (Inayaturohmat et al., 2022; Kim et al., 2020) to help increase public awareness and reduce the spread of TB.

Apart from that, health checks and screening are also important in early detection of TB infection (Kabunga et al., 2020). These measures should not be ignored as they can help break the chain of disease spread and provide timely treatment to infected individuals. By considering these various interventions, mathematical models can be an invaluable tool in planning effective strategies for controlling the spread of TB and protecting human populations from the risk of serious infection.

All articles are annotated to obtain the identity of the article and annotated to understand the research better. Based on 49 citations, some articles with more citations than others confirm that their investigative findings are useful for further research functions or even applied to real conditions. The model in each article is studied mathematically to obtain its behavior analytically and numerically. Various interventions are considered in the model to control diseases and analyze system behavior. This includes controlling and preventing infections in humans and vectors in the system both directly and indirectly.

Conclusion

The results of the analysis indicate that mathematical modeling of TB transmission can be enhanced by developing models with direct and indirect interventions for the human population. Various approaches in tuberculosis transmission dynamics, including compartmental models and spatial modelling techniques, are highlighted in this research. Evaluating the effectiveness of interventions and control measures implemented in the models also serves as a focal point to assess their impact on TB spread. This review contributes to synthesizing existing knowledge, identifying research gaps, and highlighting opportunities for future advancements in mathematical modeling for TB control strategies in the Southeast Asia region.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

* The author gratefully acknowledges Indonesia Endowment for Education for the funding through the LPDP Scholarship. Any remaining errors are the author's responsibility.

References

Ahmadin, & Fatmawati. (2014). Mathematical modeling of drug resistance in tuberculosis transmission and optimal control treatment. *Applied Mathematical Sciences*, *8*(92), 4547–4559.

Aldila, D., Latifah, S. L., & Dumbela, P. A. (2019). Dynamical analysis of mathematical model for Bovine Tuberculosis among human and cattle population. *Communication in Biomathematical Sciences*, *2*(1), 55–64.

Bhatia, V., Rijal, S., Sharma, M., Islam, A., Vassall, A., Bhargava, A., Thida, A., Basri, C., Onozaki, I., Pai, M., Rezwan, M. K., Arinaminpathy, N., Chandrashekhar, P., Sarin, R., Mandal, S., & Raviglione, M. (2023). Ending TB in South-East Asia: Flagship priority and response transformation. *The Lancet Regional Health - Southeast Asia*, *18*, 1–9.

Biswas, M. H. A., Samad, S. A., Parvin, T., Islam, M. T., & Supriatna, A. K. (2022). Optimal control strategy to Reduce the infection of pandemic HIV associated with tuberculosis. *Communication in Biomathematical Sciences*, *5*(1), 20–39.

Campo, M., & Kawamura, L. M. (2017). What is tuberculosis (TB)?. *American Journal of Respiratory and Critical Care Medicine*, *195*(4), 7–8.

Chukwu, C. W., Bonyah, E., Juga, M. L., & Fatmawati. (2023). On mathematical modeling of fractional-order stochastic for tuberculosis transmission dynamics. *Results in Control and Optimization*, *11*, 1–17.

Fatmawati, Khan, M. A., Bonyah, E., Hammouch, Z., & Shaiful, E. M. (2020). A mathematical model of tuberculosis (TB) transmission with children and adults groups: A fractional model. *AIMS Mathematics*, *5*(4), 2813–2842.

Garnett, G. P. (2002). An introduction to mathematical models in sexually transmitted disease epidemiology. *Sexually Transmitted Infections*, *78*, 7–12.

Gomes, M. G. M., Oliveira, J. F., Bertolde, A., Ayabina, D., Nguyen, T. A., Maciel, E. L., Duarte, R., Nguyen, B. H., Shete, P. B., & Lienhardt, C. (2019). Introducing risk inequality metrics in tuberculosis policy development. *Nature Communications*, *10*(2480), 1–12.

Han, W. M., Mahikul, W., Pouplin, T., Lawpoolsri, S., White, L. J., & Pan-Ngum, W. (2021). Assessing the impacts of short-course multidrug-resistant tuberculosis treatment in the Southeast Asia Region using a mathematical modeling approach. *PLoS One*, *16*(3), 1–14.

Inayaturohmat, F., Anggriani, N., & Supriatna, A. K. (2022). A mathematical model of tuberculosis and COVID-19 coinfection with the effect of isolation and treatment. *Frontiers in Applied Mathematics and Statistics*, *8*(958081), 1–13.

Kasbawati, Tungga, R. A., Jaya, A. K., & Kalondeng, A. (2021). A mathematical study of tuberculosis infections using a deterministic model in comparison with continuous Markov chain model. *Communications in Mathematical Biology and Neuroscience*, *2021*(25), 1–22.

Kabunga, S. K., Goufo, E. F. D., & Tuong, V. H. (2020). Analysis and simulation of a mathematical model of tuberculosis transmission in Democratic Republic of the Congo. *Advances in Difference Equations*, *2020*(642), 1–19.

Kim, S., De Los Reyes V, A. A., & Jung, E. (2020). Country-specific intervention strategies for top three TB burden countries using mathematical model. *PLoS One*, *15*(4), 1–18.

Menzies, N. A., Wolf, E., Connors, D., Bellerose, M., Sbarra, A. N., Cohen, T., Hill, A. N., Yaesoubi, R., Galer, K., White, P. J., Abubakar, I., & Salomon, J. A. (2018). Progression from latent infection to active disease in dynamic tuberculosis transmission models: a systematic review of the validity of modelling assumptions. *The Lancet Infectious Diseases*, *18*(8), e228–e238.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., Antes, G., Atkins, D., Barbour, V., Barrowman, N., Berlin, J. A., Clark, J., Clarke, M., Cook, D., D'Amico, R., Deeks, J. J., Devereaux, P. J., Dickersin, K., Egger, M., Ernst, E., Gøtzsche, P. C., … & Tugwell, P. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine*, *6*(7), 1–6.

Muhafzan, Zulakmal, Narwen, Baqi, A. I., Lestari, A. G., & Oktaviani, M. (2023). A fractional SITR MODEL for dynamıc of tuberculosıs spread. *Communications in Mathematical Biology and Neuroscience*, *2023*(14), 1–9.

Nuraini, N., Fauzi, I. S., Lestari, B. W., & Rizqina, S. (2022). The impact of COVID-19 quarantine on tuberculosis and diabetes mellitus cases: A Modelling Study. *Tropical Medicine and Infectious Disease*, *7*(407), 1–20.

Padma, T. V. (2010). Health officials warn of poor tuberculosis detection. *Nature Medicine*, *16*(4), 351.

Pramono, J. S. (2021). A literature review: risk factors of increasing tuberculosis incidence. *Jurnal Ilmiah PANNMED (Pharmacyst, Analyst, Nurse, Nutrition, Midwivery, Environment, Dentist)*, *16*(1), 106–113.

Qu, H., Rahman, M. U., Arfan, M., Laouini, G., Ahmadian, A., Senu, N., & Salahshour, S. (2022). Investıgatıng fractal-fractıonal mathematıcal model of tuberculosıs (tb) under fractal-fractıonal caputo operator. *Fractals*, *30*(5), 1–14.

Vo, L. N. Q., Forse, R. J., Tran, J., Dam, T., Driscoll, J., Codlin, A. J., Creswell, J., Sidney-Annerstedt, K., Van Truong, V., Thi Minh, H. D., Huu, L. N., Nguyen, H. B., & Nguyen, N. V. (2023). Economic evaluation of a community health worker model for tuberculosis care in Ho Chi Minh City, Viet Nam: a mixed-methods Social Return on Investment Analysis. *BMC Public Health*, *23*(945), 1–13.

Rahman, M. U., Arfan, M., Shah, Z., Kumam, P., & Shutaywi, M. (2021). Nonlinear fractional mathematical model of tuberculosis (TB) disease with incomplete treatment under Atangana-Baleanu derivative. *Alexandria Engineering Journal*, *60*, 2845–2856

Revelle, C. S., Lynn, W. R., & Feldmann, F. (1967). Mathematıcal models for the economıc allocatıon of tuberculosıs control actıvities in developıng natıons. *American Review of Respiratory Disease*, *96*(5), 893–909.

Russell, D. G. (2007). Who puts the tubercle in tuberculosis?. *Nature Reviews Microbiology*, *5*(1), 39–47.

Singh, R., Rehman, A. ul, Ahmed, T., Ahmad, K., Mahajan, S., Pandit, A. K., Abualigah, L., & Gandomi, A. H. (2023). Mathematical modelling and analysis of COVID-19 and tuberculosis transmission dynamics. *Informatics in Medicine Unlocked*, *38*, 1–13.

Stovold, E., Beecher, D., Foxlee, R., & Noel-Storr, A. (2014). Study flow diagrams in Cochrane systematic review updates: An adapted PRISMA flow diagram. *Systematic Reviews*, *3*(54), 1–5.

Sulayman, F., Abdullah, F. A., & Mohd, M. H. (2021). An SVEIRE model of tuberculosis to assess the effect of an imperfect vaccine and other exogenous factors. *Mathematics*, *9*(327), 1–23.

Tamhaji, N. H., & Hamdan, N. 'Izzati. (2023). The dynamics of tuberculosis through BSEIR model with immigration in Malaysia. *Malaysian Journal of Fundamental and Applied Sciences*, *19*, 1176–1189.

Turner, R. D., Chiu, C., Churchyard, G. J., Esmail, H., Lewinsohn, D. M., Gandhi, N. R., & Fennelly, K. P. (2017). Tuberculosis infectiousness and host susceptibility. *Journal of Infectious Diseases*, *216*, S636–S643.

Vinh, D. N., Ha, D. T. M., Hanh, N. T., Thwaites, G., Boni, M. F., Clapham, H. E., & Thuong, N. T. T. (2018). Modeling tuberculosis dynamics with the presence of hyper-susceptible individuals for Ho Chi Minh City from 1996 to 2015. *BMC Infectious Diseases*, *18*(494), 1–13.

Waaler, H., Geser, A., & Andersen, S. (1962). The use of mathematıcal models ın the study of the epıdemıology of tuberculosıs. *American Journal of Public Health and the Nation's Health*, *52*(6), 1002–1013.

Weerasuriya, C. K., Harris, R. C., McQuaid, C. F., Bozzani, F., Ruan, Y., Li, R., Li, T., Rade, K., Rao, R., Ginsberg, A. M., Gomez, G. B., & White, R. G. (2021). The epidemiologic impact and cost-effectiveness of new tuberculosis vaccines on multidrug-resistant tuberculosis in India and China. *BMC Medicine*, *19*(60), 1–13.

Author Information

Zulfaidil Zulfaidil
Institut Teknologi Bandung
Bandung, Indonesia
Contact e-mail: *20122025@mahasiswa.itb.ac.id*

Haryanto Haryanto
Universitas Pendidikan Indonesia
Bandung, Indonesia

Sri Redjeki Pudjaprasetya
Institut Teknologi Bandung
Bandung, Indonesia

Warsoma Djohan
Institut Teknologi Bandung
Bandung, Indonesia

To cite this article:

Zulfaidil, Z., Haryanto, H. Pudjaprasetya, S. R., & Djohan, W. (2024). Mathematical models for tuberculosis disease transmission in Southeast Asia: A systematic literature review. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 9-19.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 20-31

ICGeHeS 2024: International Conference on General Health Sciences

Bibliometric Analysis of the Impact of COVID-19 on Athlete Performance: Publication Trends and Implications for Future Research

Muh. Aswar
Universitas Pendidikan Indonesia

Jajat Darajat Kusumah Nagara
Universitas Pendidikan Indonesia

Haryanto Haryanto
Universitas Pendidikan Indonesia

Syaipul Hari Baharuddin
Universitas Pendidikan Indonesia

Daniel Assetiawan Iriana
Universitas Pendidikan Indonesia

Abstract: The COVID-19 pandemic has significantly affected various sectors, including the realm of sports. The importance of researching the impact of COVID-19 on athlete performance lies in the urgency to prevent similar repercussions in the future. Therefore, this study aims to explore publication trends related to the influence of COVID-19 on athlete performance from 2020 to 2023, using bibliometric analysis methods. In this analysis, VOSviewer software and the bibliometric analysis application biblioshiny were utilized to analyze bibliographic data. From the Scopus database, a total of 977 documents were analyzed after undergoing the screening process. The results indicate fluctuations in publication trends, with the International Journal of Environmental Research and Public Health being the highest contributor with 87 documents. The United States ranks first in the number of documents, with a primary focus on athlete performance, totaling 907. Semmelweis University emerged as the most productive affiliation with 25 documents. Research findings highlight contributions from 19 Indonesian researchers, with the highest number of authors originating from the United States with 14 documents. The most globally cited document, published in the British Journal of Sports Medicine, has reached 387 citations. Visualization of research trends reveals popular topics aligning with the research, including "human," "Covid-19," "male," "female," "athlete," "adult," and "pandemic." The implications of these findings provide significant benefits to researchers by offering guidance for future analysis and serving as considerations in determining research themes. Furthermore, this supports global efforts to enhance understanding of the pandemic's impact on athlete performance and design more effective strategies to protect their health and well-being in the future.

Keyword: Biblimetric analysis, Covid-19, Athlete performance

Introduction

The COVID-19 outbreak has changed the way people engage in physical activity, making it difficult for many to meet recommended levels due to major changes in their lifestyles. (Ha & Dauenhauer, 2020). The importance of physical activity in preventing and treating COVID-19, as well as improving psychological well-being, has been clearly proven (Yang et al., 2022). Especially in the middle of the quarantine phase, the interrelationship

between physical activity and mental health becomes more pronounced (Khosravi, 2020). Therefore, it is important to find methods to maintain physical activity levels during the pandemic for the general well-being.

Throughout this pandemic, athletes have faced many obstacles in maintaining their performance. Home workouts during lockdown have resulted in irregular changes in physical performance and decreased motivation, especially among elite athletes. (Paludo et al., 2022). The performance of the top 10 athletes has changed due to a significant decrease in their performance levels. (Schipman J., 2022). The financial side of the sports industry has also suffered, adding to the difficulties experienced by athletes (Wiltshire et al., 2022). However, retraining has shown effectiveness in mitigating the adverse impact of the pandemic on the performance load and performance of elite athletes, with performance returning to normal levels within a period of time (Valenzuela et al., 2021).

The psychological impact of COVID-19 on athletes deserves attention. Research shows that athletes who contract the virus tend to face increased levels of depression, anxiety, and stress, especially those who are losing income and belong to certain age groups. (Sanborn et al., 2021: Factors such as age, having children, and smoking are also associated with a higher risk of virus transmission among athletes (Lopes et al., 2021). Therefore, elite athletes are advised to undergo regular RT-PCR tests to monitor and curb the spread of the virus. (Rankin et al., 2021).

Studies on the impact of COVID-19 on athlete performance show significant variations. Some studies find that athletes experience various symptoms, with women and those experiencing severe symptoms tending to experience exercise disturbances (Kim et al., 2023). There are also reports of inconsistent effects from home training during lockdowns, with significant decreases in motivation and perceived effort (Paludo et al., 2022). However, there are also interesting findings that the vertical jump performance of volleyball players can improve even when they are infected with COVID-19, highlighting the complexity of the pandemic's impact on athlete performance (Orscelik et al., 2022).

The primary aim of this study is to conduct a thorough examination of how the COVID-19 pandemic has affected athlete performance, with a specific focus on various aspects such as publication trends, international collaboration, document sources, and research dissemination. By delving into these areas, we aim to gain a comprehensive understanding of the impact of COVID-19 on athletes and the broader research landscape surrounding this topic.

To achieve this overarching goal, we have formulated a series of research questions that will guide our investigation:

1. Understanding Publication Productivity Trends: Through the utilization of VOSviewer analysis, we seek to unravel the trends in publication productivity pertaining to the impact of COVID-19 on athlete performance. By employing this method, we aim to discern how research output in this area has evolved over time and identify any notable patterns or fluctuations.
2. Analyzing Publication Trajectories: By analyzing publication trajectories spanning the past decade, we aim to uncover patterns and fluctuations in the number of publications related to the impact of COVID-19 on athletes. This analysis will provide insights into the trajectory of research in this field and highlight any significant shifts in focus or intensity over time.
3. Insights into International Collaboration and Knowledge Dissemination: We aim to explore the dynamics of international collaboration and the dissemination of knowledge among the global research community in COVID-19 impact research. By examining collaboration patterns and knowledge-sharing networks, we seek to understand how researchers from different countries collaborate and disseminate their findings in this field.
4. Examining Document Sources and Journal Contributions: Our study will delve into the document sources and journal contributions in analyzing athlete performance amid the COVID-19 pandemic. Through this examination, we aim to identify the key sources of research and assess the contributions of different journals to the advancement of knowledge in this area.
5. Dispersal of Research across Disciplines: We will investigate the dispersal of research on the impact of COVID-19 on athletes across various disciplines. This analysis will provide insights into the interdisciplinary nature of research in this field and shed light on the diverse perspectives and methodologies employed by researchers.
6. Identifying Leading Countries and Institutions: Our study aims to identify the leading countries and institutions in analyzing the impact of COVID-19 on athletes and assess their contributions to the research.

By identifying the most prolific countries and institutions, we aim to understand the global distribution of research activity in this field.

7. Key Authors and Their Contributions: We will map the landscape of COVID-19 impact research on athletes by identifying key authors and assessing their contributions. This analysis will highlight the pivotal role played by individual researchers in shaping the direction and progress of research in this area.
8. Network Visualization and Cluster Analysis: Through network visualization and cluster analysis, we aim to gain a deeper understanding of research focuses and trends in the field of COVID-19 impact on athletes. By visualizing the connections between key terms and concepts, we seek to uncover underlying patterns and thematic clusters within the research literature.
9. Overlay and Density Visualizations: We will track the evolution of research focuses and trends using overlay and density visualizations. These visualizations will enable us to identify changes in research interests over time and track the emergence of new areas of focus within the field.
10. Utilizing VOSviewer for Research Planning and Identifying Future Directions: Finally, we will explore how VOSviewer can be utilized for research planning and identifying future directions in the study of COVID-19 impact on athletes. By leveraging this tool, we aim to identify gaps in the existing literature and pinpoint areas that warrant further investigation.

Through a comprehensive exploration of these research questions, we seek to contribute to a deeper understanding of the impact of COVID-19 on athlete performance and provide valuable insights for future research directions in this rapidly evolving field.

Method

Data Source

A structured literature review is an important instrument for identifying gaps in knowledge as well as research needs. (García-Peñalvo, 2022). When conducting a bibliometric review, it is important to outline precisely the scope of the study and describe the methods used to monitor related sources. The current investigation extracted bibliometric information from the Scopus database, which is known for its wide coverage of scientific journal articles, including abstracts and citations. (Mongeon & Paul-Hus, 2016 ; Sweileh, 2020 ; Kussainova et al., 2024). Traditionally, Scopus and Web of Science are the two databases most commonly utilized for bibliometric analysis. (Singh et al., 2021).

Bibliometric analysis techniques are employed to condense bibliographic datasets. This approach reveals the structural, social, and author networks, along with the prevailing analytical focuses within particular research domains. (Alsolbi et al., 2022; Öztürk et al., 2024 ; Donthu et al., 2021; (Sweileh, 2020). Through examination of citation patterns, publication trends, and co-authoring networks, bibliometric analysis offers valuable insights into the research landscape and knowledge dissemination. (Oliveira et al., 2019). This helps in finding lead authors, important papers, and new research directions, as well as areas of literature that require further study. (Alsolbi et al., 2022) In addition, bibliometric analysis can evaluate the influence and prominence of research by analyzing the number of citations and publication metrics. This helps in assessing authors, institutions, and funding bodies. (Alsolbi et al., 2022) . Figure 1 illustrates the implementation of these five steps, emphasizing bibliometric methods

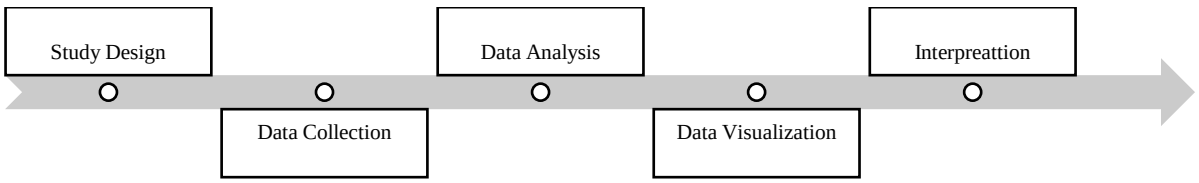


Figure 1. Bibliometric methodology

Study Design

The study was structured by creating research inquiries and choosing relevant keywords and databases. It focused on six key aspects: yearly publication patterns, document origins, subject fields, and countries; institutional affiliations; leading authors; contributions from Indonesian specialists; extensively referenced articles; and potential future research directions.

Data Collection

The search approach employed the title keywords "COVID 19" AND "ATHLETE" within the timeframe of 2020-2023 to gather data from the Scopus database. This yielded 977 documents meeting the criteria, all in their final publication stage and written in English. Additionally, the documents were downloaded in both Comma-Separated Values (CSV) and Research Information System (RIS) formats to capture article titles, author names, references, and keywords.

Data Analysis

The data analysis commenced by importing CSV and RIS data retrieved from the Scopus database into Microsoft Excel and VOSviewer. This step was pivotal for facilitating subsequent data manipulation. The analysis aimed to uncover trends and patterns, such as publication characteristics, document origins, country affiliations, institutional distributions, subject category breakdowns, and prominent authors and citations. This data was meticulously dissected and comprehensively examined to gain deeper insights into the prevailing research landscape, specifically concentrating on the timeframe spanning from 2020 to 2023.

Data Visualization

Following data processing and analysis, the subsequent stage involved data visualization. Visualization was carried out utilizing VOSviewer and Microsoft Excel. With VOSviewer, the processed RIS metadata was transformed into network visualizations, overlays, and densities. Meanwhile, Microsoft Excel was utilized to present the data in tables and diagrams, aiming to offer a more straightforward and comprehensible overview of the identified research patterns.

Interpretation

Ultimately, the data visualized through VOSviewer underwent additional analysis and interpretation. This process entailed examining the quantity of formed clusters, charting both past and recent studies, and scrutinizing densities to unveil insights regarding saturation levels and potential future research avenues. Diligent interpretation of these visualization outcomes was essential for extracting profound insights into the patterns and trajectories of research within the field.

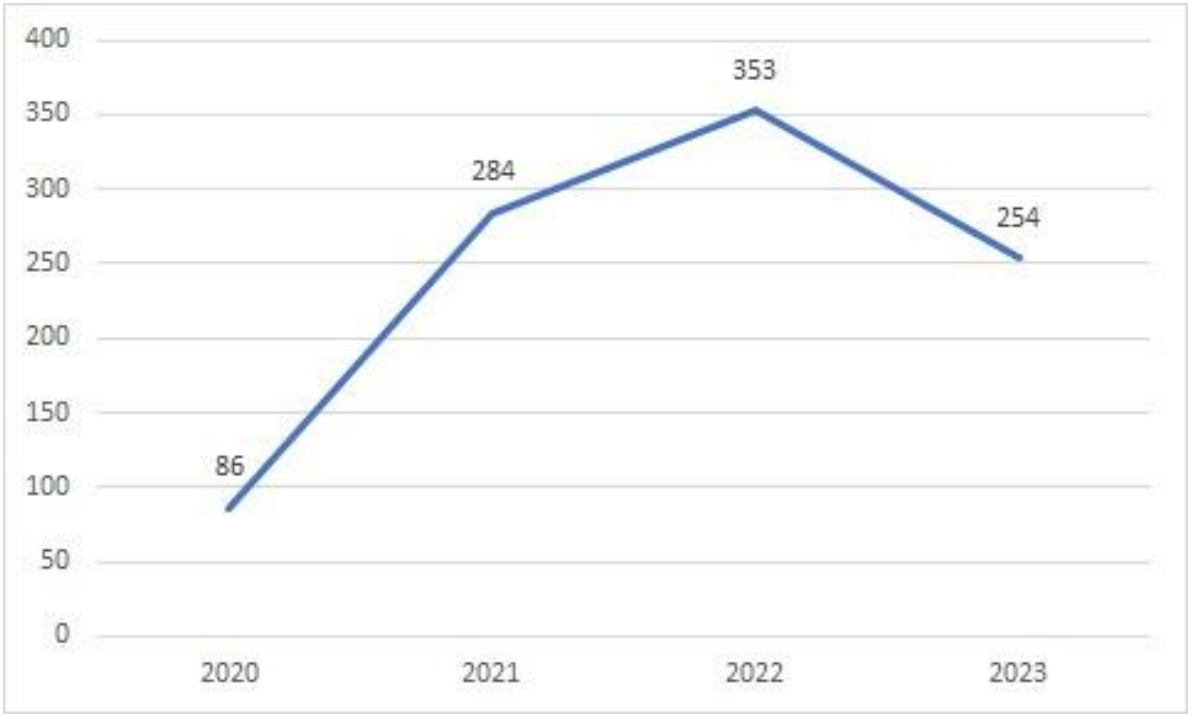


Figure 2. Annual publication trends for the period 2020-2023

Results

The pattern of publication output over a three-year period (2020-2023), as indicated by the Scopus database, reveals notable fluctuations. Specifically, the analytical trend concerning the impact of COVID-19 on athlete performance across different disciplines exhibited variations over this timeframe. This is evidenced by the rise in the number of publications in 2020, 2021, 2022, and 2023, with 86, 284, and 353 publications respectively,followed by a decrease in 2023 with 254 publications. Figure 2 depicts the annual publication trends.

Main Source Document

The articles were sourced from academic journals, with a predominant focus on examining the influence of COVID-19 on athlete performance across diverse disciplines such as environmental science, engineering, energy, and social sciences. Table 1 showcases the ten most prolific journals in publishing articles concerning the impact of COVID-19 on athlete performance.

Table 1. Distribution of documents by related sources from 2020-2023

SOURCES	**ARTICLES**	SJR INDEX (SCİMAGOJR 2022)
INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH AND PUBLIC HEALTH	87	0.83 (Q2)
FRONTIERS IN PSYCHOLOGY	50	0.89 (Q2)
FRONTIERS IN SPORTS AND ACTIVE LIVING	28	0.62 (Q1)
BRITISH JOURNAL OF SPORTS MEDICINE	23	4.76 (Q1)
JOURNAL OF SPORTS MEDICINE AND PHYSICAL FITNESS	18	0.5 (Q2)
PLOS ONE	16	0.89 (Q1)
ORTHOPAEDIC JOURNAL OF SPORTS MEDICINE	15	1.11 (Q1)
JOURNAL OF ATHLETIC TRAINING	13	1.43 (Q1)
SUSTAINABILITY (SWITZERLAND)	13	0.66 (Q1)
JOURNAL OF PHYSICAL EDUCATION AND SPORT	11	0.31 (Q3)

Top Publications by Country

According to country categorization, 977 documents were distributed among different nations, with the majority originating from the USA, Italy, United Kingdom, Spain, and Brazil, with 907, 360, 257, 221, and 182 articles respectively. Figure 4 provides a visual representation of the top 5 countries contributing to publications on the impact of COVID-19 on athlete performance.

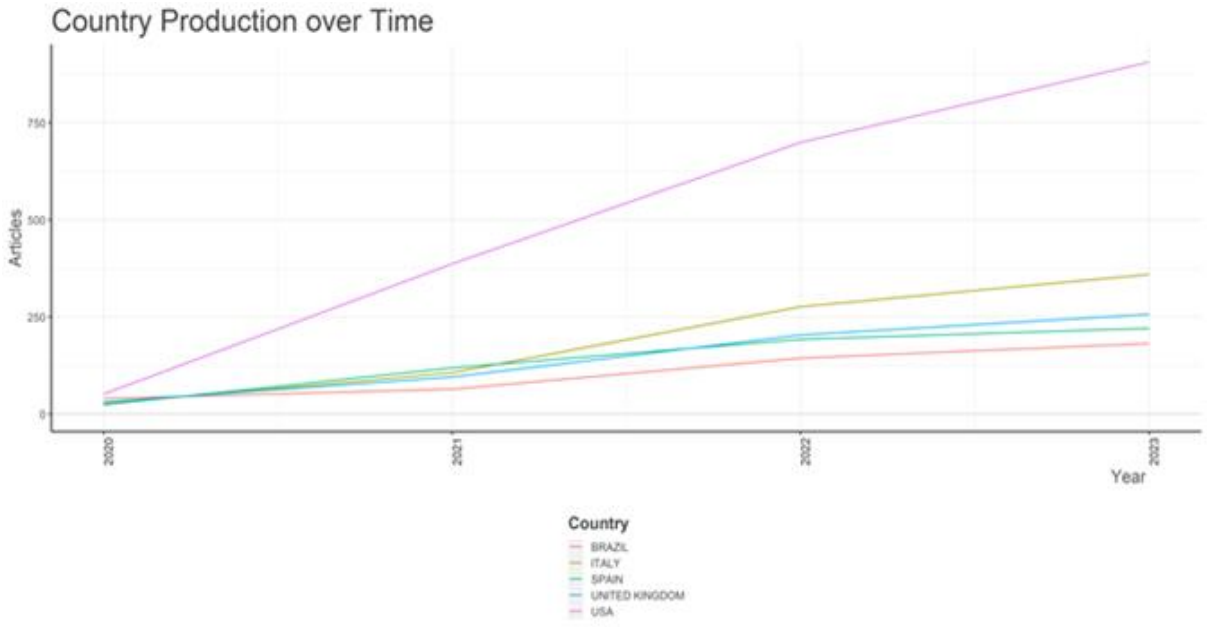


Figure 3. Country production over time

University Affiliation

Figure 5 depicts the distribution of the top 5 university affiliations, highlighting their analytical focus on the impact of COVID-19 on athletes. The publication distribution originates from various university affiliations, namely: Semmelweis University, University of Wisconsin, University of Wisconsin-Madison, University of Calgary, and University of Washington. Among these, Semmelweis University emerges as the most prolific institution, with 25 documents published in the Scopus database. It is followed by the University of Wisconsin and University of Wisconsin-Madison, each with 21 articles, and the University of Calgary and University of Washington, both contributing 20 articles each.

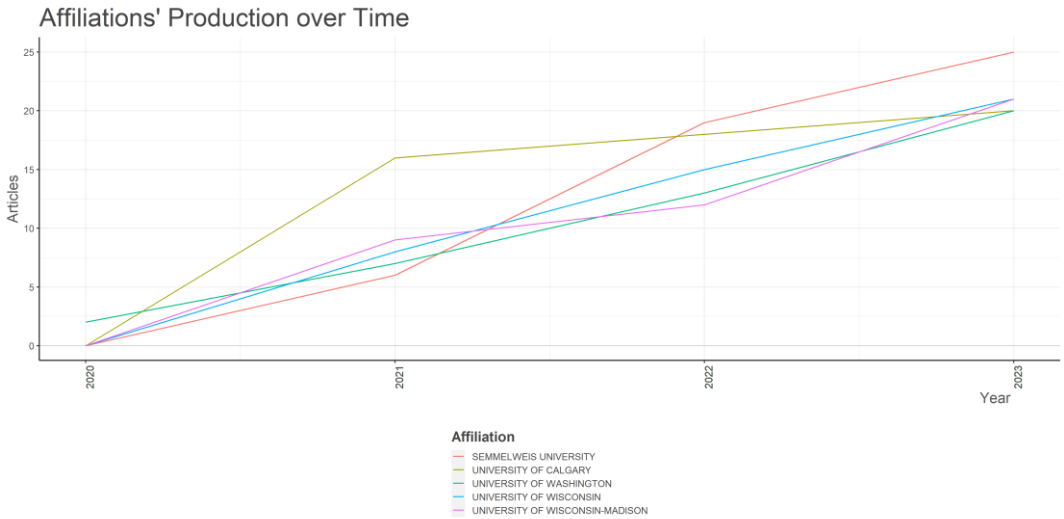


Figure 4. Most relevant Affiliation

Among the most significant authors in this field, Baggish Al and Washif Ja are notable, each contributing 14 documents. They are closely followed by Ammar A, Chamari K, Drezner Ja, and Trabelsi K, with 12 documents each. Furthermore, Farooq A, Harmon KG, and Mujika I each have 11 documents, while Chtourou H has 10 documents, as depicted in Figure 6

Document Citation

According to data extracted from Scopus, the top 10 most frequently cited documents per year are as follows: Daniels CJ leads the list with a total citation count (TC) of 203, closely followed by Martinez MW with a TC of 182. Miljoen H's research in Heal Sci Rep ranks third with a TC of 178, while Starekova J's contribution in Jama. Cardiol holds the fourth position with a TC of 152. Abbasi J's study follows with a TC of 35, while Dixon BC's work in Jama Netw OpeN and Tsao J's contribution have TCs of 13 and 10, respectively. Schultz EA's research has a TC of 5, and both Hamburger RF's document in Clin Cardiol and Kasashi K's work in Jaccp Jam Coll Clin Pharm have a TC of 0.

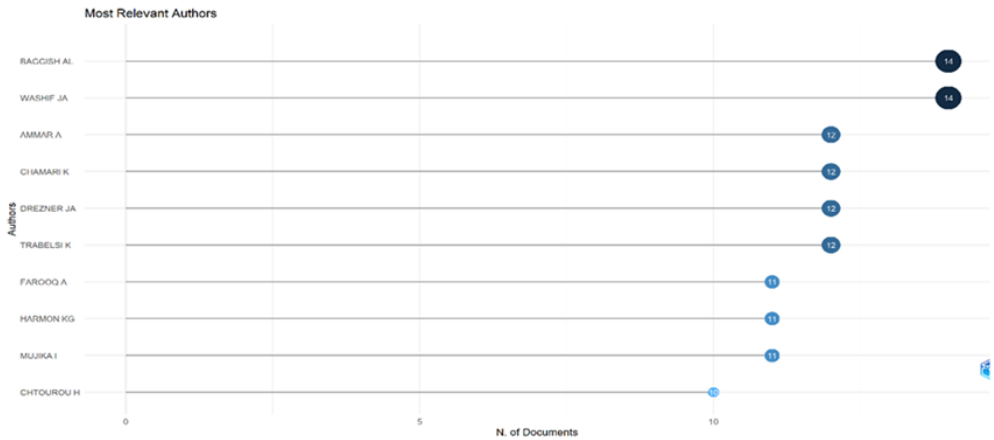


Figure 5. Top 10 authors

Table 2. A breakdown of the top 10 documents on Scopus by number of citation

Author/Year	Title	DOI	Total Citations	TC per Year
Abbasi, (2021)	Researchers Investigate What COVID-19 Does to the Heart	10.1001/jama.2021.0107	35	8.75
Starekova et al., (2021)	Evaluation for Myocarditis in Competitive Student Athletes Recovering From Coronavirus Disease 2019 With Cardiac Magnetic Resonance Imaging	10.1001/jamacardio.2020.7444	152	38.00
Martinez et al., (2021)	Prevalence of Inflammatory Heart Disease Among Professional Athletes With Prior COVID-19 Infection Who Received Systematic Return-to-Play Cardiac Screening	10.1001/jamacardio.2021.0565	182	45.50
Daniels et al., (2021)	Prevalence of Clinical and Subclinical Myocarditis in Competitive Athletes With Recent SARS-CoV-2 Infection	10.1001/jamacardio.2021.2065	203	50.75
Dixon et al., (2021)	Contact and SARS-CoV-2 Infections Among College Football Athletes in the Southeastern Conference During the COVID-19 Pandemic	10.1001/jamanetworkopen.2021.35566	13	3.25
Schultz et al., (2022)	Comparison of SARS-CoV-2 Test Positivity in NCAA Division I Student Athletes vs Nonathletes at 12 Institutions	10.1001/jamanetworkopen.2021.47805	5	1.67
Tsao et al., (2022)	Accuracy of Rapid Antigen vs Reverse Transcriptase–Polymerase Chain Reaction Testing for SARS-CoV-2 Infection in College Athletes During Prevalence of the Omicron Variant	10.1001/jamanetworkopen.2022.17234	10	3.33
Hamburger et al., (2023)	Longitudinal cardiac remodeling in collegiate American football players as assessed by echocardiography during their collegiate career	10.1002/clc.24121	0	0.00
Miljoen et al., (2022)	Effect of BNT162b2 mRNA booster vaccination on VO2 max in recreational athletes A prospective cohort study	10.1002/hsr2.929	2	0.67
Kasashi et al., (2023)	Clinical Pharmacy Services at the Tokyo Olympic and Paralympic Games and the Sports Pharmacist Training Program	10.1002/jac5.1869	0	0.00

Mapping Impact of COVID-19 on Athlete Performance with VOSviewer

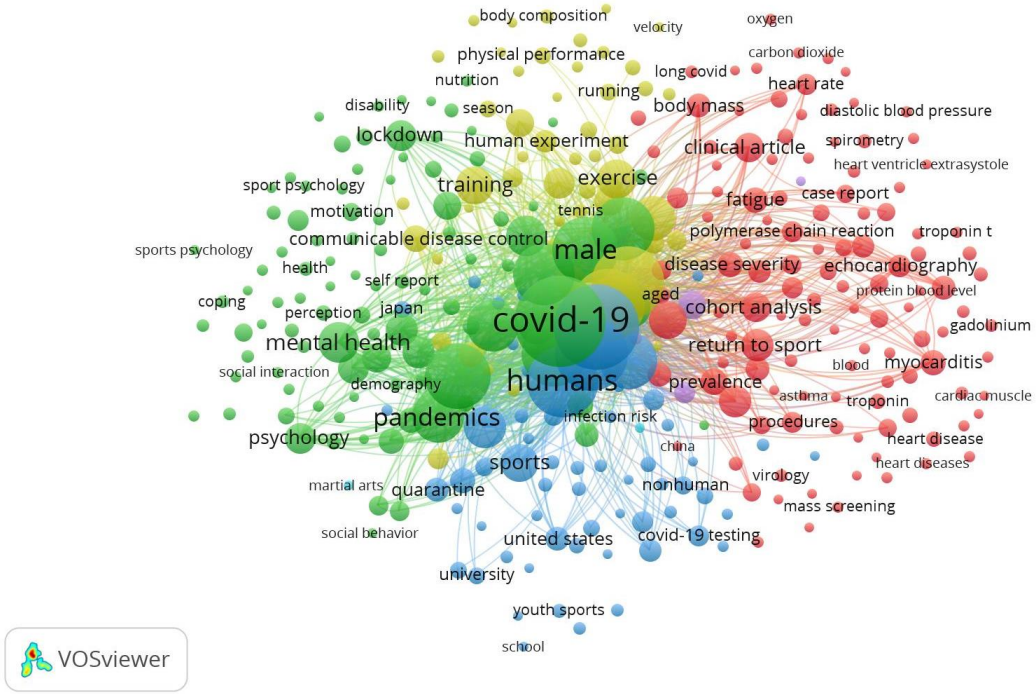


Figure 6 . Networking visualization

The classification of research subjects related to the Impact of COVID-19 on Athlete Performance deserves attention, with a minimum of 5054 keywords identified, 398 of which meet the threshold. Network visualization is categorized into 5 clusters, comprising 31969 links with a total binding strength of 151804. In this context, the red, green, blue, and yellow clusters prioritize COVID-19 and athletes. Based on these results, larger circles indicate higher keyword frequencies within the documents. Figure 6 displays network visualization using VOSviewer, highlighting the most frequently occurring keywords and networks.

Overlay Visualization

The overlay visualization is represented by colors signifying annual trends in experimentation, where yellow, green, and purple highlight recent investigations and analyses pertaining to COVID-19 and athletes. These recent trends center around terms such as retrospective study, athletic injuries, cross-sectional study, incidence, sport injuries, heart left ventricle ejection, headache, transthoracic echocardiography, and outcome assessment.. Co-occurrence overlay visualization is displayed in Figure 7.

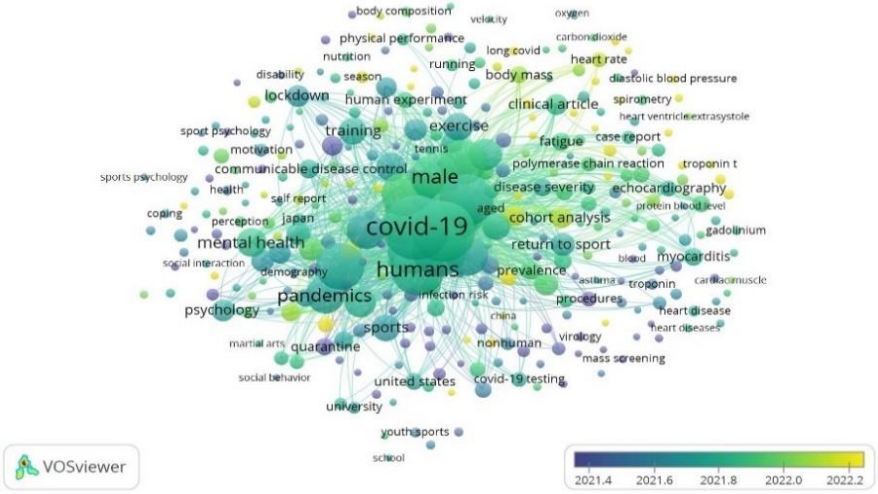


Figure 7. Overlay visualization

Density Visualization

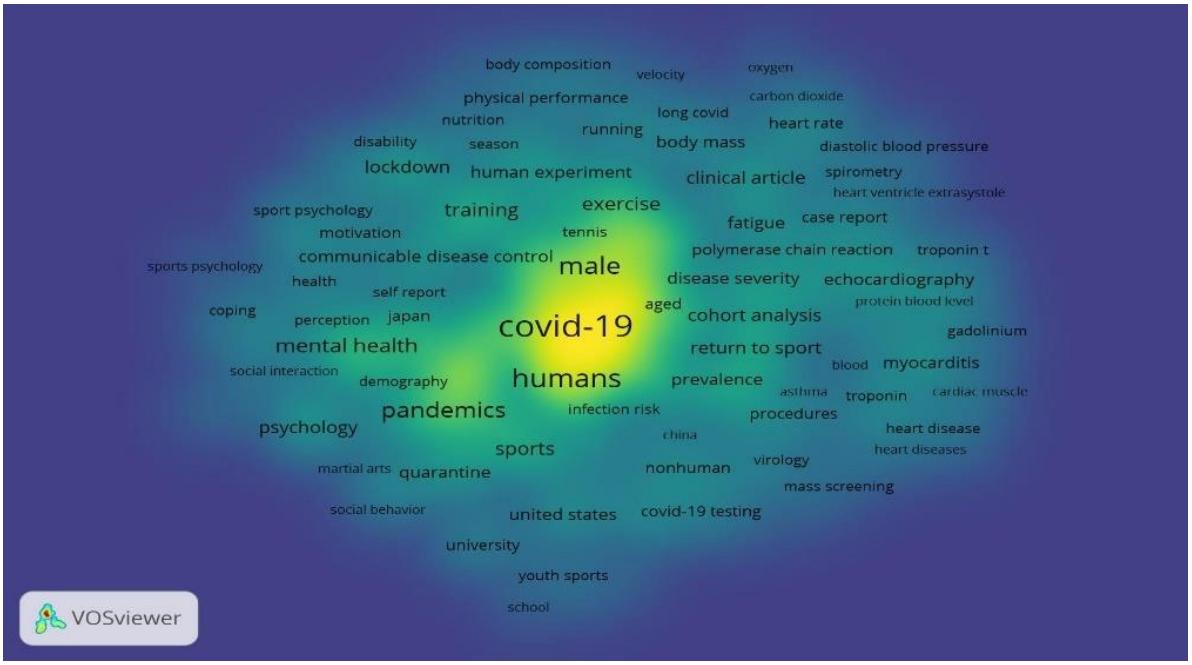


Figure 8. Density visualization

Density visualization plays a crucial role in assessing the concentration of a topic, where red, blue, yellow, and green colors denote unexplored, rarely studied, and highly researched areas, respectively. Brighter colors in the image signify more frequent usage of the analyzed terms in relevant studies. Additionally, keyword density distribution illustrates the frequency of research topics. (Hoang et al., 2020). For instance, COVID-19 and humans are frequently examined analytically, whereas terms related to athletic performance are less commonly studied. Figure 8 illustrates the visualization of the density of decision-making and problem-solving skills based on keyword indices.

Discussion

The utilization of VOSviewer for bibliometric analysis has proven to be an invaluable tool for exploring research trends and contributions in the realm of Impact of COVID-19 on Athlete Performance. This approach allows us to delve deeper into understanding the evolution of publication productivity over the past decade, spanning from 2020 to 2023. Through this analysis, notable fluctuations in publication numbers from year to year become evident. Certain years exhibit an uptick in publication productivity, notably observed during the period from 2020 to 2022. These increases may signify a growing interest and research emphasis on the analytical impact of COVID-19 during those intervals. Factors such as the development of novel technologies or emerging environmental concerns could contribute to heightened research activity in this domain. However, there is also a decline in the number of publications noted in 2023. While this decline may seem unexpected, it's essential to recognize that this dataset only encompasses the early months of 2024. Therefore, this decrease might reflect early-year trends and may not accurately represent the overall publication trajectory for that year. It is imperative to scrutinize these trends more comprehensively and gather additional data to ascertain whether this decline is enduring or transitory.

Moreover, bibliometric analysis enables us to explore the contributions of different countries and institutions in this field of research. By identifying leading countries and academic institutions, we gain insights into international collaboration dynamics and knowledge dissemination among research communities. In essence, bibliometric analysis using VOSviewer offers a comprehensive overview of research trends and contributions in analyzing the impact of COVID-19 on athletes. It aids in understanding the evolution of research in this area over time and provides valuable guidance for future research planning and identifying areas requiring further investigation. Notably, the primary sources of documents predominantly originate from scientific journals, indicating a robust scientific foundation for research in this domain. These journals cover diverse fields such as environmental science, engineering, and energy. Table 2, which outlines the top twenty sources, offers a clear representation of these journals' contributions to advancing knowledge in analyzing the impact of COVID-19 on athletes.

Moreover, examination by fields of study reveals that this research is extensively dispersed across different disciplines, encompassing areas such as environmental and social sciences. This indicates that the analytical impact of COVID-19 on athletes is a prominent area of interest across multiple fields. Visualizing document distribution by fields of study offers a nuanced perspective of research dispersion across diverse disciplines. Notably, when scrutinizing contributions by specific countries, it becomes apparent that nations in the Americas and Spain have made substantial contributions to this research. This underscores the significance of the analytical impact of COVID-19 in those regions.

In terms of university affiliations, we observe significant participation from institutions across various countries in this research. Numerous leading universities from these nations actively contribute to publications regarding the analytical impact of COVID-19 on athletes. The table detailing the top university affiliations offers a more comprehensive understanding of these institutions' involvement in this research. It is evident that collaborative efforts spanning countries and institutions greatly propel knowledge advancement in this field. Furthermore, notable contributions are also observed from top authors in this area. Authors hailing from diverse countries such as Brazil, Italy, Spain, and the United Kingdom are actively engaged in research pertaining to the impact of COVID-19 on athletes. The visual representation provided by the graph underscores the substantial contributions of these authors to the advancement of knowledge in this domain. This analysis also underscores the significance of the most frequently cited publications, indicating the broad influence of specific research in this field. Several researchers and authors play pivotal roles in shaping the trajectory and progress of this research.VOSviewer network visualization is an extremely useful tool in understanding the structure and relationships between the most frequently occurring keywords in research. Using cluster analysis techniques, VOSviewer helps us identify groups of keywords that often appear together in literature. This provides valuable insights into the main focuses and research trends in the field of the analytical impact of COVID-19 on athletes.

Overlay and density visualizations also provide additional information about experimental trends and ongoing research focuses. By using different colors to highlight annual trends and density levels, we can see how research interests evolve over time and where research centers are located. For example, an increase in yellow color may indicate increased interest in specific topics in recent research. Meanwhile, the blue color may indicate that the topic is less studied. This information is crucial for planning future research. By understanding existing research trends and focuses, researchers can identify extensively studied areas and areas that require further attention. For example, if we see that there are underexplored keyword groups, this may provide opportunities for researchers to further explore these topics and make meaningful contributions to scientific literature. Thus, network, overlay, and density visualizations provide a holistic view of research trends and directions in the field of the analytical impact of COVID-19 on athletes.

Conclusion

The utilization of VOSviewer for bibliometric analysis has emerged as a very useful tool for exploring research trends and contributions in the domain of COVID-19's impact on athlete performance. This analysis facilitates a deeper comprehension of how publication productivity has evolved over the past decade, spanning from 2020 to 2023, revealing noticeable fluctuations in publication numbers from year to year. While certain years exhibit an upsurge in publication productivity, particularly observed during the period from 2020 to 2022, a decline is observed in 2023. However, it's essential to acknowledge that this dataset only covers the early months of 2024, suggesting that this decrease may reflect early-year trends and might not accurately represent the overall publication trajectory for that year.

Moreover, bibliometric analysis enables the examination of contributions from various countries and institutions in this research, underscoring the crucial role of cross-country and cross-institutional collaborations in advancing knowledge within this domain. Additionally, this analysis identifies contributions from top authors and the most frequently cited publications, offering insights into the structure and relationships between the most recurrently occurring keywords in literature. Such insights aid in planning future research endeavors by comprehending existing trends and focal points. Thus, bibliometric analysis utilizing VOSviewer offers a comprehensive overview of research trends and contributions in the field of analyzing the impact of COVID-19 on athletes, furnishing valuable insights for future research planning and pinpointing areas warranting further attention.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

* The authors express their heartfelt appreciation and gratitude to the Lembaga Pengelola Dana Pendidikan LPDP (Indonesia Endowment Fund for Education) under the Ministry of Finance of the Republic of Indonesia for their invaluable assistance in facilitating this publication and promoting collaboration.

References

Abbasi, J. (2021). Researchers investigate what COVID-19 does to the heart. *JAMA*, *325*(9), 808–811.

Alsolbi, I., Wu, M., Zhang, Y., Joshi, S., Sharma, M., Tafavogh, S., Sinha, A., & Prasad, M. (2022). Different approaches of bibliometric analysis for data analytics applications in non-profit organisations. *Journal of Smart Environments and Green Computing*, *2*(3), 90-104.

Daniels, C. J., Rajpal, S., Greenshields, J. T., Rosenthal, G. L., Chung, E. H., Terrin, M., Jeudy, J., Mattson, S. E., Law, I. H., Borchers, J., Kovacs, R., Kovan, J., Rifat, S. F., Albrecht, J., Bento, A. I., Albers, L., Bernhardt, D., Day, C., Hecht, S., … Investigators, B. T. C.-19 C. R. (2021). Prevalence of clinical and

subclinical myocarditis in competitive athletes with recent SARS-CoV-2 infection: Results from the big ten covid-19 cardiac registry. *JAMA Cardiology*, *6*(9), 1078–1087.

Dixon, B. C., Fischer, R. S. B., Zhao, H., O'Neal, C. S., Clugston, J. R., & Gibbs, S. G. (2021). Contact and SARS-CoV-2 infections among college football athletes in the Southeastern conference during the Covid-19 pandemic. *JAMA Network Open*, *4*(10), e2135566–e2135566.

Donthu, N., Kumar, S., Mukherjee, D., Pandey, N., & Lim, W. M. (2021). How to conduct a bibliometric analysis: An overview and guidelines. *Journal of Business Research*, *133*, 285–296.

García-Peñalvo, F. J. (2022). *Developing robust state-of-the-art reports: Systematic literature reviews*. *Education in Knowledge Society*, *23*(2), e8600.

Ha, T. H., & Dauenhauer, B. (2020). Physical activity participation during the covid-19 pandemic. Changes in the frequency of sports and walking among people in the second half of life. In A*geing times of the covid-19.* (pp.119-141).

Hamburger, R. F., Taha, Y., Ruzieh, M., Clugston, J. R., Handberg, E. M., Reifsteck, F., Martinez, M. W., Pepine, C. J., & Edenfield, K. M. (2023). Longitudinal cardiac remodeling in collegiate American football players as assessed by echocardiography during their collegiate career. *Clinical Cardiology*, *46*(9), 1090–1096.

Hoang, N. H., Ishigaki, T., Kubota, R., Yamada, M., & Kawamoto, K. (2020). A review of construction and demolition waste management in Southeast Asia. *Journal of Material Cycles and Waste Management*, *22*(2), 315–325.

Kasashi, K., Suzuki, S., Sato, A., & Kawaguchi-Suzuki, M. (2023). Clinical pharmacy services at the tokyo olympic and paralympic games and the sports pharmacist training program. *Jaccp: Journal of the American College of Clinical Pharmacy*, *6*(12),1336-1345.

Kim, S.-H., Park, J.-M., Kim, Y.-H., Kim, T., & Park, J.-C. (2023). Impact of COVID-19 infection on sports performance of elite university athletes. *The Journal of Sports Medicine and Physical Fitness*, *63*(5), 650—659.

Kussainova, R. E., Urazbayeva, G. T., Kaliyeva, A. B., & Denst-Garcia, E. (2024). Innovative teaching: A bibliometric analysis from 2013 to 2023. *European Journal of Educational Research*, *13*(1), 233-247.

Lopes, L., Miranda, V., Goes, R., Souza, G., Souza, G., Rocha, J., Cossich, V., & Perini, J. (2021). Repercussions of the covid-19 pandemic on athletes: a cross-sectional study. *Biology of Sport*, *38*(4), 703–711.

Martinez, M. W., Tucker, A. M., Bloom, O. J., Green, G., DiFiori, J. P., Solomon, G., Phelan, D., Kim, J. H., Meeuwisse, W., Sills, A. K., Rowe, D., Bogoch, I. I., Smith, P. T., Baggish, A. L., Putukian, M., & Engel, D. J. (2021). Prevalence of inflammatory heart disease among professional athletes with prior covid-19 infection who received systematic return-to-play cardiac screening. *JAMA Cardiology*, *6*(7), 745–752.

Miljoen, H., Bekhuis, Y., Roeykens, J., Taha, K., Frankinouille, R., Michielsen, M., Van de Heyning, C. M., Cornelissen, V., Favere, K., Eens, S., Gielen, J., Goetschalckx, K., Heidbuchel, H., & Claessen, G. (2022). Effect of BNT162b2 mRNA booster vaccination on VO(2) (max) in recreational athletes: A prospective cohort study. *Health Science Reports*, *5*(6), e929.

Mongeon, P., & Paul-Hus, A. (2016). The journal coverage of Web of Science and Scopus: a comparative analysis. *Scientometrics*, *106*(1), 213–228.

de Oliveira, O. J., da Silva, F. F., Juliani, F., Barbosa, L. C. F. M., & Nunhes, T. V. (2019). Bibliometric method for mapping the state-of-the-art and identifying research gaps and trends in literature: An essential instrument to support the development of scientific projects. In *Scientometrics recent advances*. IntechOpen.

Orscelik, A., Kaya, I., Karaaslan, B., Agiragac, B., & Ceyhan, C. (2022). Is COVID-19 infection decreasing the sports performance of the volleyball players? A pilot study. *Journal of Men's Health*, *18*(4), 1–6.

Ozturk, O., Kocaman, R., & Kanbach, D. K. (2024). How to design bibliometric research: an overview and a framework proposal. *Review of Managerial Science*, 1-29.

Paludo, A., Karpinski, K., Silva, S., Kumstát, M., Sajdlová, Z., & Milanovic, Z. (2022). Effect of home training during the COVID-19 lockdown on physical performance and perceptual responses of team-sport athletes: a mini-review. *Biology of Sport*, *39*(4), 1095–1102.

Rankin, A., Massey, A., Falvey, É. C., Ellenbecker, T., Harcourt, P., Murray, A., Kinane, D., Niesters, B., Jones, N., Martin, R., Roshon, M., McLarnon, M. E. D., Calder, J., Izquierdo, D., Pluim, B. M., Elliott, N., & Heron, N. (2021). Infographic. covid-19 RT-PCR testing for elite athletes. *British Journal of Sports Medicine*, *55*(14), 818–820.

Sanborn, V., Todd, L., Schmetzer, H., Manitkul-Davis, N., Updegraff, J., & Gunstad, J. (2021). Prevalence of covid-19 anxiety in division i student-athletes. *Journal of Clinical Sport Psychology*, *15*(2), 162–176.

Schipman J. (2022). The COVID-19 pandemic impact on the best performers in athletics and swimming among Paralympic and non-disabled athletes. *The Journal of Sports Medicine and Physical Fitness*, *62*(12),

1605–1614.
Schultz, E. A., Kussman, A., Jerome, A., Abrams, G. D., & Hwang, C. E. (2022). Comparison of SARS-CoV-2 test positivity in NCAA division i student athletes vs nonathletes at 12 institutions. *JAMA Network Open*, *5*(2), e2147805–e2147805.
Singh, V. K., Singh, P., Karmakar, M., Leta, J., & Mayr, P. (2021). The journal coverage of Web of Science, Scopus and Dimensions: A comparative analysis. *Scientometrics*, *126*(6), 5113–5142.
Starekova, J., Bluemke, D. A., Bradham, W. S., Eckhardt, L. L., Grist, T. M., Kusmirek, J. E., Purtell, C. S., Schiebler, M. L., & Reeder, S. B. (2021). Evaluation for myocarditis in competitive student athletes recovering from coronavirus disease 2019 with cardiac magnetic resonance imaging. *JAMA Cardiology*, *6*(8), 945–950.
Sweileh, W. M. (2020a). Bibliometric analysis of scientific publications on "sustainable development goals" with emphasis on "good health and well-being" goal (2015-2019). *Globalization and Health*, *16*(1), 1–13.
Sweileh, W. M. (2020b). Bibliometric analysis of scientific publications on "sustainable development goals" with emphasis on "good health and well-being" goal (2015–2019). *Globalization and Health*, *16*(1), 68.
Tsao, J., Kussman, A. L., Costales, C., Pinsky, B. A., Abrams, G. D., & Hwang, C. E. (2022). Accuracy of Rapid Antigen vs Reverse Transcriptase–Polymerase Chain Reaction Testing for SARS-CoV-2 Infection in College Athletes During Prevalence of the Omicron Variant. *JAMA Network Open*, *5*(6), e2217234–e2217234
Valenzuela, P. L., Rivas, F., & Sánchez-Martínez, G. (2021). Effects of COVID-19 Lockdown and a Subsequent Retraining Period on Elite Athletes' Workload, Performance, and Autonomic Responses: A Case Series. *International Journal of Sports Physiology and Performance*, *16*(11), 1707–1711.
Wiltshire, H. D., Supriya, R., & Baker, J. S. (2022). COVID-19 Impact on the Sport Sector Economy and Athletic Performance. *Journal of Risk and Financial Management*, *15*(4).
Yang, J., Li, X., He, T., Ju, F., Qiu, Y., & Tian, Z. (2022). Impact of Physical Activity on COVID-19. *International Journal of Environmental Research and Public Health*, *19*(21)

Author Information

Muh. Aswar
Universitas Pendidikan Indonesia
Bandung, Indonesia
Contact e-mail: *aswar6442@upi.edu*

Jajat Darajat Kusumah Nagara
Universitas Pendidikan Indonesia
Bandung, Indonesia

Haryanto Haryanto
Universitas Pendidikan Indonesia
Bandung, Indonesia

Syaipul Hari Baharuddin
Universitas Pendidikan Indonesia
Bandung, Indonesia

Daniel Assetiawan Iriana
Universitas Pendidikan Indonesia
Bandung, Indonesia

To cite this article:

Aswar, M., Nagara, J.D.K., Haryanto, H., Baharuddin, S.H., & Iriana, D.A. (2024). Bibliometric analysis of the impact of COVID-19 on athlete performance: Publication trends and implications for future research. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 20-31.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 32-39

ICGeHeS 2024: International Conference on General Health Sciences

The Effect of Corn Cob Powder and Oat Fibre Incorporation in Physicochemical Properties and Sensory Acceptance of *Otak-Otak*

Fisal Ahmad
Universiti Malaysia Terengganu

Muhamat Hafiz Sapiee
Universiti Malaysia Terengganu

Abstract: *Otak-otak* is well-known as a traditional local food in Malaysia. It is made from fish meat, tapioca flour, and many spices. This study investigates the effect of corn cob powder and oat fibre incorporation on the physicochemical properties and sensory acceptance of *otak-otak* with different formulations. The formulations used control A (100% tapioca flour), B (0% CCP: 100% oat fibre), C (25% CCP: 75% oat fibre), D (50% CCP: 50% oat fibre), E (75% CCP: 25% oat fibre), and F (100% CCP: 0% oat fibre). The proximate analysis showed that *otak-otak* incorporated with CCP and oat fibre significantly differed (p<0.05) regarding crude fibre and fat. Physical analysis showed that the colour of the otak-otak for L* value ranges from 53.71 to 57.58, a* value ranges from 13.15 to 15.10, b* value ranges from 32.80 to 35.21 and the ΔE 0.96 to 4.86. For texture profile analysis, the hardness ranges from 2147.60 to 5006.00 (g), springiness ranges from 0.39 to 0.66 (mm), cohesiveness ranges from 0.35 to 0.48, and chewiness ranges from 370.40 to 1459.90 (gf). For sensory evaluation, the control formulation still the highest chosen by the panellist but has no significant difference (p<0.05) with formulation E (75% CCP: 25% oat fibre) and formulation C (25% CCP: 75% oat fibre). Therefore, from this study, we successfully developed *otak-otak* by adding corn cob powder and oat fibre.

Keywords: Local food, Fibre, Healthy foods, Fish product

Introduction

Otak-otak is well-known as a traditional local food in Malaysia. *Otak-otak* commonly can be taken as a side dish or as a snack. *Otak-otak* is made from a fish-based product with high protein and moisture content. Not only does it contain fish meat, but *otak-otak* also has many ingredients, such as spices, that have been added. Adding spices and other ingredients to the fish meat will enhance the flavour of otak-otak, making the otak-otak different from other food. The type of fish used to make *otak-otak* varies, but mackerel is most commonly used in Malaysia because it contains low fat and good gel-forming capability. In Malaysia, *otak-otak* is a popular street food among tourists and locals, especially in Kelantan and Terengganu, Malaysia.

In this study, corn cob powder and oat fibre will be added to the fish mixture by replacing tapioca flour. This is because corn cob powder (CCP) and oat fibre are known to have a high fibre content. Corn cob is commonly known as a waste product from corn. This invention also helps reduce waste production in the agricultural industry. It has been stated that corn cob has 33.33 grams or 0.21% of crude fibre content (Abubakar et al., 2016). Oats are also known for their high crude fibre content. Oat is high in protein and fibre, and its fatty acid composition is beneficial (Liukkonen et al., 1992). Adding oats fibre with the combination of CCP can increase the fibre content in food production. Besides, it also helps to enhance the taste of the product. It helps improve the texture and colour of the food product. In this study, a newly formulated *otak-otak* will be produced. Combining the new CCP and oat fibre formulation will replace tapioca flour in *otak-otak* production. Using corn cob as a powder can help reduce the waste from corn production, where corn cob is the leading waste

produced. Food that has high fibre content can help to smooth the digestive system and help diabetic people consume the food product, as the fibre helps to slow down the blood sugar response after a meal. Thus, this study aimed to determine the effect of CCP and oat fibre incorporation on physicochemical properties and sensory acceptance of *otak-otak.*

Method

Corn Cob Powder (CCP) and Oat Fibre Preparation

The corn cob was cut using a slicer to get a thin corn cob for drying. Then, the corn cob undergoes steam blanching to stop the enzymatic reaction. Then, the corn cob is placed in the cabinet dryer for approximately 15 hours at 60°C (final moisture content 8%). After the drying process was done, the corn cob was ground using the blender. Then, the corn cob powder will be sieved using the sieve shaker 125µm to get the fine powder. Next, for the oat fibre powder production, the oat fibre was ground using the dry blender and was sieved using the sieve shaker at 125µm (Hamzah and Wong, 2012).

Otak-Otak Preparation

In the first step in *otak-otak* preparation, the dried chillies were soaked in hot water for 10 minutes. The seeds were discarded to reduce the spiciness and then set aside. Then, chop the rest of the spices and herbs into smaller pieces. Then, all the ingredients were blended until they became smooth. The oil was added to the blender to make the blending process easier. Add some oil to sauté blended spices in a pan over medium to low heat until cooked. The process was continued by stirring the ingredients to prevent the paste from burning. Then, the paste was completely cooked and set aside to cool.

The excess oil was discarded once the chilli paste was no longer warm. Then, chilli paste was added with the fish fillet, coconut milk, water, salt, sugar, and tapioca flour and blended together until it became a fine paste. Scoop 40 grams of fish paste onto a nipah leaf. Then, fold and wrap the fish paste. Secure the ends with toothpicks. The final step was repeated until the all *otak-otak* paste was finished. All samples were frozen before being used and grilled using the standard procedure for coking before further analyses and sensory evaluations. The treatment is as follows: control A (100% tapioca flour), B (0% CCP: 100% oat fibre), C (25% CCP: 75% oat fibre), D (50% CCP: 50% oat fibre), E (75% CCP: 25% oat fibre), and F (100% CCP: 0% oat fibre) and the others ingredients were constant.

Texture Profile Analysis

The texture profile analysis was determined using the texture analyser TA-XT-plus (Stable Micro System, United Kingdom) (Samakradhamrongthai et al., 2017). In this method, the pre-test speed was 2mm/sec, the test speed was 1mm/sec, and the post-test speed was 2mm/sec. The percentage of strain used was 50%, and the load cell was 30kg. The distance from the platform was 30.0mm, and double compression was performed. The attributes measured were hardness, springiness, cohesiveness, and chewiness.

Colour Profile Analysis

Colour measurement was performed using the Minolta Chroma Meter (Konica Minolta, CR-400, Japan). The measurement was taken three times for each sample. Data were stored in the CIE L*a*b* colour model, and colour changes were evaluated. The Total Colour Difference (ΔE*) was generated based on differences of colour space L*, a*, b* and a single number metric. The colour difference was calculated using the Equation 1 (Purlis and Salvadori, 2007). The Minolta CR-400 Chroma Meter D65 calibration plate was used for calibration. All measurements were repeated in three replicates.

$$\Delta E^* = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2} \quad \ldots\ldots (1)$$

Where L_o*, a_o* and b_o* indicate colour parameters of control, parameter L* refers to the lightness of the different formulations and ranges from black (L=0) to white (L=100), and a negative value of parameter a* indicates green. A positive one indicates red-purple colour, a positive value of parameter b* indicates yellow, while a negative value indicates blue.

Proximate Analysis

The proximate analysis of the control and different formulations of *otak-otak* products were measured using AOAC methods (AOAC, 2000). Oven drying and weighing methods (926.12, 41.1.02) were used to measure the moisture content. Ash content was measured by weighing and furnace methods at 600°C for 3-5 h (942.05, 4.1.10). Fat extraction using soxhlet distillation and chloroform as a solvent was used to measure the fat content (948.22, 40.1.05). The protein content was measured using Kjeldahl distillation, and the nitrogen value was converted to protein value using conversion factors (960.52, 12.1.07). The carbohydrate content was measured using different methods.

Sensory Evaluation

The sensory evaluation used the acceptance test (Samakradhamrongthai et al., 2017). The evaluation involved 35 semi-trained panelists. The panellists were not allergic to seafood and fibre. The seven-point hedonic scale was used with 1 for extremely dislike and 7 for extremely like. The characteristics evaluated were appearance, colour, aroma, texture, taste, and overall acceptance. Each of the formulations was packed individually, and the sample was given a three-digit random number code.

Statistical Analysis

The data obtained after completing the experiments was reported as a mean ± standard deviation. The data for physical analysis was subjected to a one-way analysis of variance (One-way ANOVA) using Minitab 21. The significance difference of mean values (p<0.05) is examined using Tukey's test for all responses.

Results and Discussion

Texture Profile (TPA)

The texture profile analysis (TPA) results in Table 1 provide valuable insights into the textural attributes of the different formulations (A to F). Hardness, springiness, cohesiveness, and chewiness were the parameters assessed in this study. Formulation F exhibited the highest hardness at 5006.00g, significantly surpassing the other formulations. This suggests that formulation F may possess a denser or more compact structure. The water-starch-protein interactions of the different flours significantly impact the textural properties of o*tak-otak* (Fustier et al., 2008). The gradual reductions in protein and starch content, combined with a significant increase in fibre content, resulted in accelerated increases in textural hardness. Additionally, formulation F also demonstrated the highest chewiness value at 1459.90 g, indicating that it requires more effort to masticate and potentially contains components with higher resistance to deformation. Conversely, the control (formulation A) exhibited the lowest hardness and chewiness values among the formulations, suggesting it may have a softer and less resilient texture.

Regarding springiness, Formulation F again stood out with a springiness value of 0.66, indicating that it quickly returns to its original shape after deformation, likely due to its elastic properties. In contrast, Formulation C displayed the lowest springiness value at 0.39, suggesting it may exhibit a more sluggish recovery after compression. The capacity of a product to rebound after deformation is represented by resilience, and a decrease in resilience post supplementation of corn cob powder may be related to the product's denser matrix (Baixauli et al., 2008). Cohesiveness, which measures the degree of internal bonding within the formulation, varied but generally fell within a narrow range. Notably, Formulation F demonstrated the highest cohesiveness and remarkable hardness and chewiness values, indicating a well-integrated structure resistant to breakdown during chewing.

Control A (100% tapioca flour), Formulation B (0% CCP: 100% oat fibre), Formulation C (25% CCP: 75% oat fibre), Formulation D (50% CCP: 50% oat fibre), Formulation E (75% CCP: 25% oat fibre), and Formulation F (100% CCP: 0% oat fibre). Values are expressed as mean ± SD of triplicate measurement. Superscripts with different letters are significantly different at $p<0.05$ in the same column.

Table 1.Results for texture profile analysis

Formulation	Hardness (g)	Springiness (mm)	Cohesiveness	Chewiness (gf)
A	2421.00±6.71^{c}	0.46±0.05^{cd}	0.37±0.04^{ab}	414.10±3.26^{c}
B	2147.60±5.37^{c}	0.50±0.04^{bc}	0.45±0.04^{ab}	477.70±2.18^{c}
C	2727.00±7.86^{c}	0.39±0.08^{d}	0.35±0.05^{b}	370.40±2.42^{c}
D	3891.40±8.31^{b}	0.51±0.09^{bc}	0.40±0.06^{ab}	773.63±2.58^{b}
E	3527.70±6.45^{b}	0.59±0.07^{ab}	0.48±0.03^{a}	984.20±2.66^{b}
F	4006.00±5.22^{a}	0.66±0.08^{a}	0.45±0.08^{ab}	1459.90±3.11^{a}

Colour Profile

The results presented in Table 2 depict the colour profile analysis of six distinct formulations (A to F), characterized by their respective L*, a*, and b* values and the corresponding ΔE values. L*, a*, and b* values represent the lightness, greenness-redness, and blueness-yellowness components of colour, respectively, while ΔE quantifies the overall colour difference between the two samples. Notably, formulation F exhibited the highest L* value at 57.58, indicating its lighter appearance than the other formulations. The results show that the lightness of the formulation was increased when the amount of corn cob powder was increased. Therefore, the formulation with corn cob powder shows brighter in colour compared to the formulation that contained a high amount of oat fibre. Conversely, formulation F displayed the lowest a* value at 13.15, suggesting a shift towards greenness in its colour profile. Formulation E closely followed formulation F in terms of L* and a* values, indicating similarities in their lightness and greenness characteristics.

Interestingly, formulation D and E exhibited similar L* values but demonstrated variations in their b* values, with formulation E displaying a slightly higher b* value (34.83) than formulation D (34.76), indicating a slightly more yellowish hue. These nuanced differences are reflected in their respective ΔE* values, with formulation E displaying an ΔE* value of 3.77, indicative of a moderate colour difference compared to formulation D, with an ΔE* value of 3.45. Overall, the analysis highlights subtle variations in the colour profiles of the formulations, with formulation F standing out as the lightest and most greenish, while formulation D presents a slightly less yellowish hue compared to formulation E. The value of ΔE* between 0 and 1.5 is thought to imply that the formulations are almost similar to the naked eye. The colour difference may already be seen when the AE* value is between 1.5 and 5, and it gets more noticeable when the AE* value is larger than 5 (Obón et al., 2009).

Table 2. Results for colour profile analysis

Formulation	L*	a*	b*	ΔE*
A	53.71±0.04^{c}	14.87±0.52^{a}	32.80±0.20^{c}	0.00±0.00^{c}
B	54.38±0.38^{bc}	15.10±0.81^{a}	33.43±0.65^{bc}	0.96±0.49^{c}
C	55.16±0.26^{b}	14.68±0.44^{ab}	33.96±0.16^{abc}	1.86±0.03^{c}
D	56.56±0.49^{a}	14.87±0.63^{a}	34.76±0.47^{ab}	3.45±0.65^{b}
E	56.88±0.57^{a}	15.04±0.73^{a}	34.83±0.74^{ab}	3.77±0.72^{ab}
F	57.58±0.54^{a}	13.15±0.47^{b}	35.21±0.75^{a}	4.86±0.36^{a}

Control A (100% tapioca flour), Formulation B (0% CCP: 100% oat fibre), Formulation C (25% CCP: 75% oat fibre), Formulation D (50% CCP: 50% oat fibre), Formulation E (75% CCP: 25% oat fibre), and Formulation F (100% CCP: 0% oat fibre). Values are expressed as mean ± SD of triplicate measurement. Superscripts with

Proximate Compositions

The proximate analysis results presented in Table 3 provide valuable insights into the nutritional composition of the formulations analysed. The parameters evaluated include moisture content, protein content, fat content, fibre content, ash content, and carbohydrate content for six different samples labelled from A to F. Moisture content, an important indicator of the water content in the formulations, ranged between 51.48% to 52.11%, with

minimal variation observed among the formulations. This consistency suggests that the formulations were processed or stored under similar conditions, maintaining uniformity in moisture levels. The trend in this study shows the moisture content of the *otak-otak* increasing as the amount of corn cob increased. This shows that the corn cob has the ability to hold the water molecules in the food product compared to the oat fibre and tapioca flour. However, based on statistical analysis, there was no significant difference (p>0.05) among the formulations. Thus, oat fibre and CCP additions did not affect moisture content.

Protein content, a crucial nutrient for growth and repair, displayed relatively consistent values across all formulations, ranging from 14.03% to 14.33%. This consistency suggests that the protein content of the formulations was maintained during processing, indicating the reliability of the production process in preserving this essential nutrient. The trend of the crude protein content in this study shows the decreasing amount of crude protein when the amount of corn cob powder was increased. However, based on statistical analysis, there was no significant difference (p>0.05) among the formulations. Thus, oat fibre and CCP additions did not affect protein content.

Fat content varied slightly among the formulations, ranging from 5.64% to 6.32%. Formulation A exhibited the lowest fat content, while sample F displayed the highest. The total fat content of corn cob powder was 4.72% (Abubakar et al., 2016), while the amount of fat content in oat fibre was 6.91% (Syed et al., 2020) and tapioca flour's fat content is only 0.60% (Balogun et al., 2012). This variation could be attributed to differences in the amount of oat fibre and CCP in the formulations. Therefore, the additions of oat fibre and CCP resulted in a significant difference between treated formulations and control.

Fibre content ranged from 0.49% to 0.86%. Control A exhibited the lowest fibre content, while formulation F had the highest. This variation suggests differences in the fibre content of the raw materials used or variations in processing methods affecting fibre retention. Adding com cob powder and oat fibre to the otak-otak formulation increased crude fibre content. This was because the crude fibre content of corn cob powder was higher, giving it a higher fibre content in the food products incorporated with corn cob powder (Aniola et al., 2009). According to (Abubakar et al., 2016), the amount of fibre in the corn cob powder was 33.33%, which shows that the corn cob contains a high amount of fibre. This is also supported by Ahmad et al. (2021), who found that adding corn cob powder and oats into *putu piring* increased its total crude fibre content. This shows that the ability of com cob powder to replace the tapioca flour in *otak-otak* has successfully increased the total crude fibre content. Therefore, corn cob powder is also known to have insoluble fibre (Kuan et al., 2011).

Ash content, representing the inorganic mineral content of the formulations, showed minimal variability, ranging from 5.18% to 5.39%. Table 4.3 shows that the total ash content of *otak-otak* incorporated with corn cob powder and oat fibre did not have a significant difference at p>0.05 with the control sample. This was because the total ash content in the corn cob powder, oat fibre and tapioca flour was almost the same, where corn cob powder had 2.49% ash (Abubakar et al., 2016), oat fibre had 1.97% ash (Syed et al., 2020) and tapioca flour had 2.20% of ash (Balogun et al., 2012). This is also supported by the fact that the mineral content of the product determined the ash content of the food product. The higher the mineral, the higher the ash content in the food product. Hence, substituting corn cob powder and oat fibre with tapioca flour did not affect the total ash content of the *otak-otak*. This study was supported by the finding by Hamzah and Wong (2012), where the percentage of ash did not show any significant difference when the amount of corn cob powder increased in the formulation of high-fibre bread.

Table 3. Results for proximate analysis

Formulation	Moisture	Protein	Fat	Fibre	Ash	Carbohydrates
A	51.50±1.00^{a}	14.33±0.29^{a}	5.64±0.05^{b}	0.49±0.04^{b}	5.18±0.12^{a}	21.85±0.59^{a}
B	51.66±1.06^{a}	14 32±0.07^{a}	6.14±0.04^{a}	1.78±0.06^{a}	5.38±0.10^{a}	20.72±1.21^{a}
C	51.48±0.33^{a}	14.23±0.11^{a}	6.28±0.03^{a}	1.80±0.02^{a}	5.20±0.10^{a}	21.01±0 49^{a}
D	51.60±0.66^{a}	14.17±0.09^{a}	6.22±0.04^{a}	1.82±0.04^{a}	5.39±0.02^{a}	20.79±0.68^{a}
E	52.01±0.26^{a}	14.10±0.08^{a}	6.05±0.04^{a}	1.84±0.03^{a}	5.20±0.16^{a}	20.84±0.15^{a}
F	52.11±0.36^{a}	14.03±0.09^{a}	6.32±0.03^{a}	1.86±0.03^{a}	5.36±0.03^{a}	20.32±0.31^{a}

Carbohydrate content ranged from 21.32% to 22.85%, with slight variations observed among the formulations. This consistency suggests that carbohydrates constitute a significant portion of the formulation composition, contributing to their energy content. Based on Table 3, the results obtained from this study show no significant difference (p>0.05) between all formulations.

The carbohydrates remain the same as the total amount of each substituted ingredient, such as corn cob and oat fibre, which contain almost the same amount of carbohydrates. This was supported by the study by Ahmad et al. (2021), where the amount of carbohydrates in putu piring does not change when the CCP and oat fibre are in the *putu piring* formulation. Overall, the proximate analysis results indicate that the formulations exhibit relatively consistent nutritional profiles, with minor fat and fibre content variations.

Control A (100% tapioca flour), Formulation B (0% CCP: 100% oat fibre), Formulation C (25% CCP: 75% oat fibre), Formulation D (50% CCP: 50% oat fibre), Formulation E (75% CCP: 25% oat fibre), and Formulation F (100% CCP: 0% oat fibre). Values are expressed as mean ± SD of triplicate measurement. Superscripts with different letters are significantly different at $p<0.05$ in the same column.

Sensory Evaluation

The sensory acceptance results presented in Table 4 provide valuable insights into the perceived qualities of different samples based on appearance, colour, aroma, texture, taste, and overall acceptance. Respondents evaluated each sample, labelled A through F, providing ratings on a scale accompanied by standard deviations to indicate the variability within each assessment. There was no significant difference ($p>0.05$) among all formulations regarding appearance, colour, aroma and taste. Thus, adding oat fibre and CCP will not affect these attributes.

Meanwhile, texture, an important aspect of food sensory evaluation, showed some divergence among the samples. Formulations A, E, C, D and B scored higher in texture, suggesting they possessed desirable mouthfeel characteristics. Conversely, Formulation F received lower scores in this category, implying potential issues with texture that may have detracted from the overall enjoyment. This result also showed similar trends for overall acceptability. Formulations A and F significantly differed from formulation F, which was added with 100% CCP. Thus, the higher percentage of CCP will affect the texture and overall acceptability of the *otak-otak*.

Table 4. Results for sensory acceptance

Formulation	Appearance	Color	Aroma	Texture	Taste	Overall Acceptance
A	4.87 ± 1.63^{a}	4.90 ± 1.65^{a}	4.43 ± 1.52^{a}	5.03 ± 0.89^{a}	4.67 ± 0.84^{a}	4.90 ± 0.85^{a}
B	4.43 ± 1.31^{a}	4.43 ± 1.25^{a}	4.67 ± 1.56^{a}	4.13 ± 1.52^{ab}	4.00 ± 1.49^{a}	4.07 ± 1.36^{ab}
C	4.83 ± 1.34^{a}	4.80 ± 1.54^{a}	4.93 ± 1.34^{a}	4.43 ± 1.48^{ab}	4.23 ± 1.74^{a}	4.57 ± 1.48^{ab}
D	4.40 ± 1.63^{a}	4.73 ± 1.39^{a}	4.50 ± 1.76^{a}	4.23 ± 1.46^{ab}	3.70 ± 1.69^{a}	3.87 ± 1.72^{ab}
E	4.97 ± 1.45^{a}	4.80 ± 1.61^{a}	5.00 ± 1.49^{a}	4.80 ± 1.52^{a}	4.47 ± 1.57^{a}	4.73 ± 1.72^{a}
F	4.43 ± 1.48^{a}	4.57 ± 1.31^{a}	4.17 ± 1.76^{a}	3.77 ± 1.50^{b}	3.60 ± 1.61^{a}	3.60 ± 1.48^{b}

Control A (100% tapioca flour), Formulation B (0% CCP: 100% oat fibre), Formulation C (25% CCP: 75% oat fibre), Formulation D (50% CCP: 50% oat fibre), Formulation E (75% CCP: 25% oat fibre), and Formulation F (100% CCP: 0% oat fibre). Values are expressed as mean ± SD of triplicate measurement. Superscripts with different letters are significantly different at $p<0.05$ in the same column.

Conclusion

From this study, it was found that the use of corn cob powder and oat fibre can help in increasing the crude fibre content in *otak-otak*. The sample produced contained more crude fibre than the control sample. The other nutrients were maintained the same way, and adding corn cob powder and oat fibre did not affect any nutritional value other than fibre and fat. Thus, corn cob powder and oat fibre are suitable for other food formulations as they do not change the nutritional composition.

The physical analysis found that the hardness of the sample was directly proportional to the amount of corn cob powder used. Even though the hardness of the sample increased when the corn cob powder and oat fibre were added, the panellists still accepted it. Last but not least, increasing fibre in food products strengthens the ability of food by-products to be transformed into other ingredients that can help increase the nutritional value of the food. Thus, from this study, we have successfully improved the nutritional value of our local product to compete with other food products and meet consumer needs.

Recommendations

For further study, the shelf life of *otak-otak*, which contains corn cob powder and oat fibre, should be determined. This is due to the busy lifestyle of people nowadays, who always keep their food frozen and stored for a long period of time. This study can help identify whether the *otak-otak* can still be eaten if stored for a long time. Next, it is also suggested to study the effect of the type of cooking method of otak-otak on the texture and sensory acceptance of *otak-otak*. The cooking methods used are steaming, oven and air frying. This is because people nowadays love to eat healthy food that does not use oil in their cooking style. Hence, all of these cooking methods have a healthy benefit and may give different appearance and taste to the *otak-otak.*

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

* We would like to express our gratitude to all those who have contributed to the completion of this research project and the preparation of this manuscript.

References

Abubakar, U. S., Yusuf, K. M., Safiyanu, I., Abdullahi, S., Saidu, S. R., Abdu, G. T., & Indee, A. M. (2016). Proximate and mineral composition of corn cob, banana and plantain peels. *International Journal of Food Science and Nutrition*, *1*(6), 25-27.

Ahmad, F., Ismail, N. E., Chilek, T. Z., Zamri, A. I., Zainol, M. K., Wahab, R. A., & Hamid, M. A. (2021). Effect incorporated of oat and corn cob powder on physicochemical and sensory properties of Putu Piring. *Bulletin of Culinary Art and Hospitality*, *1*(1), 7-12.

Aniola, J., Gawecki, J., Czarnocinska, J., & Galinski, G. (2009). Corncobs as a source of dietary fiber. *Polish Journal of Food and Nutrition Sciences*, *59*(3).

Baixauli, R., Salvador, A., Hough, G., & Fiszman, S. M. (2008). How information about fibre (traditional and resistant starch) influences consumer acceptance of muffins. *Food Quality and Preference*, *19*(7), 628-635.

Balogun, M. A., Karim, O. R., Kolawole, F. L., & Solarin, A. O. (2012). Quality attributes of tapioca meal fortified with defatted soy flour. *Agrosearch*, *12*(1), 61-68.

Fustier, P., Castaigne, F., Turgeon, S. L., & Biliaderis, C. G. (2008). Flour constituent interactions and their influence on dough rheology and quality of semi-sweet biscuits: A mixture design approach with reconstituted blends of gluten, water- solubles and starch fractions. *Journal of Cereal Science*, *48*(1), 144-158.

Hamzah, Y., & Wong, F. L., (2012). Physicochemical properties and acceptance of high fibre bread ıncorporated with corn cob flour. *Asian Journal of Food and Agro-Industry*, *5*(6), 547-553.

Kuan, C. Y., Yuen, K. H., Bhat, R., & Liong, M. T. (2011). Physicochemical characterization of alkali treated fractions from corncob and wheat straw and the production of nanofibers. *Food Research International*, *44*(9), 2822-2829.

Liukkonen, K. H., Montfoort, Ad., & Laakso, S. V. (1992). Water-induced lipid changes in oat processing. *Journal of Agricultural and Food Chemistry*, *40*(1), 126-130.

Obón, J. M., Castellar, M. R., Alacid, M., & Fernández-López, J. A. (2009). Production of a red-purple food colorant from Opuntia stricta fruits by spray drying and its application in food model systems. *Journal of Food Engineering*, *90*, 471-479.

Purlis, E., & Salvadori, V. O. (2007). Bread browning kinetics during baking. *Journal of Food Engineering*, *80*(4), 1107-1115.

Samakradhamrongthai, R., Thakeow, P., Kopermsub, P., & Utama-Ang, N. (2017). application of multi-core encapsulated Michelia alba D.C. flavor powder in Thai Steamed Dessert (Nam Dok Mai). *Journal Science, 44*(2).

Syed, S. J., Gadhe, K. S., & Katke, S. D. (2020). Studies on physical, chemical and mineral evaluation of oats (Avena sativa). *Journal of Pharmacognosy and Phytochemistry*, *9*(5), 79-82.

Author Information

Fisal Ahmad
Faculty of Fisheries and Food Science,
Universiti Malaysia Terengganu, 21030 Kuala Nerus,
Terengganu, Malaysia.
Functional Food RIG, Food Security in a Changing Climate SIG, Food Security Research Cluster, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
Contact e-mail: *fisal@umt.edu.my*

Muhamat Hafiz Sapiee
Faculty of Fisheries and Food Science,
Universiti Malaysia Terengganu, 21030 Kuala Nerus,
Terengganu, Malaysia.

To cite this article:

Ahmad, F. & Sapiee, M.F. (2024). The effect of corn cob powder and oat fibre incorporation in physicochemical properties and sensory acceptance of Otak-Otak. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 32-39.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 40-49

ICGeHeS 2024: International Conference on General Health Sciences

Menstrual Patterns and Identifying Health Trends

Rusudan Vadatchkoria
Batumi Shota Rustaveli State University

Devasi Rupal Ramesh
Batumi Shota Rustaveli State University

Abstract: Menstrual cycle is natural and one of the most important processes of female life, which gives us insight about significant hormonal and behavioral changes in female organism. In this regard, in order to have a better understanding of the different, and very specific factors, associated with menstrual health status, we conducted a survey study of some aspects of menstrual period. The survey was based on several targeted groups of the age between 8 and 49 years old, Indian and Georgian women. Various assumptions were made on behalf of the survey regarding the menstrual health and factors affecting or reliving the menstrual cycle related problems. Besides, the survey was a precious confirmation of the assumption of females conceive, that menstrual cycle affects their everyday lifestyle, the survey research method undoubtedly has some priorities to facilitate the understanding of real circumstances and to provide a basis for planning subsequent studies, which will certainly be expanded and deepened in the near future. In addition, factors that may be associated with the state of menstrual health, as well as the level of awareness of the female population about the characteristics of the menstrual cycle are shown. The final achievement of this study is a broader knowledge of the characteristics of the female body during these rather difficult days in the life of every woman, in order to properly manage this period, better adapt to everyday life and maintain normal ongoing activities.

Keywords: Menstruation, Tracking, Modifiable, Non-modifiable factor.

Introduction

Menstrual period in the female health has some natural and important physiological and psychological parameters. It is insight to hormonal balance and co-ordination, to understand menstrual cycle and its pattern and the alleviating and prevocational factor, which affect menstrual cycle is very important, since hormonal imbalance can lead to various disorder, such as: mood swings, depression and affect work life and day to day life of female, in this case, having better understanding of menstrual period features are very important. Menstrual health is not just about periods, its far way more important and deeper than understanding of your own body, ensuring the holistic care to one's body, tracking cycle, prioritizing mental, social and emotional factor affecting menstrual cycle. In various regions of Asian countries, even the speaking about Menstruation it's still a topic of stigma and embarrassment, therefore awareness regarding Menstrual health, hygiene and association with everyday lifestyle and its affect are less known by population. Apart from this, there are various modifiable and non-modifiable factors, which affect menstrual cycle, including eating habits, sleep cycle, smoking, drinking, exercise, stress, genetics, chances in environment, and etc.

Methods

To test the assumption of the "Menstrual Patterns and Identifying Health Trends" we conducted an online survey among women of menstrual age (8yrs-49yrs) (N=115) from India and Georgia. Based on the previously

developed on-line questionnaire the females' responses done remotely and were collected. The obtained data were statistically processed, summarized in the tables and presented in diagrams for further discussion, analysis and conclusions.

Result and Discussion

Menstrual Cycle Duration

In scientific term, menstrual cycle is regulated by complex interplay of hormonal changes in endocrine system. It consists of distinct phases, such as menstruation, the follicular phase, ovulation and luteal phase. Duration of menstrual cycle means the time interval between onset of one menstrual period (the first day of bleeding) and onset of next period. The average length of menstrual cycle is individual and varies from person to person. However, the healthy cycle is considered between 26 to 35 days (in average 28-30 days). Menstrual cycle duration is one of the major indicators of reproductive health, although there are various factors, which can influence menstrual cycle, namely stress, hormonal imbalance, side effect of medication, weight, exercise.

Certain Medical Condition, Hormonal Contraceptive

In order to understand the factors influencing menstrual cycle, set of an online questionnaire was prepared and were answered by female participants. Collected and analyzed responses regarding the menstrual cycle duration are demonstrated below accordingly (Figure 1).

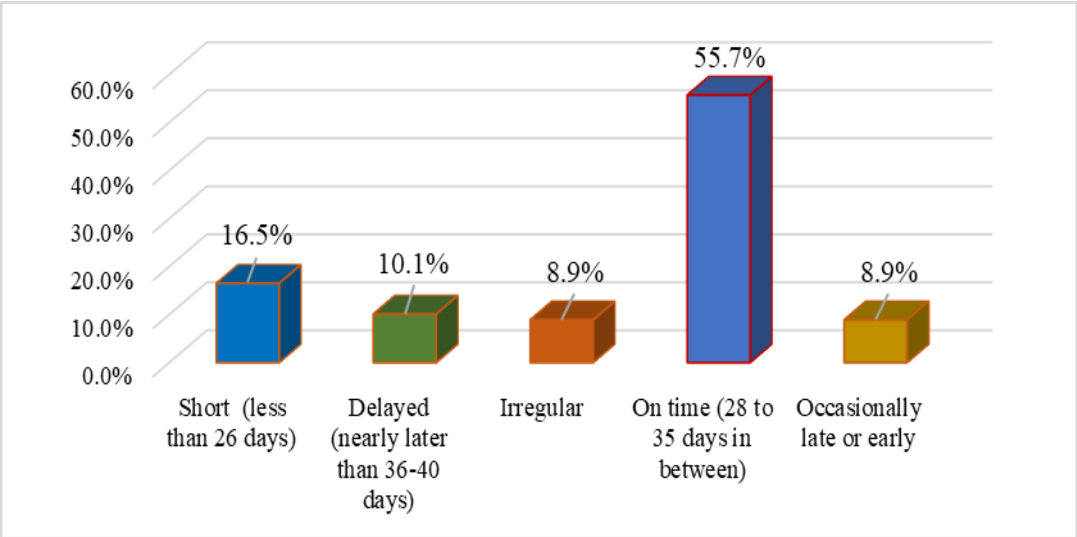


Figure 1. Character of menstrual cycle

Based on the survey results, about 55.70% of female are having healthy menstrual cycle duration falling within in range of 26 to 35 days; whereas around 16.50% of women are having early periods. From other side, roughly 10.10% of females are contend with delayed periods, lasting from 36 to 40 days, and about 8.90% participant encountered irregular menstrual cycle characterized by occasional lateness or earliness.

The data collected from online survey suggest that around 44.4% of females have imbalance in their menstrual cycle either maybe early, late, or irregular. There can be various factor affecting the cycle. Given that this study relies on observation and online surveys, its limitation lies in its inability to ascertain the root cause of irregular periods. Nonetheless, imbalance in menstrual cycle serve as a significant indicator for individuals to consider making improvements in healthy habits, lifestyle or seeking consultation with medical professionals for a thorough evaluation. The survey with similar study was conducted by Grieger J.A., which stated, that the average typical 28 days cycle length is not common for enough high percentage of female. Only 13.08% of women noticed their ovulation on 14th day, rest of them have very varied cycle, which is, again, affected by varied causative factors, and cultural and ethnic diversity tends to play a major role as well. (Grieger, 2020).

Menstrual Flow Duration Currently; Change in Flow in First Year of Menarche; Regularity in Flow

Menstrual flow usually consists of combination of blood, uterine tissue and mucus which is shedding of uterus during the period of menstruation. The amount of menstrual flow varies in every woman, and can differ in every cycle. There can be large variation in flow in females, which can also be categorize as normal flow depending on time and associated factor, unless and until flow is consistently not lighter and nor heavier.

In the conducted survey, when enquired about the women current status of flow duration as depicted in (Figure 2), around 60.30% of women experience their flow for 3-5 days and 26.90% of women indicated that their menstrual flow extended for duration of 5-7 days, while roughly 9% reported a flow lasting 2-3 days and 3.80% women reported having menstrual flow for more than 7 days.

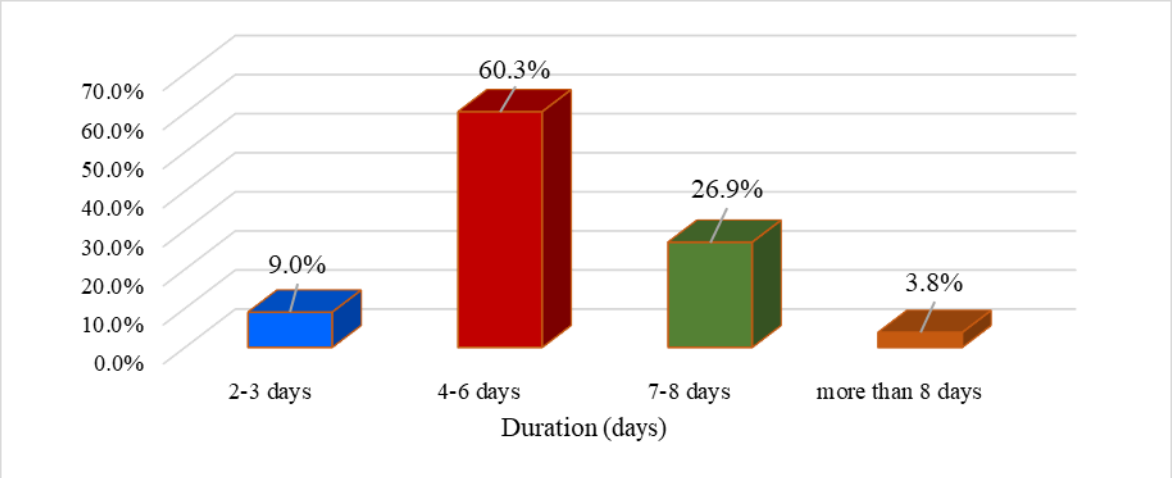


Figure 2. Duration of first year menstrual cycle

Similarly, in order to study how the flow of menstrual cycle varies among female, we drafted a set of additional questions concerning the first year of menarche character, which is done in the graph below (Figure 3).

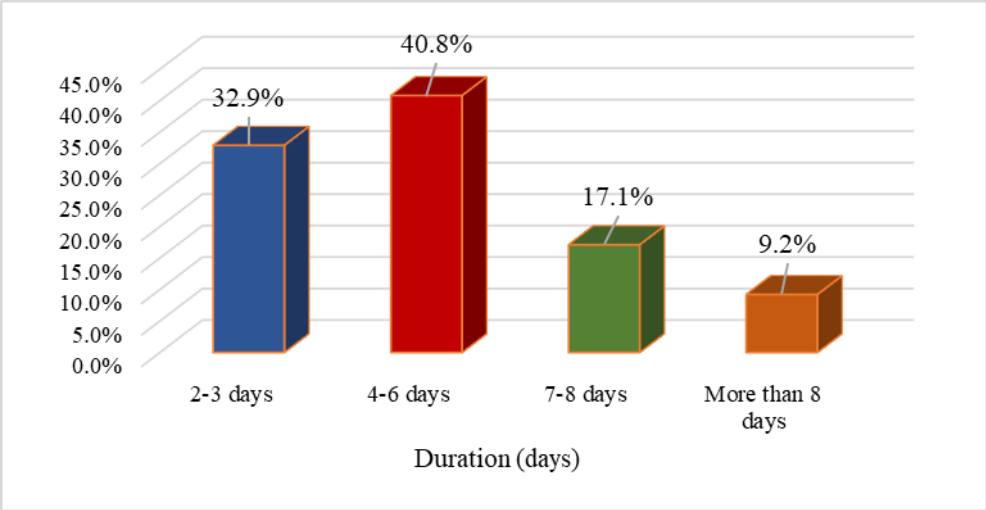


Figure 3. Duration of first menstrual period (for the period of survey)

During the first year following menarche, it was observed that moderate number of female experienced menstrual flow lasting for 5-6 days, while small proportion participants reported menstrual flow duration of 7-8 days, and roughly around 9.20% young women had a flow lasting more than 8 days. Supporting these empirical findings, a parallel study conducted in Tbilisi corroborated this observation by identifying oligomenorrhea as most prevalent menstrual cycle disorder. Oligomenorrhea is characterized by infrequent menstrual periods, typically marked by extended intervals between cycles. These investigations collectively yield valuable insights into the variation of menstrual flow duration and prevalence of menstrual cycle flow days among the participant during initial year of menarche (Dzhorbenadze, 2006), whereas amenorrhea can be caused by a broad spectrum

of causes, such as anatomic deficiencies of the reproductive tract and hormonal disorders. (Deligeoroglou, 2010).

The other set of questions which was put forward, concerned the first year of menarche regularity and demonstrated, that about 69.20% female had regular period or menstrual cycle during first year of menarche, whereas by around 30.80% females' irregularity of period was noted. One of study conducted by Williams via pediatric gynecology department stated, that menstrual disorders in adolescent girls are very common and their periods can be irregular, heavy and/or painful, especially in the first few years following menarche. (Williams, 2012).

To be exact and deepen the study in regularity of flow of women, which previously, during first year of menarche had irregular flow, some questions regarding regularity of periods was put forward further (when periods became regular and is the flow still irregular?). Received data are evaluated and reflected on the diagram below (Figure 4).

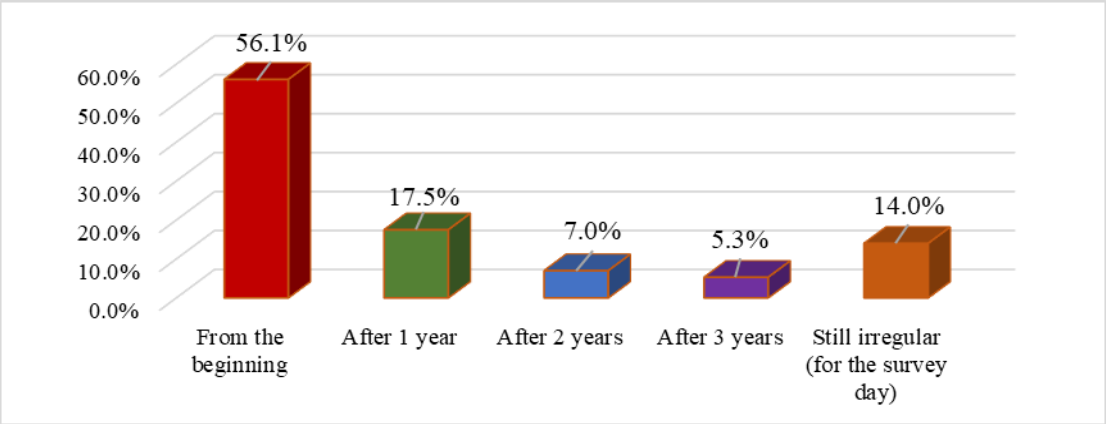


Figure 4. Stabilization period of menstruation

The evaluation of received results stated that around 14% of female are still facing irregular menstrual flow, 56.10% had regular menstrual flow during their first year, whereas 17.50% females after 1 year, 7% after 2 years, and 5.30% after 3 years. The irregular flow during first few years of menarche is considered as normal, unless other associated symptoms are absent. However, the major concern here can be seen after analyzing responses is, that 14% females are still experiencing irregular flow.

Menstrual Pain

Menstrual pain or dysmenorrhea is one of the most common symptoms among menstruating women, and usually ranges and varies from female to female. Along being one of the common symptoms, it can also be pioneer symptom to identify if there is any abnormality presented. The survey results are demonstrated below (Figure 5).

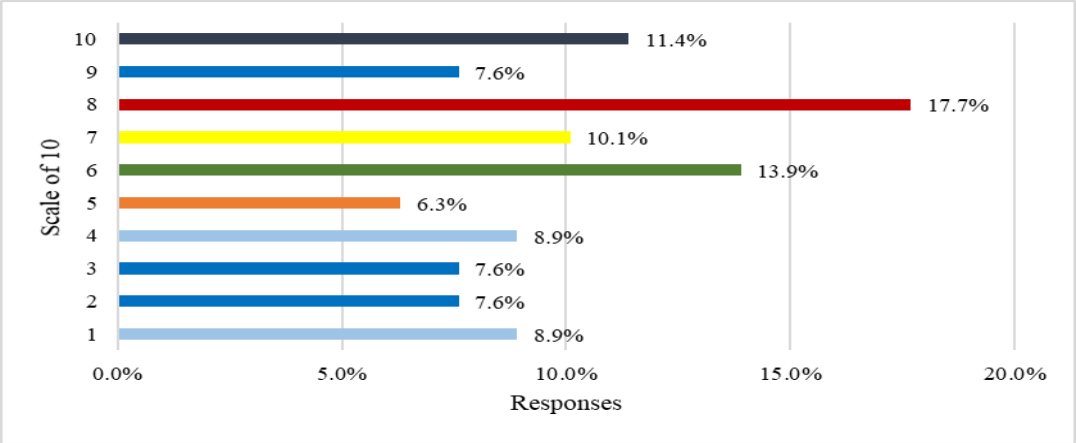


Figure 5. Rating of menstrual pain on the scale of 1 to 10

In these regards participants were asked to rate the menstrual pain on a scale of 1 to 10. According to data received, it is observed that approximately 11.40% participant report experiencing extreme menstrual pain, rating it at the highest intensity of 10 on scale 10. Additionally, 7.60% women rate their menstrual pain at level of 9, while an equal percentage, 7.60% assign a rating of 3 and 2, respectively, on the pain scale. Furthermore, 17.70% of females feel pain at an intensity of 8, 10.10% marked at level of 7 and 13.90% at 6. A lower percentage, 6.30% rate their menstrual pain at 5 and 8.90% of females assign a rating of 4 and 1 respectively.

In the context of dysmenorrhea, it is categorized in two groups. The first is known as Primary Dysmenorrhea, which entails pain associated with menstruation and is supposed to be not attributed to any underlying medical condition. The etiology of primary dysmenorrhea is often linked with hormonal factors, such as the release of substance like prostaglandins and vasopressin. On the other hand, the second category, Secondary Dysmenorrhea, is characterized by menstrual pain that is consequence of underlying medical condition. It is imperative to identify and address the root cause of dysmenorrhea for effective management. (Marjoribanks, 2015). The above (Figure 5) statistics underscore the significance of understanding the various levels of menstrual pain experienced by women, with notable portion reporting from moderate to severe discomfort.

Evaluation of menstrual pain by medical profession or doctors also was included in the current online survey. The result of the responses received and analyzed revealed around 63.30% female even after having menstrual pain never visited hospitals or been consulted to medical professionals, 13.90% females visited doctors during the starting years of pain, 21.50% females sometimes do visit a doctor, and around 1.30% female regularly visit doctor and follow medical advice regarding their menstrual pain. (Fig.6). Similar result is stated in one of the studies, which reveals the fact, that dysmenorrhea is the major health problem in adolescent, therefore, awareness about this issue should be increased (De Sanctis, 2015).

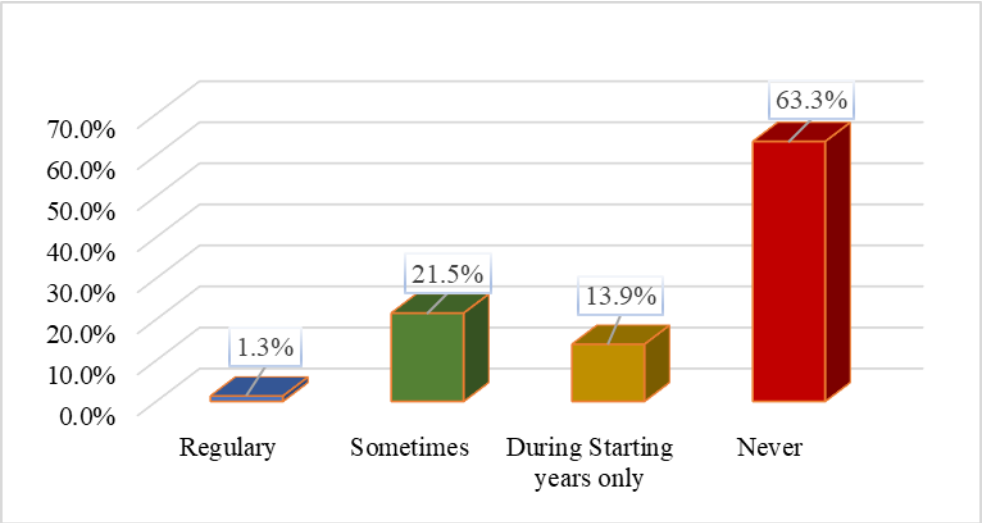


Figure 6. Visiting a doctor concerning the menstrual pain

Medications for Menstrual Pain

Menstrual pain may impede lifestyle of women. In order to avoid any interference in their day-to-day life and to gain relief from pain, many females all over the world take medication. The survey revealed, that around 41.80% of females are taking medication for menstrual pain whereas 58.20% of respondents do not need or abstain from taking of any kind of medications.

There have been contacted various studies for the aim to know the prevalence of females taking medication for menstrual pain, one of which stated that around 86.14% of female from the study participant are following pharmacological methods for menstrual pain (Tataj-Puzyna, 2021). Similar study conducted stated, that 25% women systematically take medication for relieving menstrual pain (Grandi, 2012).

The exact period of medication intake started were also determined and it was estimated that around 78% of study participants started taking medication in the recent years, whereas 22% of females were taking from start of menarche. Regarding the frequency of taking medication intake it was ascertained, that around 48.80% of

females take medications occasionally, 34.10% women just on the first day of menstruation and around 17.10% of females - on every menstrual cycle and precisely for more than two days.

More details about widely used specific pharmacological drug among participants of the study, is demonstrated on the Fig 7. In particular, the study showed, that 65.10% of women use Meftal-Spas, 12.20% uses Ibuprofen, 10.40% - Dexagin Sachet, 7.30% - Paracetamol, and 5% of women uses Cetrolac.

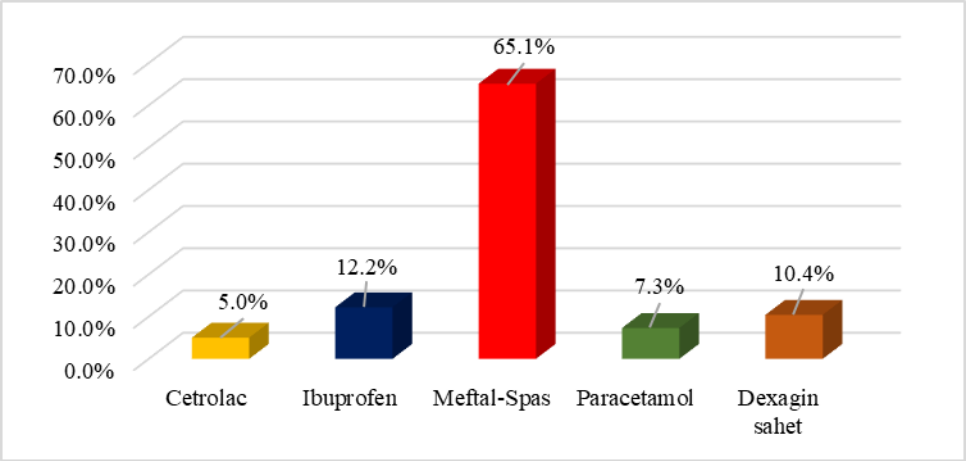


Figure 7. Type of intake medicine

Mood Swings/ Cravings during Menstrual Cycle

Mood swings and cravings are the very common premenstrual or menstrual symptoms seen in population. The symptoms widely range from female-to-female and can be experienced before the menstruation or during menses, as given below in Fig. 8. There are wide variety of feelings or emotions, which participants of online survey experience. 49% females stated that they feel irritated, 37% females feel anxious and anger, 27% females noted more fatigue, 21% females were not able to concentrate on work, 17% had the sense of hopelessness, 14% stated loss of appetite, 5% described some other kind of emotions and 6% of females did not feel any such mood swings.

Similar study conducted by Nworie among Nigerian students stated the prevalence of pre-menstrual symptoms among participant is enough higher (Nworie, 2018). Another meta-analysis study (Ramcharan, 1992) affirm that the prevalence of pre-menstrual symptoms among females have been increased during 1996-2011.

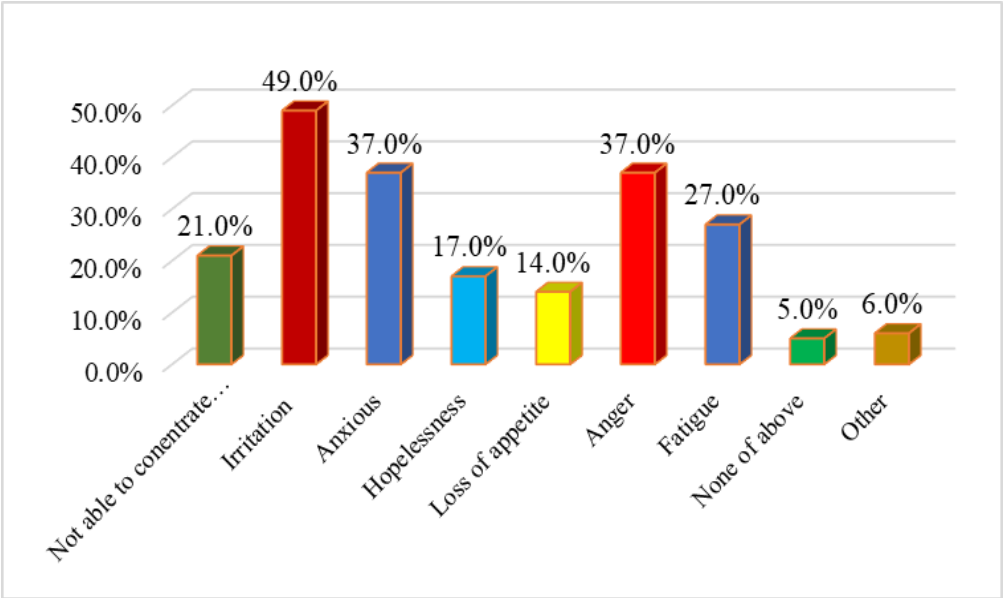


Figure 8. Mood swings or changes experience during menstrual cycle

Among the menstruating females one of the main symptoms which is seen in participants is craving or binge eating during or before menstrual cycle. As depicted in the image below (Figure 9) 41% of females stated that they crave for junk foods, 40% of women - for sweets products/deserts, 12% of females - dairy products, 11% participant - cold drinks only, or none of any such thing respectively.

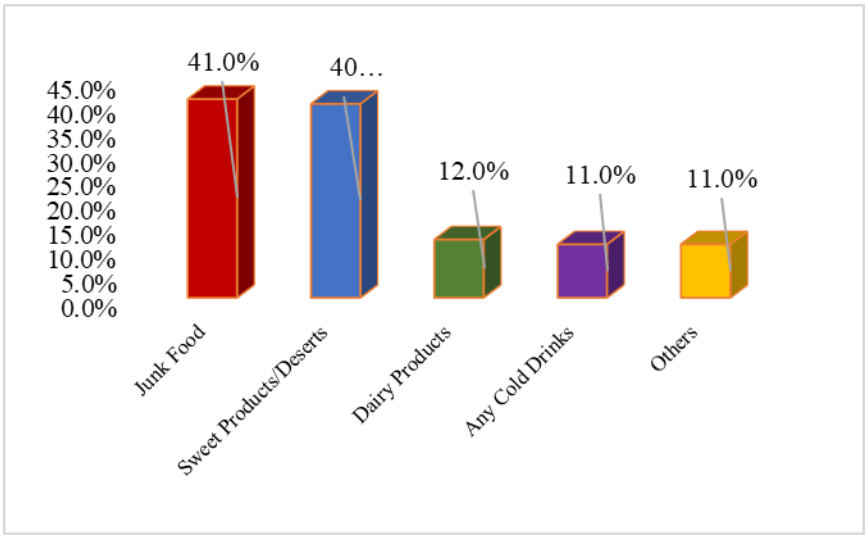


Figure 9. Craving types of food during the menstrual period

The menstrual cycle/period cramps and their effect on their day-to-day life evaluation asserted, that 49.49% of women think they have menstrual pain/mood swings which affect their life and various other activities, in contrast 39.20% participants perceive it as intermittently influencing their circumstances of day-to-day life and 11.40% of females think that menstrual pain/mood swings have no impact on their day-to-day life. Correspondent study was conducted among Mexican students, and it was deduced that dysmenorrhea constituted one of the factors contributing to short-term absenteeism among the student population (Ortiz, 2009).

Menstrual Tracking, Diet, Exercise Impact

In social media there are various kinds of technological applications, like “Flo App for menstrual cycle - calculate menstrual cycle”, “Flo tracker”, “Period tracker”, etc.,), which are developed to track menstrual cycle. The Flo App have the inbuilt feature to predict next menstrual cycle, to assess the symptoms and emotions which females are feeling, and can prognose female’s ovulation day respectively. To evaluate the utilization of menstrual cycle tracking applications, a series of questions was formulated, and findings demonstrate, that 54.40% women do track their menstrual cycle, whereas 45.60% of them do not control their menstrual cycle.

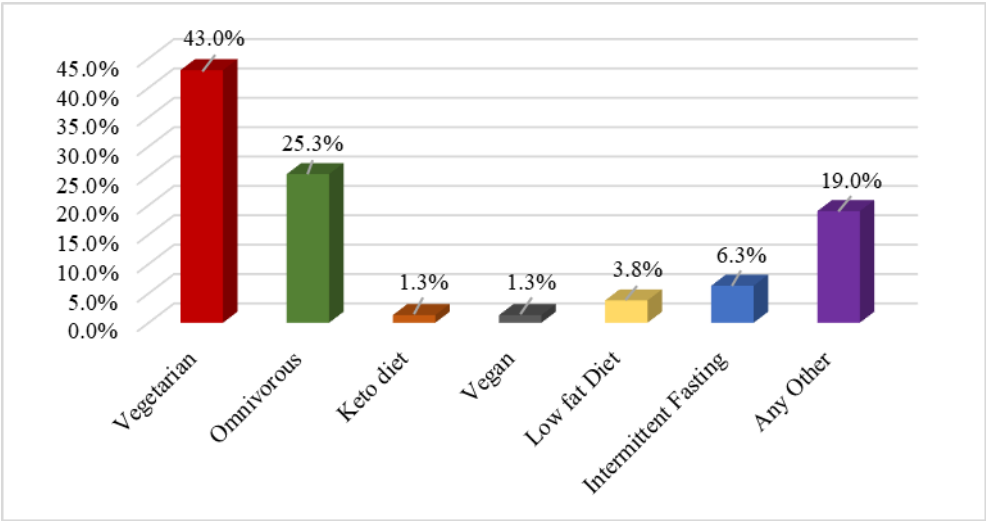


Figure 10. Type of usual diet

Diet has great impact on menstrual cycle. As it seen below (Figure 10) 43% of females choose vegetarian diet, 25.30% have opinion, that their diet have to be omnivorous, 19% review other type of diet, which are not specifically mentioned, 1.30% think that their diet should be keto and 1.30% vegan respectively, 6.30% females state that they are intermittent fasting, and 3.80% females prefer low fat diet. Due to great diversity in diet of women it cannot be highly assured, that their menstrual cycle is probably being affected or not including other various causative factors

The impact of physical activity on menstrual cycle regularity was noted in responses: for 77.20% females exercising routine or physical activity have a great impact on menstrual cycle, 22.80% females stated that physical exercise do not have any impact on their menstrual cycle.

Disorders

There is various correlation of various disorders, high body mass index (BMI), and hormonal changes, which evidently have impact on menstrual cycle (Figure 11). It was revealed, that 57% of females have no specific disorder, 9% of participants expressed, that they are suffering with depression symptoms, 6% are noted overweight, 4% stated polycystic ovary syndrome, 3% females are suffering from endometriosis, and 2% females have the thyroid related problem. Clarification the question whether there are other female close relatives with similar dysmenorrhea problem shown, that 52.90% females have other family member with similar kind of pain, and 47.10% female stated that they don't have any other female with dysmenorrhea in the same family.

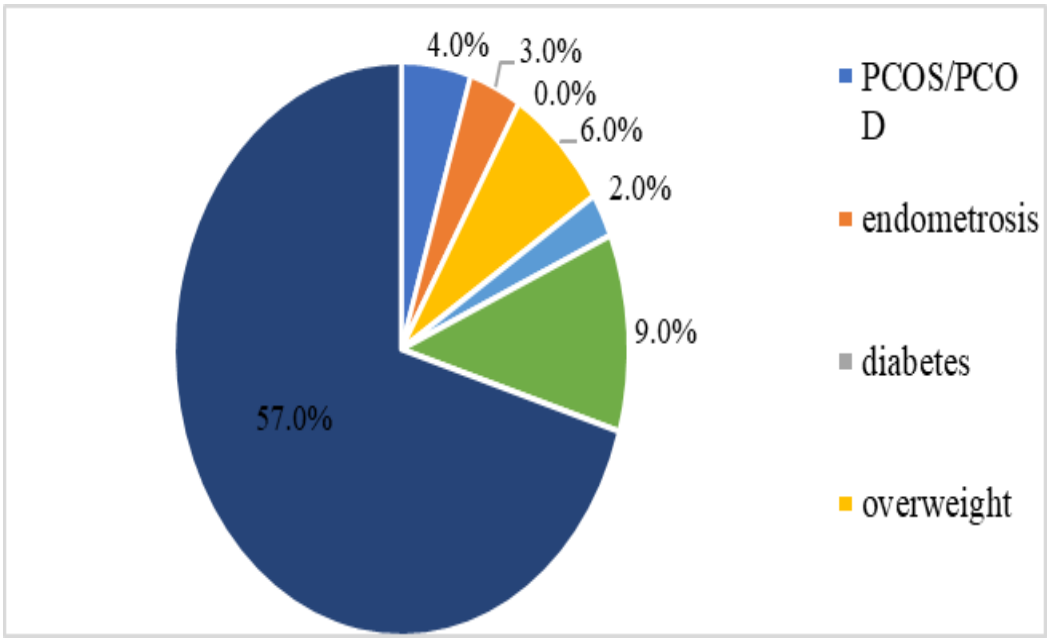


Figure11. Any past or current history of the following health problem

Conclusions and Recommendations

From all the data collected and analyzed from online survey and various studies supporting with the received information as evidence it can be concluded the followings:

1. Among the study participants the most are Indian and Georgian women with the age between 8 and 49 years old;
2. In target groups the menstrual flow duration for 3-5 days was noted, while during first year of menarche the flow lasted for 5-6 days;
3. Menstrual cycle/period cramp has a great impact on their day-to-day life, due to various pre-menstrual symptom faced by females, it has negative impacts on their work and study lives;

4. Even after having menstrual pain or dysmenorrhea majority of female population do not visit a medical profession, or do not seek medical professional help. Therefore, it's very important to increase awareness among women;
5. Women on large scale track their menstrual cycle to understand their ovulation timings and their cycle period;
6. Physical activity, diet and sleep routine has major impact on their menstrual cycle;
7. On a large-scale female has been using Meftal-Spas (NSAID) for dysmenorrhea. These are medications, which are taken largely for menstrual pain, but due to study limitation it couldn't be identify the kind of dysmenorrhea (primary or secondary);
8. Desserts/Chocolates are consumed on large scale before and during menstrual cycle, Junk food is majorly consumed by females before their menses;
9. The genetic variation, ethnicity (Indian and Georgian in study survey), diet, lifestyle and habit, are diverse, so we directly cannot conclude about the common causative factor of dysmenorrhea or menstrual cycle length variation reason, but despite this, some patterns have nevertheless been identified and research in this area is expected to be continued and in-depth.
10. Presented information is very essential to support updates of existing clinical guidelines concerning menstrual cycle length and patterns for clinical use in Indian and Georgian fertility programs, as well as to supply women with wide popular information about women's health and to increase their awareness about their specific period.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey, on May 02-05, 2024.

References

Deligeoroglou E., & Tsimaris P. (2010). Menstrual disturbances in puberty. *Clinical Obstetrics & Gynaecology. 24*(2),157- 171.

De Sanctis V., Soliman A., Bernasconi S., Bianchin, L., Bona, G., Bozzola,M., Buzi F., De Sanctis C., Tonini, G., Rigon, F., & Perissinotto, E. (2015). Primary dysmenorrhea in adolescents: Prevalence, impact and recent knowledge. *Pediatric Endocrinol Rev.,13* (2), 512-20.

Direkvand-Moghadam A., Sayehmiri K., Delpisheh A., & Kaikhavandy S. (2014). Epidemiology of premenstrual syndrome (PMS)-a systematic review and meta-analysis study. *Journal of Clinical and Diagnostic Research*, *8*(2), 106 – 109.

Dzhorbenadze, M. T., Kristesashvili, D. I., & Chopikashvili, N. A. (2006). Menstrual function in adolescent girls in Tbilisi. *Georgian Medical News*, (130), 37-40.

Grandi G., Ferrari S., Xholl, A., Cannoletta M., Palma F., Romani C., Volp, A., & Cagnacc, A. (2012). Prevalence of menstrual pain in young women: what is dysmenorrhea? *Journal of Pain Research, 5*, 169–174.

Grieger, J. A., & Norman, R. J. (2020). Menstrual cycle length and patterns in a global cohort of women using a mobile phone app: Retrospective cohort study. *Journal of Medical Internet Research*, *22*(6), e17109.

Marjoribanks J., Ayeleke R. O., Farquhar C., & Proctor M. (2015, Jul 30). Nonsteroidal anti-inflammatory drugs for dysmenorrhoea. *The Cochrane Database of Systematic Reviews, 2015*(7), 26224322.

Nworie K., Aluh D., & Onyekwum C. (2018). Assessment of premenstrual syndrome among female students in Southeast Nigeria. *Journal of Obstetrics and Gynecological Investigations, 1*(1), 55-61.

Ortiz M. I., Rangel-Flores E., Carrillo-Alarcón L. C., & Veras-Godoy H. A. (2009). Prevalence and impact of primary dysmenorrhea among Mexican high school students. *International Journal of Gynaecology and Obstetrics, 107*(3),240-243.

Ramcharan S., Love E. J., Fick G. H., & Goldfien A. (1992). The epidemiology of premenstrual symptoms in a population-based sample of 2650 urban women: attributable risk and risk factors. *Journal Of Clinical Epidemiology*, *45*(4), 377–392.

Tataj-Puzyna U., Ilczuk P., Kalita-Kurzyńska K., & Gotlib J. (2021). Women's experiences of dysmenorrhoea - preliminary study. Przeglad menopauzalny. *Menopause Review, 20*(3), 133–139.

Williams C.E., & Creighton, S.M. (2012). Menstrual disorders in adolescents: Review of current practice. *Hormone Research in Paediatrics,78*(3),135-143.

Author Information

Rusudan Vadatchkoria
Batumi Shota Rustaveli State University
Address: Georgia, Batumi, 6010,
Ninoshvili/Rustaveli str. 35/32, Georgia
Contact e-mail: rusudanvadatchkoria@gmail.com

Devasi Rupal Ramesh
Batumi Shota Rustaveli State University
Address: Georgia, Batumi, 6010,
Ninoshvili/Rustaveli str. 35/32, Georgia

To cite this article:

Vadatchkoria, R., & Ramesh, D. (2024). Menstrual patterns and identifying health trends. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13,* 40-49.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 50-54

ICGeHeS 2024: International Conference on General Health Sciences

Cytotoxic Activity of *Onobrychis Megataphros* Leaf Extract

Salih Muvakit
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract: This study aims to determine the cytotoxic effects of *Onobrychis megataphros* plant leaves. Therefore, firstly, the *O. megataphros* samples included in our study were collected from Şanlıurfa and the surrounding areas (Siverek-Hilvan) during the vegetation periods between 2021-2022. The surface parts of the plants were dried in the shade, in the open air, and then pulverised to a suitable size with the help of a scale mill. The plant material was extracted three times separately with methanol at room temperature. After filtration, the samples were subjected to methanol extraction in Soxhlet apparatus for 6-8 hours. The extract was then filtered with Whatman blue band filter paper and evaporated at 40°C to remove the solvent. The cytotoxic activity of plants is associated with the presence of bioactive compounds such as flavonoids, alkaloids and saponins. Studies have shown that these sustances have anticancer effects by inducing apoptosis that inhibits cell proliferation and preventing angiogenesis. In our study, the cytotoxic activity of *Onobrychis megataphros* was investigated. The focus of our study is to fully understand the mechanisms underlying the cytotoxic activity of *O.megataphros* and to determine its potential as a therapeutic agent in cancer treatment. In vitro cytotoxicity experiments that were carried out in the laboratory, PC-3 prostate cancer cell line (CRL-1435) was obtained commercially from ATCC. Cytotoxic activity of the *O.megataphros* was detected with MTT (3-4,5-dimetil-tiyazolil2,5-difeniltetrazolyum bromid) test. The results have shown that the sample has not decreased vitality in all cell tests. The vitality was 100%. In conclusion, *O. megataphros* is a promising plant as a natural source of cytotoxicity with potential anticancer activity, and further research on this topic may contribute to the development of new cancer therapies that are both safe and effective.

Keywords: *Onobrychis megataphros*, Cytotoxicity, Cytotoxic activity

Introduction

Phytotherapy, which means the use of natural compounds found in plants, vegetables and roots on patients and is considered among complementary medicine methods, has been practiced since ancient times. The use of complementary medicine has increased steadily in recent years in many developing and industrialized countries. In developed countries, the use of complementary medicine is 42.1% in the USA, 48.2% in Australia, 49.3% in France, 70.4% in Canada, 71% in Chile, 70% in China, 40% in Colombia and 80% in African countries (Ozcelik & Toprak, 2015).

In our nation, as in the rest of the world, many plant species have long been utilized by the populace for a variety of reasons (Awuchi, 2019; Muvakit & Ozaslan, 2023). Roughly 20,000 medicinal plants are utilized for therapeutic purposes, according to publications on medicinal plants, 91 nations' pharmacopoeias, and the World Health Organization (WHO). Since 1926, researchers have studied the crucial aspects of plants for human health in the lab (Mazzoleni & Nelson, 2005).

There are two subgenera within the genus Onobrychis, which grows natively in Turkey: Onobrychis and Sisyrosema. Dendobrychis, Laphobrychis, and Onobrychis are the three known divisions of Onobrychis among

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

these subgenera. With a variety of applications among humans, the Fabaceae family is the second largest family in Turkey (Muvakit and Ozaslan, 2023), following Asteraceae. For instance, Vicia faba is used to treat gastrointestinal disorders, Onobrychis gracilis to cure colds and flu, Vicia cracca subsp. stenophylla to treat colds, and Vicia ervilia to treat diabetes (Demirci & Ozhatay, 2012; Sargın et al., 2013; Hayta et al., 2014).

Plant extracts are compounds obtained from different parts of the plant (root, leaf, flower, etc.) using various solvents. These extracts can contain many bioactive compounds, including various compounds such as alkaloids, flavonoids, terpenoids, phenolic compounds and lignans. These compounds can inhibit cell growth, trigger apoptosis (programmed cell death) and stop the proliferation of cancer cells (Ingle et al., 2017).

Cytotoxic activity refers to the ability of a substance to inhibit the growth of cells or trigger cell death. By targeting cancer cells, this activity can stop tumor growth or kill cancer cells. Plant extracts may have cytotoxic activity and are therefore being investigated as potential therapeutic agents for cancer treatment (Habli et al., 2017).

Although studies have revealed that plant extracts are extremely important for health, studies in this direction continue rapidly. Although studies on the determination of these potentials of plants belonging to the genus Onobrychis, which are widely available in our country, have accelerated in recent years, studies on Onobrychis megataphros species have been limited.. The aim of this study was to determine the cytotoxic activity of *Onobrychis megataphros* leaf extracts.

Method

Collection and Extraction of Plant Samples

The Onobrychis megataphros plant utilized in the study was gathered in the vegetative phases of 2021 and 2022 from Şanlıurfa and the surrounding area (Siverek-Hilvan). The plant samples were dried in the shade, and then the leaves were ground into a fine powder. In a sokslet device, the samples were exposed to methanol extraction for six to eight hours. Subsequently, the extract was filtered through Whatman blue band filter paper and the solvent was evaporated at 40 °C.

Cell Culture Studies

Cultivation of Cells

In our study, PC-3 prostate cancer cell line (CRL-1435) was obtained commercially from ATCC. Cells stored at -80 °C were thawed before the study and placed in medium (DMEM containing 10% FBS, 1% penicillin-streptomycin and 1% L-glutamine). Centrifuged at 800 rpm for 5 min, the cells settled to the bottom. The pellet was dissolved in 5 mL of medium, transferred to a 25 cm2 cell culture dish and incubated at 37°C with 5% CO2. Cultured cells were observed daily under an inverted microscope and the medium was changed every other day. When the cells covered 80% of the cell culture dish, they were trypsinized and seeded into new culture media and cultured until sufficient cell numbers were reached.

For trypsinization, the medium of the cells was removed. Cells were washed with DPBS to remove serum and dead cells. Afterwards, DPBS was aspirated and 0.5 mL of 0.1% Trypsin-EDTA solution was added to separate the cells from the culture dish and incubated at 37 °C with 5% CO2 for 4 min. At the end of 4 min, the cells were checked under a microscope to see if they had detached from the culture dish. After making sure that all cells were removed, 5ml of medium was added to the cells and this cell suspension was transferred to a falcon tube. After centrifugation at 800 rpm for 5 min, 5 mL of medium was added to the pellet to ensure homogeneous mixing of the cells and then the cell suspension was transferred to 25cm2 cell culture dishes.

Cell Counting

For cell counting, trypsinized cells were used. After trypsinization, the cells were centrifuged and the pellet was thawed in 5 mL medium. A 10µL volume of the cells was taken and an equal amount of 0.5% trypan blue dye was added. After pipetting, 10 µL of this mixture was placed on a Thoma slide and cell counting was performed

under an inverted microscope. The number found was multiplied by the dilution coefficient and the number of cells in 1 mL of medium was calculated.

Cytotoxic Activity Test

The cytotoxic effects of the sample were determined by MTT (3-4,5-dimethyl-thiazolyl-2,5-diphenyltetrazolium bromide) test. The MTT assay is a standard colorimetric assay that measures cell proliferation. It is based on the principle that the yellow MTT dye is converted into purple formazan crystals by the dehydrogenase enzyme in the active mitochondria of living cells and then this color change is measured spectrophotometrically (Mosmann, 1983). With this method, it is aimed to determine the growth profiles of cell cultures and to determine the cytotoxic effect depending on the application times and doses of the substances.

For MTT assay, PC-3 cells were grown in medium and counted before the study. Each well of the 96-well plate was seeded with 5×103 cells and left to incubate for 24 hours at 37 °C containing 5% CO2. After incubation, the sample was added to the cells at different doses (500-0 µg/mL) in 3 replicates. At the end of 48 hours of incubation, 40µL of MTT dye was added to each well and the cells were incubated for another 4 hours to ensure the formation of formazan crystals. After the presence of blue-violet formazan crystals was detected on the microscope, the medium was replaced with 80 µL DMSO to solubilize these crystals and formazan crystals were dissolved for 20 min. The color intensity of the cells was measured using a spectrophotometer at 570 nm wavelength. The results were calculated as mean ± standard deviation and % viability of the cells was determined.

Results and Discussion

According to the results of cytotoxic activity, it was observed that the sample did not decrease the viability from the highest dose to the lowest dose as seen in Figure 1. It is seen that the viability is above 100% at all doses.

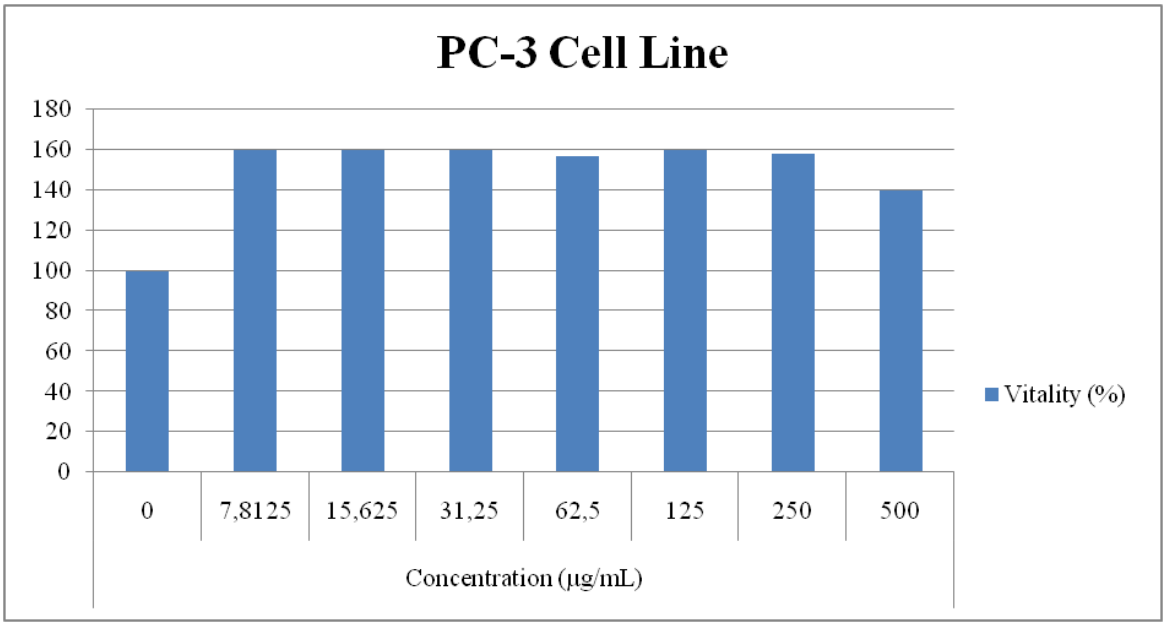


Figure 1. The vitality (%) of the sample in PC-3 cell line

The term "cytotoxic activity" describes a substance's capacity to stop cell growth or cause cell death (Akter et al., 2014). This action can inhibit tumor growth or destroy cancer cells by specifically targeting them. Because plant extracts may be cytotoxic, they are being studied as possible therapeutic agents for the treatment of cancer (Suffredini et al., 2006). Numerous investigations have assessed the cytotoxic properties of various plant extracts against cancerous cells. Both in vitro (cell culture) and in vivo (on living organisms) models are typically used in these investigations (Itharat et al., 2004; Prakash and Gupta, 2013 Liang et al., 2017). Plant extracts have lethal effects on cancer cells through a variety of methods, such as cell cycle regulation, antioxidant activity, induction of apoptosis, and blockage of cellular signaling pathways (Almatroodi et al., 2021). In studies on the determination of cytotoxic activity on different species belonging to the genus

Onobrychis (Karakoca et al., 2015; Clericuzio et al., 2020; Amin et al., 2023; Yeniçeri et al., 2024;), it was observed that Onobrychis species have cytotoxic activity and the results obtained from our study are compatible with the literature.

Conclusion

In conclusion, *O. megataphros* is a promising plant as a natural source of cytotoxicity with potential anticancer activity, and further research on this topic may contribute to the development of new cancer therapies that are both safe and effective.

Recommendations

Further mechanistic studies should be conducted to determine the effective mechanisms for the anticancer activity of O. megataphros. This is necessary to understand which components of the plant target cancer cells and how they act. Chemical analyses should be carried out to identify the active components responsible for the plant's anticancer activity. This is important to isolate the plant's potential anticancer agents and obtain their pure forms. Dose response studies should be conducted to evaluate the cytotoxic effects of different doses of the plant against cancer cells. In addition, toxicity studies in animal models are necessary to evaluate the toxicity of the plant on humans. Clinical trials should be conducted to evaluate the anticancer efficacy and safety of the herb. This is important to assess the usability of the herb in the treatment of cancer in humans. Besides the potential anticancer activity of O. megataphros, other biological activities of the plant should also be investigated. This would allow us to more comprehensively evaluate the potential health benefits of the plant. Methods for collecting and using the plant in a sustainable manner should be developed. This helps to preserve the plant's natural habitat and make it accessible for future generations. These recommendations could enable progress in the evaluation of O. megataphros as a natural source of cytotoxicity with potential anticancer activity and help us better understand the plant's usability in cancer treatment.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

References

Akter, R., Uddin, S. J., Grice, I. D., & Tiralongo, E. (2014). Cytotoxic activity screening of Bangladeshi medicinal plant extracts. *Journal of natural medicines*, *68*, 246-252.

Almatroodi, S. A., Alsahli, M. A., Almatroudi, A., Verma, A. K., Aloliqi, A., Allemailem, K. S., & Rahmani, A. H. (2021). Potential therapeutic targets of quercetin, a plant flavonol, and its role in the therapy of various types of cancer through the modulation of various cell signaling pathways. *Molecules*, *26*(5), 1315.

Amin, H. I. M., Abdoulrahman, K., Sadraddin, A. S., Smail, H. A., Jawhar, Z. H., Dilawer Issa, K., ... & Vidari, G. (2023). Chemical composition and ın vitro evaluation of antioxidant and antiproliferative effects of volatile oils hydrodistilled from onobrychis carduchorum CC Towns a Kurdish traditional plant. *Plants*, *12*(16), 3013.

Awuchi, C. G. (2019). Medicinal plants: the medical, food, and nutritional biochemistry and uses. *International Journal of Advanced Academic Research*, *5*(11), 220-241.

Clericuzio, M., Hussain, F. H., Amin, H. I. M., Bona, E., Gamalero, E., Giorgia, N., ... & Cornara, L. (2020). Cytotoxic, anti-bacterial, and wound-healing activity of prenylated phenols from the Kurdish traditional

medicinal plant Onobrychis carduchorum (Fabaceae). *Planta Medica International Open*, *7*(03), e106-e113.

Demirci, S., & Ozhatay, N. (2012). An ethnobotanical study in Kahramanmaras (Turkey); Wild plants used for medicinal purpose in Andirin, Kahramanmaras. *Turk J Pharm Sci*, *9*(1), 75-92.

Habli, Z., Toumieh, G., Fatfat, M., Rahal, O. N., & Gali-Muhtasib, H. (2017). Emerging cytotoxic alkaloids in the battle against cancer: Overview of molecular mechanisms. *Molecules*, *22*(2), 250.

Hayta, S., Polat, R., & Selvi, S. (2014). Traditional uses of medicinal plants in Elazıg (Turkey). *Journal of Ethnopharmacology*, *154*(3), 613-623.

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P., & Khelurkar, V. C. (2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry*, *6*(1), 32-36.

Itharat, A., Houghton, P. J., Eno-Amooquaye, E., Burke, P. J., Sampson, J. H., & Raman, A. (2004). In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *Journal of Ethnopharmacology*, *90*(1), 33-38.

Karakoca, K., Asan-Ozusaglam, M., Cakmak, Y. S., & Teksen, M. (2015). Phenolic compounds, biological and antioxidant activities of Onobrychis armena Boiss. & Huet flower and root extracts. *Chiang Mai University Journal of Natural of Sciences*, *42*(2), 376-392.

Liang, C., Pan, H., Li, H., Zhao, Y., & Feng, Y. (2017). In vitro anticancer activity and cytotoxicity screening of phytochemical extracts from selected traditional Chinese medicinal plants. *JBUON*, *22*(2), 544-551.

Mazzoleni, R., & Nelson, R. R. (2005). *The roles of research at universities and public labs in economic catch-up* (No. 2006/01). LEM Working Paper Series.

Muvakit, S., & Ozaslan, M. (2023). Investigation of total phenolic, flavonoid content and antioxidant activity of onobrychis megataphros leaf extract. *The Eurasia Proceedings of Science Technology Engineering and Mathematics*, *25*, 42-49.

Özçelik, G., & Toprak, D. (2015). Why is Phytotherapy Preferred?. *Ankara Medical Journal*, *15*(2).

Prakash, E., & Gupta, D. K. (2013). Cytotoxic activities of extracts of medicinal plants of euphorbiacae family studied on seven human cancer cell lines. *Univers J Plant Sci*, *1*, 113-117.

Sargın, S. A., Akçicek, E., & Selvi, S. (2013). An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. *Journal of Ethnopharmacology*, *150*(3), 860-874.

Suffredini, I. B., Paciencia, M. L., Nepomuceno, D. C., Younes, R. N., & Varella, A. D. (2006). Antibacterial and cytotoxic activity of Brazilian plant extracts-Clusiaceae. *Memorias do Instituto Oswaldo Cruz*, *101*, 287-290.

Yeniçeri, E., Altay, A., Koksal, E., Altın, S., Taslimi, P., Yılmaz, M. A., ... & Kandemir, A. (2024). Phytochemical profile by LC-MS/MS analysis and evaluation of antioxidant, antidiabetic, anti-Alzheimer, and anticancer activity of Onobrychis argyrea leaf extracts. *European Journal of Integrative Medicine*, *66*, 102337.

Author Information

Salih Muvakit
Gaziantep University
Gaziantep University, Department of Biology. 27310
Gaziantep - Turkiye

Mehmet Ozaslan
Gaziantep University
Gaziantep University, Department of Biology. 27310
Gaziantep - Turkiye
Contact e-mail: *ozaslanmd@yahoo.com*

To cite this article:

Muvakit, S. & Ozaslan, M.(2024). Cytotoxic activity of *Onobrychis Megataphros* leaf extract. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 50-54.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 55-66

ICGeHeS 2024: International Conference on General Health Sciences

Bibliometric Analysis of Mental Health in Athletes

Syaipul Hari Baharuddin
Universitas Pendidikan Indonesia

Agus Mahendra
Universitas Pendidikan Indonesia

Haryanto Haryanto
Universitas Pendidikan Indonesia

Muh. Aswar
Universitas Pendidikan Indonesia

Herdiansyah Herdiansyah
Universitas Pendidikan Indonesia

Abstract Mental health in athletes is crucial as it significantly impacts sports performance, overall well-being, and the daily lives of athletes. The importance of mental health in the athlete context indicates that attention to this aspect not only affects sports performance but also the overall well-being and lives of athletes. This research aims to understand publication trends related to mental health in athletes through bibliometric analysis. In this analysis, VOSviewer software and the bibliometric analysis application biblioshiny were used to graphically analyze bibliographic data. After filtering, 729 documents were analyzed from the Scopus database. The results show fluctuating trends in the number of publications, with the International Journal of Environmental Research and Public Health as the highest contributor with 125 documents. The USA ranks first in documents focusing on mental health in athletes, with 637 documents. Additionally, Ulster University is the most prolific affiliation, contributing the highest number of publications, each with 30 documents. The globally most cited document, published in the Journal of Science and Medicine in Sport, has been cited 177 times. Visualization of research trends reveals popular topics aligned with research and discussions, including mental health, male, human, female, athlete, adult, psychology. This research can significantly contribute to understanding and treating the mental health of athletes, as well as stimulate further research and interventions in the future.

Keywords: Mental health, Athlete. Bibliometric analysis

Introduction

Mental health is a complex and multifaceted aspect of overall well-being, encompassing cognitive, emotional, and social elements (Adam & Johnston, 1971; Richardson, 2020). It is not just the absence of mental illness, but also the ability to cope with life's challenges and contribute to one's community (Adam & Johnston, 1971). The promotion of mental health is crucial, particularly in the face of adverse conditions such as poverty and discrimination (Herrman, 2008). Factors such as stress, depression, and anxiety can significantly impact mental health, underscoring the importance of maintaining a balance in life activities and responsibilities (James, 2020). Research in the field of mental health in sport has highlighted the need for improved early intervention knowledge and confidence among elite sport staff (Sebbens et al., 2016). This is particularly important given the

significant impact of mental health on sports performance (Yaffé, 1981). The growing momentum in this area is evident in the increasing research and commentary on mental health in sport (Vella et al., 2020).Elite athletes are at a comparable risk of mental health disorders as the general population, with factors such as injury, overtraining, and burnout contributing to these issues (Rice et al., 2016). Participation in sport, particularly team-based activities, has been associated with improved psychological and social health (Eime et al., 2013)

Mental health symptoms and disorders are common among elite athletes, with a need for evidence-based guidelines for diagnosis and management (Reardon et al., 2019). A study on Australian elite athletes found high levels of mental health problems, particularly in injured athletes, highlighting the need for support and access to mental health professionals (Gulliver et al., 2015). However, there is a need for further development of sports psychiatric care in competitive sports, including the promotion of mental health and the safe handling of mental problems and illnesses (Claussen et al., 2021).Research indicates that athletes, particularly elite and young athletes, are at a heightened risk for mental health concerns such as anxiety, depression, eating disorders, and substance abuse (Putukian, 2021). These issues are often exacerbated by the unique stressors and demands of the athletic environment, including the pressure to perform, injuries, and the transition from sport (Putukian, 2021).

The prevalence of these mental health problems in elite athletes is similar to that in the general population, with injured athletes reporting higher levels of symptoms (Gulliver et al., 2015). Furthermore, athletes are also at risk of suicide, with a review of the literature revealing a significant number of cases (Baum, 2005). These findings underscore the need for proactive mental health support and education tailored to the unique needs of athletes. The International Olympic Committee has recognized the need for proactive mental health education and tailored support services for this population (Reardon et al., 2019). However, there is a lack of evidence-based guidelines for diagnosis and management of mental health symptoms in elite athletes, highlighting the need for further research in this area (Reardon et al., 2019).

The mental health of athletes is a critical aspect of their overall well-being and performance. (Puri & Sood, 2018) emphasizes the importance of positive mental health in student athletes, while (Purcell et al., 2019a; Putukian, 2021) both underscore the need for early detection and intervention in elite athletes, particularly in the context of mental health concerns and injury risk. (Rogers et al., 2023) further highlights the bidirectional relationship between mental health and injury risk and outcomes in athletes, emphasizing the need for comprehensive support systems. These studies collectively underscore the significance of mental health in athletes and the need for proactive measures to support their well-being.

The primary objective of this study is to conduct a comprehensive examination of mental health in athletes, with a specific focus on various aspects such as publication trends, international collaboration, document sources, and research dissemination. By delving into these areas, we aim to achieve a thorough understanding of mental health in athletes and the broader research landscape surrounding this topic. To accomplish this overarching goal, we have devised a series of research questions that will guide our investigation:

1 Understanding Publication Productivity Trends: Through the utilization of VOSviewer analysis, we aim to unravel the trends in publication productivity pertaining to mental health in athletes. By employing this method, we aim to discern how research output in this area has evolved over time and identify any notable patterns or fluctuations.
2. Analyzing Publication Trajectories: By analyzing publication trajectories spanning the past decade, we aim to uncover patterns and fluctuations in the number of publications related to mental health in athletes. This analysis will provide insights into the trajectory of research in this field and highlight any significant shifts in focus or intensity over time.
3. Insights into International Collaboration and Knowledge Dissemination: We aim to explore the dynamics of international collaboration and the dissemination of knowledge among the global research community in mental health in athlete impact research. By examining collaboration patterns and knowledge-sharing networks, we seek to understand how researchers from different countries collaborate and disseminate their findings in this field.
4. Examining Document Sources and Journal Contributions: Our study will delve into the document sources and journal contributions in analyzing mental health amid athletes. Through this examination, we aim to identify the key sources of research and assess the contributions of different journals to the advancement of knowledge in this area.
5. Dispersal of Research across Disciplines: We will investigate the dispersal of research on mental health in athletes across various disciplines. This analysis will provide insights into the interdisciplinary nature of research in this field and shed light on the diverse perspectives and methodologies employed by researchers.

6. Identifying Leading Countries and Institutions: Our study aims to identify the leading countries and institutions in analyzing mental health in athletes and assess their contributions to the research. By identifying the most prolific countries and institutions, we aim to understand the global distribution of research activity in this field.
7. Key Authors and Their Contributions: We will map the landscape of mental health in athlete research by identifying key authors and assessing their contributions. This analysis will highlight the pivotal role played by individual researchers in shaping the direction and progress of research in this area.
8. Network Visualization and Cluster Analysis: Through network visualization and cluster analysis, we aim to gain a deeper understanding of research focuses and trends in the field of mental health in athletes. By visualizing the connections between key terms and concepts, we seek to uncover underlying patterns and thematic clusters within the research literature.
9. Overlay and Density Visualizations: We will track the evolution of research focuses and trends using overlay and density visualizations. These visualizations will enable us to identify changes in research interests over time and track the emergence of new areas of focus within the field.
10. Utilizing VOSviewer for Research Planning and Identifying Future Directions: Finally, we will explore how VOSviewer can be utilized for research planning and identifying future directions in the study of mental health in athletes. By leveraging this tool, we aim to identify gaps in the existing literature and pinpoint areas that warrant further investigation.

Through a comprehensive exploration of these research questions, we seek to contribute to a deeper understanding of mental health in athletes and provide valuable insights for future research directions in this rapidly evolving field.

Method

Data Source, Study Boundary and Search Strategy

Bibliometric analyses play a crucial role in systematic literature reviews, marked by their clarity, transparency, and reproducibility in constructing review databases. It is essential to clearly define the study's scope and elaborate on the methodologies used to identify relevant sources. In this study, bibliometric data is sourced from the Scopus database, known for its comprehensive coverage, including abstracts and citations of scholarly journal articles. (Ellegaard & Wallin, 2015). Scopus covers a wide array of academic fields, such as medical, technical, social, and scientific studies, all of which are highly pertinent to exploring and improving mental health among athletes. (Colangelo et al., 2023). Scopus provides more extensive coverage than Web of Science, with approximately 20% broader coverage. However, results from Google Scholar may vary in terms of accuracy. (Singh et al., 2021).

Bibliometric analysis techniques are employed to derive insights from bibliographic datasets. These methods unveil structural, social, and author networks, along with prevailing analytical interests within a particular research domain. (Lim & Buntine, 2016)). This method also entails examining statistics of published articles and their citations to assess their significance and scrutinize emerging gaps or subjects (Lim & Buntine, 2016). Additionally, bibliographic analysis utilizes pertinent data sourced from online databases, facilitating scientific investigations and offering a comprehensive outlook on related areas of interest from a global standpoint. (Blakeman, 2018). Figure 1 depicts the execution of these five steps, highlighting the utilization of bibliometric techniques.

Figure 1.Bibliometric methodology

Study Design

The research design was developed by formulating research inquiries and choosing keywords and databases. It encompasses six investigations into the following aspects: annual publication trends, document sources, fields

of study, and countries; affiliations; most prolific authors; most cited documents; and potential future research opportunities regarding Mental Health in athletes. The design also confirms that the search keywords used were "Mental Health" AND "athletes," with the Scopus database selected as the bibliometric source.

Data Collection

A search strategy involving the title "Mental Health" AND "athletes" within the timeframe of 2018 to 2023 was employed during the data collection phase from the Scopus database. This yielded a total of 729 documents spanning the years 2018 to 2023, sourced exclusively from globally published journals and meeting the criteria of final publication stage and English language usage. Furthermore, the documents were acquired in Comma-Separated Values (CSV) and RIS formats to extract article titles, authors, references, and keywords.

Data Analysis

The data analysis began by importing CSV and RIS data from the Scopus database into Microsoft Excel and VOSviewer software. This step was essential for facilitating subsequent data processing. The analysis sought to identify and understand various prevailing trends, including characteristics of publication output, document sources, distribution of country and institution affiliations, dissemination of subject categories, as well as top authors and citations. This data was thoroughly examined and analyzed to gain a deeper understanding of the current research landscape, with a focus on the period from 2018 to 2023.

Data Visualization

After completing data processing and analysis, the next step entailed data visualization. Visualization was conducted using VOSviewer and Microsoft Excel software. With VOSviewer, processed RIS metadata was utilized to generate visual representations of networks, overlays, and densities. In parallel, Microsoft Excel was employed to present data in tables and diagrams, providing a more lucid and understandable depiction of the identified research trends.

Interpretation

Finally, the data visualized using VOSviewer underwent further analysis and interpretation. This involved examining the number of clusters formed, mapping both old and recent studies, and conducting density analysis to uncover insights about saturation and potential future research opportunities. Diligent interpretation of these visualizations is vital for extracting deeper insights into research patterns and directions within the field.

Results

The pattern of publication output spanning 5 years (2018-2023) sourced from the Scopus database reveals notable fluctuations. Specifically, the analysis of Mental Health in athletes across diverse disciplines displays varying trends throughout this period. There was a progressive increase in the number of documents from 2018 to 2022, with figures rising to 34, 50, 89, 160, and 206 publications, respectively. However, there was a downturn in 2023, with a decrease of 190 publications. Figure 1 provides a visual representation of the annual publication trend.

Main Source Document

The pattern of publication output over a span of 5 years (2018-2023) is primarily derived from journal articles. The majority of these sources center around the topic of mental health in athletes, covering a wide array of fields such as medicine, health professions, psychology, environmental science, and social sciences. Table 1 presents the top ten most prolific sources in terms of publishing articles pertaining to mental health in athletes. Table 1. Distribution of documents by relevant sources from 2018-2023

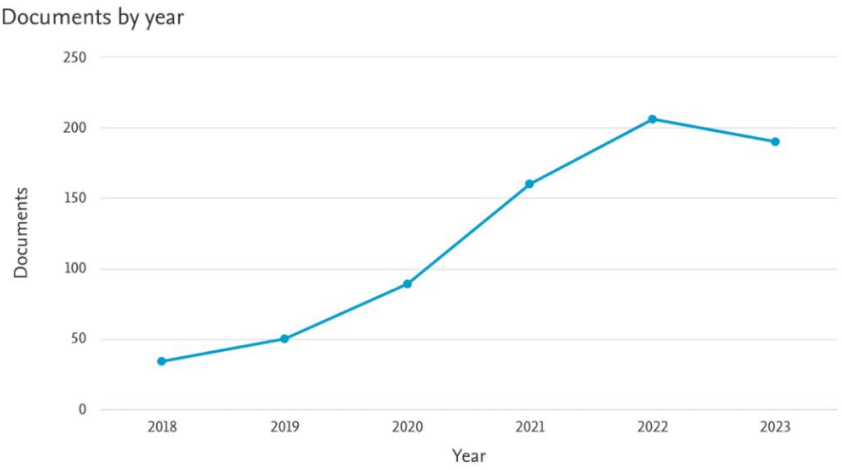


Figure 2.Annual publication trends for the period 2018-2023

Table 1. The top ten most prolific sources

Sources	**Articles**	**SJR Index (Scimagojr 2022)**
International Journal Of Environmental Research And Public Health	125	0.83 (Q2)
Frontiers In Psychology	64	0.89 (Q2)
Journal Of Athletic Training	35	1.43 (Q1)
Journal Of Clinical Sport Psychology	23	0.6 (Q2)
Frontiers In Sports And Active Living	22	0.6 (Q1)
Journal Of Physical Education And Sport	20	0.31 (Q3)
Bmj Open Sport And Exercise Medicine	18	1.25 (Q1)
Plos One	18	0.89 (Q1)
Psychology Of Sport And Exercise	15	1.35 (Q1)
Sustainability	12	0.66 (Q1)

Document Based on Subject Area

A collection of 729 publications focusing on mental health in athletes was compiled from documents covering the years 2018-2023, with a notable emphasis on subject areas within the mental health domain. This highlights contributions from various fields, with medicine, health professions, psychology, environmental science, and social sciences contributing 467, 211, 170, 142, and 81 publications respectively. Figure 3 provides a visual representation of the classification of documents highlighting subject areas.

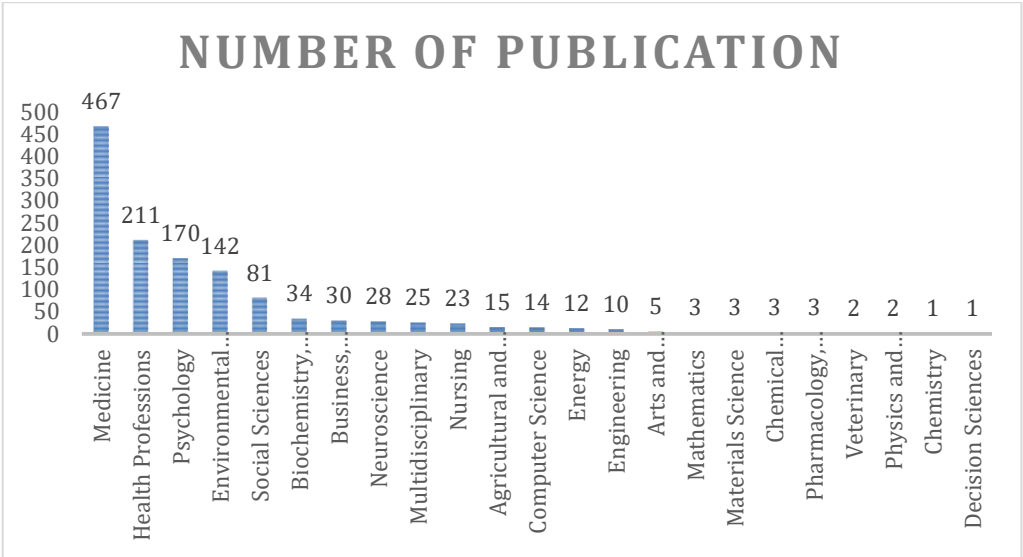


Figure 3. Document by subject area

Top Publications by Country

According to the country classification, a total of 729 documents were distributed, with 193, 139, 73, 62, and 62 articles originating from the United States, United Kingdom, Australia, Canada, and Spain respectively. Figure 4 presents a visual representation of the top 15 countries worldwide in terms of publications concerning Mental Health in athletes.

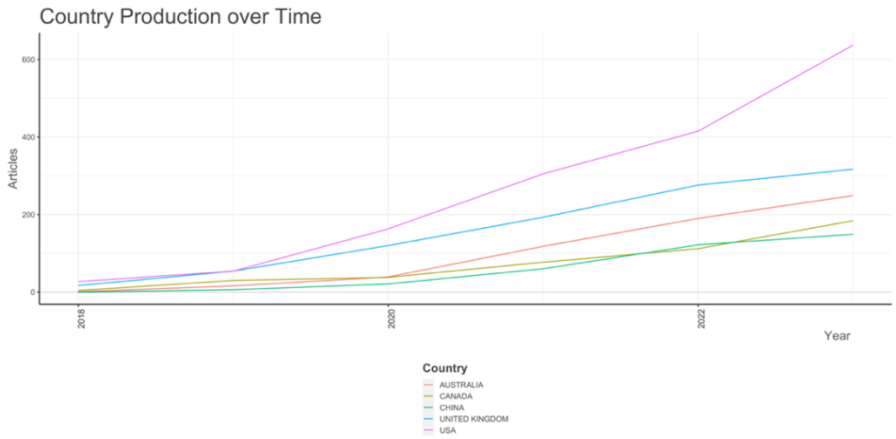


Figure 4. Country over time

University Affiliation

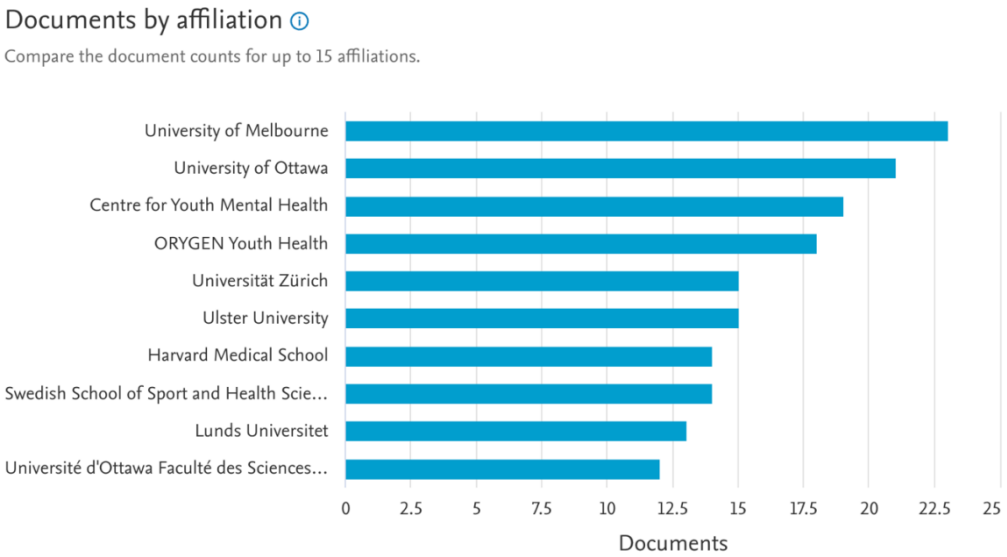


Figure 5. Most relevat affiliation

Figure 4 illustrates the distribution of the top 10 university affiliations focusing on mental health in athletes. The publication distribution spans various university affiliations, with the University of Melbourne emerging as the most productive institution with 23 documents published in the Scopus database. Following closely are the University of Ottawa with 21 documents and the Centre for Youth Mental Health with 19 articles.

Top 10 Authors

In terms of the most pertinent authors in this field, Purcell R leads with 16 documents, followed by Breslin G with 13, and Kentta G with 11 documents. The top 10 authors are depicted in Figure 6.

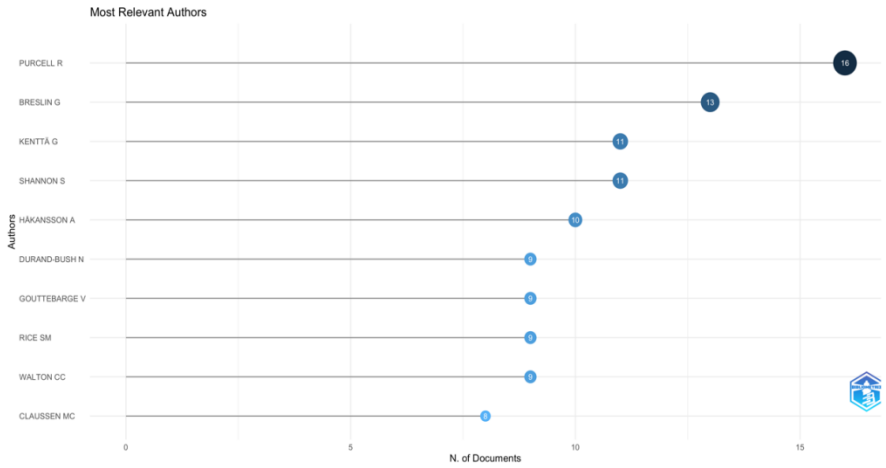


Figure 6. Top 10 Authors

Table 2. The top 10 most cited documents

Author/Year	Tittle	DOI	Total Citations	TC per Year
Pillay et al., (2020)	Nowhere to hide: The significant impact of coronavirus disease 2019 (COVID-19) measures on elite and semi-elite South African athletes	10.1016/j.jsams. 2020.05.016	177	35,40
Purcell et al., (2019b)	Mental Health In Elite Athletes: Increased Awareness Requires An Early Intervention Framework to Respond to Athlete Needs	10.1186/s40798-019-0220-1	159	26,50
McGuine et al., (2021)	Mental Health, Physical Activity, and Quality of Life of US Adolescent Athletes During COVID-19-Related School Closures and Sport Cancellations: A Study of 13 000 Athletes	10.4085/1062-6050-0478.20	129	32,25
Gouttebarge et al., (2021)	International Olympic Committee (IOC) Sport Mental Health Assessment Tool 1 (SMHAT-1) and Sport Mental Health Recognition Tool 1 (SMHRT-1): towards better support of athletes' mental health	10.1136/bjsports-2020-102411	125	31,25
Chang et al., (2020)	Mental health issues and psychological factors in athletes: detection, management, effect on performance and prevention: American Medical Society for Sports Medicine Position Statement-Executive Summary	10.1136/bjsports-2019-101583	121	24,20
Graupensperger et al., (2020)	Social (Un)distancing: Teammate Interactions, Athletic Identity, and Mental Health of Student-Athletes During the COVID-19 Pandemic	10.1016/j.jadohealth. 2020.08.001	111	22,20
Hull et al., (2020)	Respiratory health in athletes: facing the COVID-19 challenge	10.1016/S2213-2600(20)30175-2	110	22,00
Bhatia et al., (2020)	Exercise in the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) era: A Question and Answer session with the experts Endorsed by the section of Sports Cardiology & Exercise of the European Association of Preventive Cardiology (EAPC)	10.1177/20474 87320930596	95	19,00
Snedden et al., (2019)	Sport and Physical Activity Level Impacts Health-Related Quality of Life Among Collegiate Students	10.1177/08901 17118817715	83	13,83
Gerber et al., (2018)	Effects of stress and mental toughness on burnout and depressive symptoms: A prospective study with young elite athletes	10.1016/j.jsams. 2018.05.018	79	11,29

Document Citation

Based on the data extracted from Scopus, the top 10 most cited documents annually are as follows: Pillay (TC=177), Purcell (TC=159), McGuine (TC=129), Gouttebarge (TC=125), Chang (TC=121), Graupensperger (TC=111), Hull (TC=110), Bhatia (TC=95), Snedden (TC=83), and Gerber (TC=79), as listed in the Table 2 below.

Mapping Mental Health in Athletes with VOSviewer

The categorization of subject studies related to mental health in athletes is significant, with a minimum of 4007 keywords identified, of which 464 words meet the threshold. Network visualization is also segmented into 8 clusters, comprising 32847 links and a total binding strength of 123095. Notably, the blue, green, and red clusters prioritize mental health, human, athlete, and content analysis with 463, 463, and 461 occurrences, respectively. Larger circles in the visualization indicate a higher frequency of keywords in documents (Zhang et al., 2022).

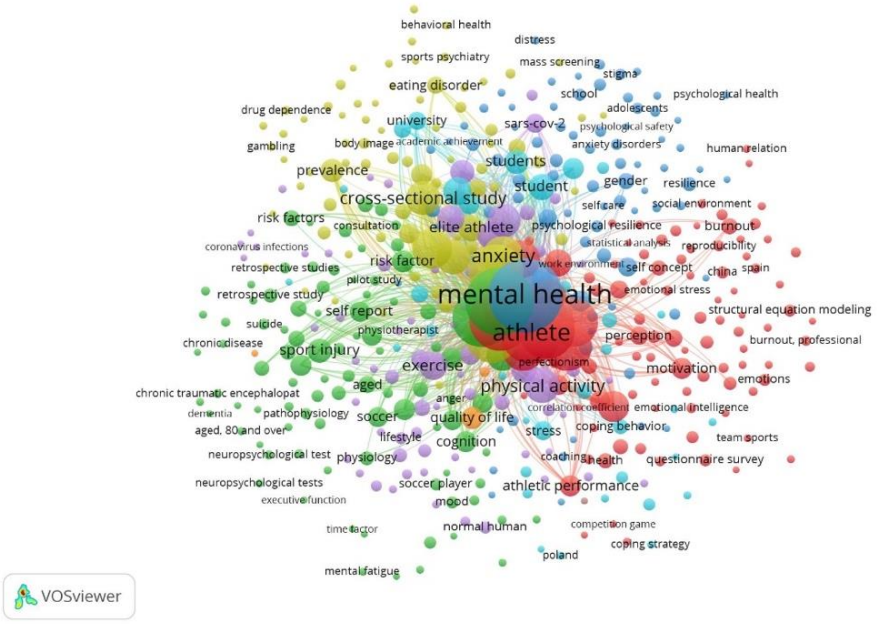


Figure 7. Network visualization of co-occurrences with index keywords

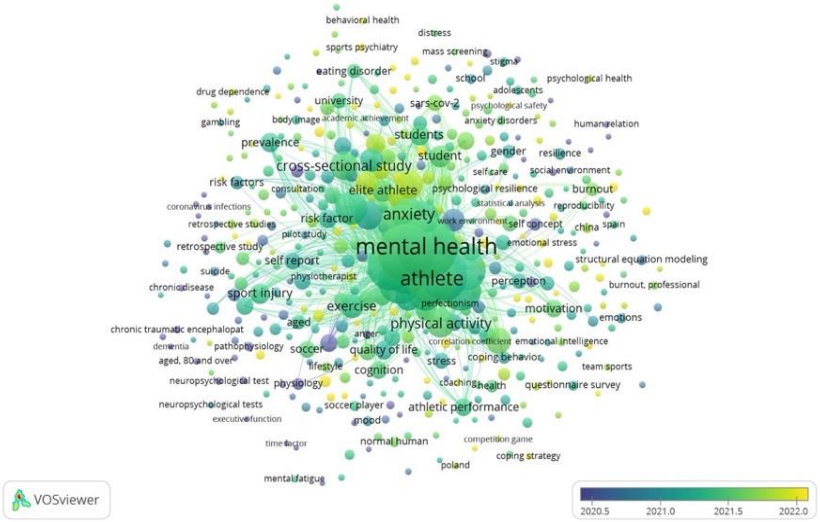


Figure 8. Overlay of visualization of co-occurrences with index keywords

Overlay Visualization

The overlay visualization is depicted using colors that symbolize annual experimental trends, with purple and yellow highlighting recent years of study and analysis, respectively. These contemporary trends are centered around terms such as mental health, humans, sport, athlete, physical activity, exercise, and anxiety. The co-occurrence overlay visualization is showcased in Figure 8

Density Visualization

Density visualization plays a crucial role in saturating a topic, with colors like red, blue, yellow, and green highlighting areas yet to be analyzed, rarely studied, and highly evaluated, respectively. Brighter colors indicate more frequent usage of terms analyzed in relevant experiments. Keyword density distribution also indicates the frequency level of research topics. For example, mental health and athlete are frequently analyzed, while psychological health, sport psychiatry, coping strategy, mental fatigue, and behavioral health are less commonly studied. Figure 9 presents the visualization of decision-making and problem-solving skill density based on keyword indices.

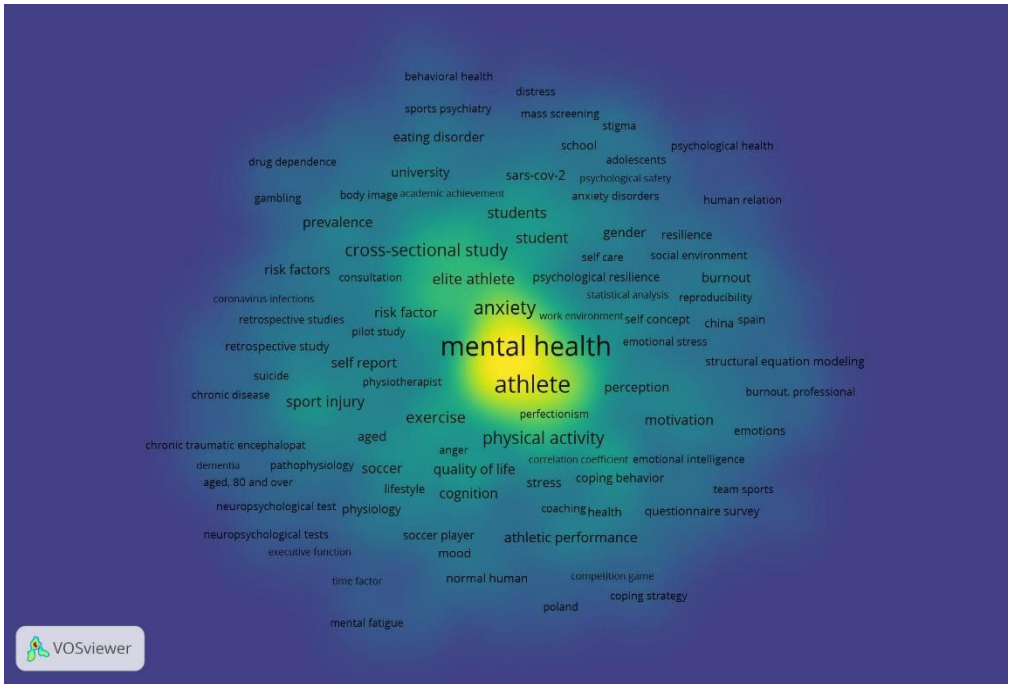


Figure 9. Density visualization of topic decision-making and problem-solving based on keywords

Discussion

The research findings indicate significant trends in publication productivity spanning a five-year period (2018-2023) as per Scopus data, highlighting notable fluctuations. Examining mental health trends among athletes across various disciplines reveals fluctuation over this duration, with a rise in document numbers from 2018 to 2022 (34, 50, 89, 160, and 206 publications, respectively), succeeded by a decrease in 2023 (190 publications). This pattern is visually depicted in Figure 1. The variability in publication productivity may signify shifts in research focus, emerging issues, or alterations in funding availability within the realm of mental health in athletes.

The bulk of the document articles originate from journals, delving into mental health among athletes across various fields like medicine, health professions, psychology, environmental science, and social sciences. This interdisciplinary approach highlights the intricate nature of mental health concerns among athletes and emphasizes the necessity for comprehensive strategies to tackle them. The reliance on journal articles underscores a sturdy bedrock of scientific evidence guiding research endeavors in this area, guaranteeing thoroughness and reliability in the outcomes.

A total of 729 publications concerning mental health in athletes were collected spanning the years 2018-2023, accentuating the focus on mental health issues. This distribution showcases diverse contributions from various disciplines, with medicine, health professions, psychology, environmental science, and social sciences accounting for 467, 211, 170, 142, and 81 publications, respectively. The dissemination of publications across disciplines underscores the interdisciplinary character of mental health research in athletes, with each field offering distinct viewpoints and approaches to examine this intricate topic.

Moreover, the categorization by country demonstrates the dispersion of 729 documents, with 193, 139, 73, 62, and 62 articles originating from the United States, United Kingdom, Australia, Canada, and Spain, respectively. Figure 3 depicts the leading 15 countries worldwide in publications concerning mental health in athletes. The global spread of research output underscores the international importance and relevance of mental health issues among athletes, signaling widespread acknowledgment of the necessity to tackle these concerns on a global level.

Moreover, the examination showcases the top 10 university affiliations emphasizing mental health in athletes, led by the University of Melbourne, trailed by the University of Ottawa and the Centre for Youth Mental Health. The prominence of these institutions underscores their leadership in mental health research within the athletic community, potentially serving as hubs for collaboration and knowledge exchange among researchers and practitioners..

Prominent authors contributing significantly to this subject area include Purcell R, Breslin G, and Kentta G. Their extensive contributions suggest expertise and influence in the field of mental health in athletes, with their research likely influencing research agendas, interventions, and policy decisions. Additionally, the most cited documents per year, sourced from Scopus data, include works by Pillay, Purcell, McGuine, Gouttebarge, Chang, Graupensperger, Hull, Bhatia, Snedden, and Gerber. These highly cited documents likely represent pivotal contributions to the field, providing foundational knowledge and shaping future research directions.

Conclusion

The comprehensive examination of mental health trends among athletes over the last five years, covering aspects such as publication output, interdisciplinary contributions, global representation, institutional affiliations, influential authors, and highly cited papers, offers valuable insights into the dynamic landscape of research in this area. The results highlight the multifaceted nature of mental health challenges faced by athletes, emphasizing the necessity for collaborative, interdisciplinary strategies to effectively address these issues. The prominence of specific countries, institutions, authors, and publications underscores their pivotal role in advancing mental health research within the athletic community, while fluctuations in publication output suggest evolving research priorities and emerging areas of interest. Overall, this study lays a strong foundation for future research endeavors, guiding efforts to enhance mental health outcomes and support athletes' well-being on a global scale.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

* The authors would like to express their heartfelt thanks and appreciation to the Lembaga Pengelola Dana Pendidikan (LPDP), Indonesia's Endowment Fund for Education, under the Ministry of Finance of the Republic of Indonesia, for their invaluable support in facilitating this publication and fostering collaboration.

References

Adam, A., & Johnston, M. K. (1971). Mental health and mental illness. In *Organising care around patients*. JB Lippincott.

Baum, A. L. (2005). Suicide in athletes: a review and commentary. *Clinics in Sports Medicine*, *24 4*, 853–869, ix.

Bhatia, R. T., Marwaha, S., Malhotra, A., Iqbal, Z., Hughes, C., Borjesson, M., Niebauer, J., Pelliccia, A., Schmied, C., Serratosa, L., Papadakis, M., & Sharma, S. (2020). Exercise in the severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) era: A question and answer session with the experts endorsed by the section of sports cardiology & exercise of the European association of preventive cardiology (EAPC). *European Journal of Preventive Cardiology*, *27*(12), 1242–1251.

Blakeman, K. (2018). Bibliometrics in a digital age: Help or hindrance. *Science Progress*, *101*, 293–310.

Chang, C., Putukian, M., Aerni, G., Diamond, A., Hong, G., Ingram, Y., Reardon, C. L., & Wolanin, A. (2020). Mental health issues and psychological factors in athletes: Detection, management, effect on performance and prevention: American medical society for sports medicine position statement-executive summary. *British Journal of Sports Medicine*, *54*(4), 216–220.

Claussen, M. C., Hofmann, C. G., Schneeberger, A., Seifritz, E., Schorb, A., Allroggen, M., Freyer, T., Helmig, F., Niebauer, J., Hefert, J., Klostermeier, E., Frohlich, S., & Scherr, J. (2021). Position paper: sports psychiatric care provision in competitive sports. *Deutsche Zeitschrift Fur Sportmedizin/German Journal of Sports Medicine*, *72*(6),316-322.

Colangelo, J., Smith, A., Buadze, A., Keay, N., & Liebrenz, M. (2023). Mental health disorders in ultra endurance athletes per ICD-11 classifications: A review of an overlooked community in sports psychiatry. *Sports*, *11*(3), 52.

Eime, R. M., Young, J. A., Harvey, J. T., Charity, M. J., & Payne, W. R. (2013). A systematic review of the psychological and social benefits of participation in sport for children and adolescents: informing development of a conceptual model of health through sport. *International Journal of Behavioral Nutrition and Physical Activity*, *10*(1), 98.

Ellegaard, O., & Wallin, J. (2015). The bibliometric analysis of scholarly production: How great is the impact? *Scientometrics*, *105*.

Gerber, M., Best, S., Meerstetter, F., Walter, M., Ludyga, S., Brand, S., Bianchi, R., Madigan, D. J., Isoard-Gautheur, S., & Gustafsson, H. (2018). Effects of stress and mental toughness on burnout and depressive symptoms: A prospective study with young elite athletes. *Journal of Science and Medicine in Sport*, *21*(12), 1200–1205.

Gouttebarge, V., Bindra, A., Blauwet, C., Campriani, N., Currie, A., Engebretsen, L., Hainline, B., Kroshus, E., McDuff, D., Mountjoy, M., Purcell, R., Putukian, M., Reardon, C. L., Rice, S. M., & Budgett, R. (2021). International olympic committee (IOC) Sport mental health assessment tool 1 (SMHAT-1) and sport mental health recognition tool 1 (smhrt-1): Towards better support of athletes' mental health. *British Journal of Sports Medicine*, *55*(1), 30–37.

Graupensperger, S., Benson, A. J., Kilmer, J. R., & Evans, M. B. (2020). Social (Un)distancing: Teammate ınteractions, athletic ıdentity, and mental health of student-athletes during the Covid-19 pandemic. *Journal of Adolescent Health*, *67*(5), 662–670.

Gulliver, A., Griffiths, K. M., Mackinnon, A. J., Batterham, P. J., & Stanimirovic, R. (2015). The mental health of Australian elite athletes. *Journal of Science and Medicine in Sport*, *18 3*, 255–261.

Hull, J. H., Loosemore, M., & Schwellnus, M. (2020). Respiratory health in athletes: Facing the COVID-19 challenge. *The Lancet Respiratory Medicine*, *8*(6), 557–558.

James, R., Savage, M. J.,..& Hennis, P. J. (2020). Mental health and movement behavior during Covid-19 pandemic in UK university students: Prospective cohort study. *Mental Health and Physical Activity*, *19*(2020), 100357.

Lim, K. W., & Buntine, W. (2015Bibliographic analysis with the citation network topic model. In *Asian conference on machine learning* (pp. 142-158). PMLR.

McGuine, T. A., Biese, K. M., Petrovska, L., Hetzel, S. J., Reardon, C., Kliethermes, S., Bell, D. R., Brooks, A., & Watson, A. M. (2021). Mental health, physical activity, and quality of life of us adolescent athletes during COVID-19-related school closures and sport cancellations: A study of 13 000 athletes. *Journal of Athletic Training*, *56*(1), 11–19.

Pillay, L., Janse van Rensburg, D. C. C., Jansen van Rensburg, A., Ramagole, D. A., Holtzhausen, L., Dijkstra, H. P., & Cronje, T. (2020). Nowhere to hide: The significant impact of coronavirus disease 2019 (COVID-19) measures on elite and semi-elite South African athletes. *Journal of Science and Medicine in Sport*, *23*(7), 670–679.

Purcell, R., Gwyther, K., & Rice, S. M. (2019a). Mental health ın elite athletes: ıncreased awareness requires an early ıntervention framework to respond to athlete needs. *Sports Medicine - Open*, *5*(1),46.

Purcell, R., Gwyther, K., & Rice, S. M. (2019b). Mental health ın elite athletes: Increased awareness requires an early ıntervention framework to respond to athlete needs. *Sports Medicine - Open*, *5*(1),46.

Puri, D., & Sood, S. (2018). Significance of positive mental health in student athletes. *Indian Journal of Health and Wellbeing*, *9*, 609–615.

Putukian, M. (2021). *Mental health concerns in athletes* (pp. 489–507).

Reardon, C. L., Hainline, B., Aron, C. M., Baron, D. A., Baum, A. L., Bindra, A., Budgett, R., Campriani, N., Castaldelli-Maia, J. M., Currie, A., Derevensky, J. L., Glick, I. D., Gorczynski, P., Gouttebarge, V., Grandner, M. A., Han, D. H., McDuff, D., Mountjoy, M. L., Polat, A., … Engebretsen, L. (2019). Mental health in elite athletes: International olympic committee consensus statement (2019). *British Journal of Sports Medicine*, *53*, 667–699.

Rice, S. M., Purcell, R., Silva, S. De, Mawren, D., McGorry, P. D., & Parker, A. G. (2016). the mental health of elite athletes: A narrative systematic review. *Sports Medicine (Auckland, N.z.)*, *46*, 1333–1353.

Richardson, R. F. (2020). Mental health and disorders: An overview. *J Trauma Stress Disor Treat Vol: 9* Issue: 3, e122. doi: 10.37532/jtsdt.2020.9(3).e122

Rogers, D. L., Tanaka, M. J., Cosgarea, A. J., Ginsburg, R. D., & Dreher, G. M. (2023). How mental health affects ınjury risk and outcomes in athletes. *Sports Health*, *16*(2), 222-229.

Sebbens, J. P., Hassmén, P., Crisp, D. A., & Wensley, K. (2016). Mental health in sport (MHS): Improving the early ıntervention knowledge and confidence of elite sport Staff. *Frontiers in Psychology*, *7*, 911.

Singh, V. K., Singh, P., Karmakar, M., Leta, J., & Mayr, P. (2021). The journal coverage of Web of Science, Scopus and dimensions: A comparative analysis. *Scientometrics*, *126*(6), 5113–5142.

Snedden, T. R., Scerpella, J., Kliethermes, S. A., Norman, R. S., Blyholder, L., Sanfilippo, J., McGuine, T. A., & Heiderscheit, B. (2019). Sport and physical activity level ımpacts health-related quality of life among collegiate students. *American Journal of Health Promotion*, *33*(5), 675–682.

Vella, S., Swann, C., & Tamminen, K. (2020). Mental health in sport: An overview and introduction to the special issue. *Journal of Applied Sport Psychology*, *33*, 1–3.

Yaffé, M. (1981). Sport and mental health. *Journal of Biosocial Science*, *13*(7), 83–95.

Author Information

Syaipul Hari Baharuddin
Universitas Pendidikan Indonesia
Bandung, Indonesia
Contact e-mail: *syaipul.hary@upi.edu*

Agus Mahendra
Universitas Pendidikan Indonesia
Bandung, Indonesia

Haryanto Haryanto
Universitas Pendidikan Indonesia
Bandung, Indonesia

Muh. Aswar
Universitas Pendidikan Indonesia
Bandung, Indonesia

Herdiansyah Herdiansyah
Universitas Pendidikan Indonesia
Bandung, Indonesia

To cite this article:

Baharuddin, S.H., Mahendra, A. Haryanto, H., Aswar, M., & Herdiansyah, H.. (2024). Bibliometric analysis of mental health in athletes. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 55-66.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 67-75

ICGeHeS 2024: International Conference on General Health Sciences

Peptide Analysis of Protein Extracts from *Caulerpa Lentillifera* by Nano LC-ESI-Ms/Ms and Their Potential as Precursor of Biologically Active Peptides – *In Silico* Approach

Fisal Ahmad
Universiti Malaysia Terengganu

Abstract: Peptidomics research is gradually positioning in food science fields due to an increasing interest in peptides released from food proteins with health benefits and nutraceutical properties. Referable to the complexity of these compounds, their study requires advanced analytical techniques such as tandem mass spectrometry. Seaweed naturally has high nutritional value and many health-promoting effects. Hence, this work aimed to characterize the peptide and to virtual screening for their potential as a precursor of biologically active peptides. The total soluble protein was extracted from *Caulerpa lentillifera* using the phenol ammonium acetate precipitation method. In solution, digestion was carried out using trypsin on 500 µg of protein. Peptide sequencing was accomplished using nano liquid chromatography-electrospray ionization tandem mass spectrometry (nLC-ESI-MS/MS), and Peaks Studio 7 was used for the analysis of MS/MS data and *de novo* peptide sequencing using an average local confidence above 90%. The results showed that 76 peptides mapped to selected proteins and 145 were *de novo* peptide sequences. *In silico* approaches of both peptide sequences resulted in 15 types of biological activity characteristics of peptides from among 44 categories as listed in the BIOPEP-UWM database, and motifs with the ACE inhibitory activity occur most frequently. These findings are relevant to the search for bio-functional ingredients as constituents of functional foods or provide added value to nutraceutical foods. Significantly, the methodology described here might apply to discovering the potential in any organism with incomplete genome data.

Keywords: Seaweed, peptidomic, *de novo* sequencing, Nutraceuticals, ACE-inhibitor

Introduction

Proteomics and peptidomic research are progressively positioned in food science due to an increasing interest in protein or peptides released from food proteins with health benefits and nutraceutical properties. Due to the complexity of these compounds, their study requires modern analytical techniques such as tandem mass spectroscopy (MS/MS). Mass spectrometry (MS) and MS/MS are now well-established as potent analytical tools for protein and peptide analysis, possessing high sensitivity and great specificity (Chen, 2008). It also can isolate a single species, including post-translational modification of peptides from a background of thousands of co-existing species. In many cases, amino-acid sequences of 8 to 10 residues carry sufficient information to determine the protein from which the peptide is derived (Wu et al., 2006).

Bioactive proteins and peptides are derived from food and have physiological, hormone-like effects on human organisms. They act directly through their presence in the undisturbed food (Hartmann & Meisel, 2007). Bioactive peptides are encrypted in the protein's primary structure and inactive until released by enzymatic hydrolysis. It can occur during digestion by proteolytic enzymes in the gastrointestinal tract or fermentation and food processing (Udenigwe & Aluko, 2012; Udenigwe & Howard, 2013). Bioactive peptides can exhibit local effects in the gastrointestinal system or cause systemic effects after intestinal absorption and entering the circulatory system. They range in size from 2 to 50 amino acid residues (Hernández-Ledesma et al., 2011). Once

liberated, the bioactive peptides may act as regulatory compounds with hormone-like activity, exhibiting a wide range of biological functions, such as antihypertensive, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, immunomodulatory, hypoglycemic, opioid, and antiproliferative activities.

The proteomic analysis of *C. lentillifera* proteins poses two significant difficulties: First, because of the complexity of the matrices examined, it is difficult to obtain high-quality protein extracts, and second, due to the lack of sequenced genomes, the database information available is limited. Therefore, this study used the phenol extraction method because it has been shown to generate high-quality protein extracts from various plant species, including our sample, *C. lentillifera*. Only a few studies have examined the seaweed protein, and no reports have been published regarding *C. lentillifera* proteome or peptides from Sabah, Malaysia waters. Therefore, the characterization of *C. lentillifera* proteins could lead to the discovery of new bioactive molecules, thus increasing the value of this seaweed as a food product.

Method

Sample Preparation

Green seaweed *C. lentillifera* was collected from Semporna, Sabah. The seaweed identification was guardedly confirmed based on morphological characteristics. AcDP was prepared according to the method described by Awang et al. (2010) with some modifications to the first steps to eliminate the non-protein compounds efficiently. This washing step was performed five times, and the resulting polyphenol-free seaweed powder was air-dried. AcDP was stored at -80^{o}C until protein extraction.

Total Soluble Protein Extraction, Quantification and Yield

Phenol extraction methanol-ammonium acetate precipitation extraction of total soluble protein was performed according to Carpentier et al. (2005) and Awang et al. (2010) with little modifications. The total soluble protein in the seaweed samples was determined using the Bradford assay (Bradford, 1976). The total soluble protein concentration was expressed as microgram per microliter (µg/µL), and the protein yield was calculated from this result.

In-Solution Tryptic Digestion, Mass Spectrometry Analysis and Database Searches

For in-solution digestion, approximately 0.5 mg of proteins were reduced, alkylated, and digested with trypsin. After overnight incubation at 37 °C, trypsin activity was stopped with 1 µL of concentrated acetic acid. The samples were later concentrated by vacuum concentrator and kept at -80 °C until use. The samples were dissolved in 0.1% formic acid and filtered. The peptide mixtures were analyzed by on-line nanoflow liquid chromatography using the EASY-nLC II system (Thermo Scientific, San Jose, CA, USA) with 10 cm capillary columns of an internal diameter of 75 µm filled with 3 µm Easy-Column C18-A2 (Thermo Scientific, San Jose, CA, USA) and coupled with pre-column (Easy-Column, 20 0.1 mm i.d., 5 m; Thermo Scientific, San Jose, CA, USA) at flow rate of 0.3 l/min. Running buffers were (A) deionised distilled water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. Instrument control and data recording were performed using Xcalibur ver. 2.1 (Thermo Scientific, San Jose, CA, USA) with a mass tolerance threshold of 5 ppm. Data analysis was performed using PEAKS studio ver. 7.0. PEAKS were used to construct predicted peptide sequences for MS/MS data without referring to the known protein databases. The process produced a list of *de novo* peptide sequences identified from MS/MS spectra. This was followed by database matching to the UniProt-Seaweed database.

In Silico Assessment of Peptides Identified Using BIOPEP-UWM Database

The peptides identified by bioinformatics were searched against the BIOPEP-UWM database (http://www.uwm.edu.pl/biochemia) to find bioactive fragments (motifs) in both identified peptide sequences. (Minkiewicz et al., 2019). This program allows the user to evaluate the peptide sequence profiles of peptide potential with biological activity. Presently, 44 biological activity characteristics of peptides and 2609 motifs of bioactive peptide sequences were listed in the BIOPEP-UWM database. This option acquired BIOPEP ID, name of peptides, potential activity of peptide, number of peptides, and location of bioactive peptide in protein

sequences. Meanwhile, the frequency of occurrence of fragments with given activity (A) in the selected protein was taken as the evaluation parameter and calculated based on equation 1:

$$A = a/N \quad \text{……equation 1}$$

Where a = the number of bioactive peptides and N = the total number of amino acid (AA) residues in the protein chain. In addition, the total frequency of bioactive fragments (∑A) in each of the four sequences was also calculated.

Data Analysis

Data analysis was done by using Microsoft Excel 2016. Data from the BIOPEP-UWM database were placed in Microsoft Excel, where the predicted bioactive peptides and activities were sorted, analysed and tabulated into tables and figures.

Results and Discussion

Total Soluble Protein Concentration and Yield

Protein extraction typically starts with breaking the protective cell wall and plasma membrane. As such, there is a definite release of other intracellular components that interfere with subsequent proteomic analyses, such as polyphenols, polysaccharides, proteases, lipids, and numerous secondary metabolites. Obviously, these components have to be removed from the protein sample. Therefore, we applied the washing step with ice-cold acetone in this study to effectively remove the polyphenol and pigment. This resulted in acetone-dried powder (AcDP) followed by the protein extraction process. The phenol extraction method was used in this study due to excellent results reported by Carpentier et al. (2005), who compared several extraction protocols and found that phenol extraction was the most powerful. Saravanan and Rose (2004) also proved that phenol extraction is preferable when dealing with recalcitrant plant tissue.

The results showed that *C. lentillifera* had a total soluble protein concentration of 4.84 ± 0.22 µg/uL and a protein yield of 1403.65 ± 15.61 µg/g AcDP. The protein content of marine algae varies to a large extent depending on species and season. In general, the highest levels of protein are found in the red species (maximum 47%(w/w) dry weight), with moderate to low levels found in green (9–26%(w/w) dry weight) and brown (3–15%(w/w) dry weight) phyla (Fleurence, 2004). In some red seaweed, protein levels can be as high as 35%(w/w) (*Palmaria palmata* (dulse)) and 47%(w/w) (*Porphyra tenera* (nori)), while the green alga *Ulva pertusa* (anori) can contain up to 26%(w/w) protein (Fleurence, 2004; Wong & Cheung, 2000).

Mass Spectrometry Analysis and Database Searches

The tandem-mass spectra were analyzed by PEAKS studio 7 *de novo* sequencing software to generate amino acid sequences. The independence of a sequence database makes *de novo* sequencing the preferred method for identifying novel peptides and studying unsequenced organisms. Even when the peptide sequence is in a database, *de novo* sequencing can significantly help improve the database-search-based peptide identification since the match between the de novo sequence and the database sequence confirms the correctness of the identification (Zhang et al., 2012). The results showed that 76 peptides were mapped to the selected protein, and all peptides ranged from 6 to 15 amino acid residues (Table 1). Meanwhile, 145 were *de novo* peptide sequences, and all these peptides range from 5 to 12 amino acid residues (Table 2).

Potential Biological Activity Profile

Bioinformatics has become a powerful tool for peptide research (Minkiewicz et al., 2008), including *in silico* prediction of the release of bioactive peptides from food proteins (Vercruysse et al., 2009). The BIOPEP-UWM database is a bioinformatics tool enabling the detection of biologically active fragments in protein sequences, the classification of proteins as potential sources of bioactive fragments, to simulate protein hydrolysis and find peptides that can be released by a given enzyme or, as a result, of the combined action of two or three enzymes (Minkiewicz et al., 2008).

A successful prediction of the activity of protein hydrolysates using the BIOPEP-UWM database and its search engine has been recently described by Cheung et al. (2009) on the example of angiotensin I-converting enzyme inhibitory activity of oat protein hydrolysates. Data concerning angiotensin I-converting enzyme inhibitory peptide with the LQP sequence have also validated the reliability of computational predictions using the BIOPEP database. This peptide has been discovered by Miyoshi et al. (1991) in a-zein hydrolysates. It has been found in the profiles of potential biological activity of bovine b-casein and wheat a/b-gliadin, obtained in silico, published by Dziuba et al. (1999). The above peptide has been detected experimentally in cheese (Tonouchi et al., 2008) and wheat grain milling by-products (Nogata et al., 2009). Physiologically active peptides form a complex and highly diversified group of compounds with regard to their terminology, structure, and functions. Many biologically active peptides are multifunctional, performing regulatory functions and directly affecting various developmental and metabolic processes.

Table 1. List of the matched peptide sequence identified by nLC-MS/MS-Orbitrap in the protein extract of C. lentillifera after digestion with Trypsin. Spectra analysis was performed using the PEAKS Studio 7 Software using the search parameters described in the materials and methods section.

No.	Peptide	No.	Peptide	No.	Peptide
1	LSGGDHLHSGTVVGK	27	AGIMLSPTFVK	53	IPYDQQIK
2	LSGGDHIHSGTVVGK	28	DNFVEKDR	54	LYSIASSR
3	HYAHVDC(+57.02)PGHADYVK	29	VAINGFGR	55	AVSLVLPKLK
4	AQLGEIFEFDR	30	AVVISVIDNLVK	56	PDTFAELK
5	SLLGC(+57.02)TIKPK	31	IGINGFGR	57	TEDC(+57.02)VGC(+57.02)KR
6	VINTWADIINR	32	LGINGFGR	58	APGFGDR
7	VLNTWADIINR	33	ELEVIHAR	59	IAAFDGER
8	VINTWADILNR	34	EIEVIHAR	60	SVDETLR
9	DHGLLLHIHR	35	VYLGPETTR	61	SAPLGGTSGQSAAAGLR
10	LEDLRIPPAYAK	36	IQPDEISSIIR	62	APGFGDRR
11	IFGVTTLDVVR	37	ASQIASAPR	63	YGSLLR
12	LFGVTTLDVVR	38	HLPGFIEK	64	VLGFSLR
13	EVTLGFVDLMR	39	VAEYTLK	65	EAHTHIK
14	NKITITNDKGR	40	ALRLEDLR	66	GLFIIDK
15	VPLILGVWGGK	41	GNAPGAAANR	67	AVYEC(+57.02)LR
16	VHTVVLNDPGR	42	GHRQELTR	68	VVDLLAPYRR
17	VHTVVINDPGR	43	AWMAAQDQPHEK	69	AGNHEAVVK
18	AGFAGDDAPR	44	GGLDFTK	70	TAGGGGAAAVR
19	TFQGPPHGIQVER	45	LINLSGK	71	ELIIGDR
20	YRELEVIHAR	46	LGC(+57.02)TIKPK	72	IGPLGLSPK
21	YREIEVIHAR	47	RDHVLYGK	73	LGPLGLSPK
22	DVMASESAAFR	48	GSTFLDPK	74	WAKPGHFSR
23	DGVYPEKVNAGR	49	YNKKPTLTSR	75	RC(+57.02)LVCPGEQPK
24	AMHAVIDR	50	GKLNGIALR	76	NQFYVTPK
25	AMHAVLDR	51	GKLNGLAIR		
26	ESTLHLVLR	52	GKLNGLALR		

The results of evaluating peptide sequence (protein peptide) as the precursor of bioactive peptide for the protein peptide of *C. lentillifera* using BIOPEP-UWM database are shown in Figure 1. A total of 159 fragments (motifs) of peptide sequences have been detected from 76 protein peptides. Overall, they showed only 13 biological activity characteristics of bioactive peptides. The majority of matches consisted related to motifs with a potential of ACE inhibitory activity of 97 different motifs (60.00%), followed by inhibitor having 16 different motifs (11.00%), antioxidative having 18 different motifs (11.00%), stimulating having 9 different motifs (6.00%).

Meanwhile, regulating has 4 different motifs (3.00%), antiamnestic has 3 different motifs (3.00%), and antithrombotic has 2 different motifs (3.00%). The other six, neuropeptide, hypotensive, immunomodulating, opioid, bacterial permease ligand, and activating ubiquitin-mediated proteolysis, generally only 1.00% and less. The motif sequence of LG in the group of ACE inhibitors showed the most frequent (12 times).

Table 2. List of the *de novo* peptide sequence identified by nLC-MS/MS-Orbitrap in the protein extract of *C. lentillifera* after digestion with Trypsin. Spectra analysis was performed using the PEAKS Studio 7 Software using the search parameters described in the materials and methods section

No.	*de novo* Peptide	No.	*de novo* Peptide	No.	*de novo* Peptide
1	LAC(+57.02)EAC(+57.02)LQAR	50	FGPNLR	98	HVVFGLVK
2	SGPEDKFR	51	LC(+57.02)YLK	99	TKLC(+57.02)YLK
3	LEDLR	52	GYLSYHDGR	100	LSMENQR
4	SGPEDKFR	53	LLFPEEVLPR	101	LTFTGSNPR
5	LLGVTTLDVVR	54	LVNNTYAK	102	LLFPEEVLPR
6	LEDLR	55	SAAHC(+57.02)YK	103	SKPSTLSLR
7	YELLTR	56	LSYHDGR	104	YLVENQK
8	LALQM(+15.99)C(+57.02)AKK	57	NVYTGLFYGR	105	VC(+57.02)NYVSWLK
9	YELLTR	58	LC(+57.02)YLK	106	FLGLNQLGEK
10	LGMDEELLR	59	LENNFR	107	M(+15.99)LADAMKK
11	VGGAFLQR	60	VSGTC(+57.02)VGSYR	108	GYLSYHDGR
12	LLGVTTLDVVR	61	VLTDDGVALR	109	TDHDHC(+57.02)WC(+57.02)K
13	VGGAFLQR	62	HGEFSK	110	LLTTVAGFDR
14	LGMDEELLR	63	LSELVTDWHR	111	LGENSDPVSVK
15	VLGSVTVR	64	LSELVTDWHR	112	VCNYVSWLK
16	HGMHFR	65	LEFYGPNR	113	LAELLR
17	VAEYTLR	66	WFTSELESNR	114	LGHHNEHLK
18	DTLADLHAK	67	HGEFSK	115	TDHDHC(+57.02)WC(+57.02)K
19	VAEYTLR	68	LEFYGPNR	116	ASGDVTLR
20	FVLNR	69	LEFYGPNR	117	DFTC(+57.02)DTSR
21	HYTEALKR	70	ASGLGVGNAAR	118	M(+15.99)DALTKK
22	LTEC(+57.02)LER	71	WSTDGGLFLR	119	LHGMHFR
23	AVASSQSTFR	72	M(+15.99)DALTKK	120	AFKPLHDR
24	LEVGFR	73	YAC(+57.02)TVGSR	121	MEGTTVNAR
25	LLYVEK	74	YTDLLLR	122	EAGEAFAVSFAR
26	LPAQALK	75	ELLGTLR	123	MNFLLK
27	LELGTLR	76	FLADAMQK	124	YYGALFPFYVNK
28	STAQTAAR	77	TSLGHLESLR	125	VEFGLTNSVR
29	LKSAASLNSR	78	VGSDSELLEAFK	126	MHFAVR
30	VLETDLLAAFR	79	LPAQALK	127	LLVAGATGR
31	STAQTAAR	80	VLTDDGVALR	128	YVLASLGSLSK
32	YLNSEFR	81	NLYWATGVR	129	KYQMK
33	YETDFGLFPR	82	SGFLLER	130	TPLHLAALK
34	VLETDLLAAFR	83	SKPSTLSLR	131	NSLTGVTPSR
35	YETDFGLFPR	84	VSLSTC(+57.02)R	132	KYQMK
36	YTELVK	85	ESLVSYNPFK	133	LLVAGATGR
37	STAQTAAR	86	LSTALDSR	134	LTFTGSNPR
38	STAQTAAR	87	EAGEAFAVSFAR	135	VLLGHSER
39	LEANFR	88	LAGFTFGPDR	136	YLSMAK
40	TKLC(+57.02)YLK	89	VMHFAVR	137	QVLTLHK
41	LEANFR	90	LELPNEVSR	138	QYKMK
42	YTELVK	91	FGPNLR	139	HLDLSAVR
43	LALQQVR	92	GLHLGEQR	140	LGHHNEHLK
44	RFEFYAK	93	QEFSSC(+57.02)LLR	141	LEFYGPNR
45	YLNSEFR	94	SVKMEVR	142	VVHKTM(+15.99)GLK
46	WSTDGGLFLR	95	TFDLAK	143	LALQQVR
47	MHFAVR	96	TDPEFLRK	144	M(+15.99)LGLNKLGEK
48	MHAVLDR	97	SPLQVR	145	AKTVPLSAR
49	LSTALDSR				

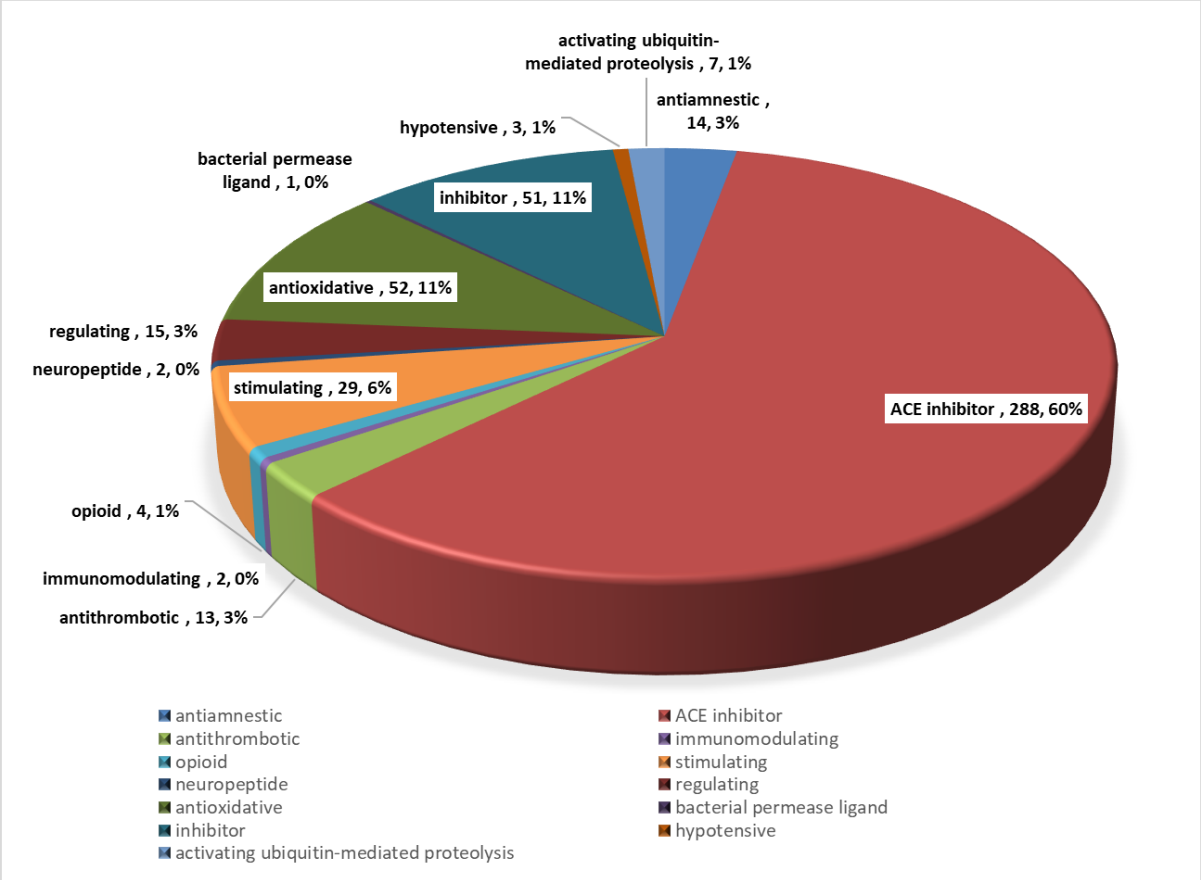


Figure 1. Distribution of bioactive peptides/motifs from *C. lentillifera* of protein-peptide according to their main activity (presented as a total number of motifs occurring and percentage).

The results of evaluating peptide sequence (*de novo* peptide) as the precursor of bioactive peptide for the protein peptide of *C. lentillifera* using BIOPEP-UWM database are shown in Figure 2. A total of 159 fragments (motifs) of peptide sequences have been detected from 145 *de novo* peptides. Overall, they showed 15 biological activity characteristics of bioactive peptides. The majority of matches consisted related to motifs with a potential of ACE inhibitory activity of 78 different motifs (55.00%), followed by inhibitor having 14 different motifs (14.00%), antioxidative having 19 different motifs (9.00%), stimulating having 7 different motifs (8.00%), regulating has 3 different motifs (2.00%), hypotensive has 2 different motifs (2.00%), antithrombotic has 2 different motifs (2.00%), neuropeptide has 2 different motifs (2.00%), and activating ubiquitin-mediated proteolysis has 2 different motifs (2.00%), The other six, antiamnestic, neuropeptide, immunomodulating, opioid, bacterial permease ligand, and antibacterial, generally only 1.00% and less. The motif sequence of LL in the group of stimulating and inhibitors showed the most frequent (23 times).

The results of computer-aided prediction of the bioactive peptide release from plant proteins indicate a relatively good possibility of obtaining biologically active peptides. The location of bioactive fragments in the hydrophilic part of a plant protein molecule can be an essential aspect in the production of functional food, i.e., food with special desired and designed features, as well as in the production of Nutraceuticals (Darewicz and Dziuba, 2000). Knowledge about the structure of bio-macro molecules can help investigate their structure-function relationship. Bioinformatic methods applied in biotechnology or biochemistry have become increasingly popular due to the short time required to obtain results, low research costs, the possibility of recording results in text files, and their good reproducibility. At the same time, as computer science develops and methods are improved.

In view of the Figure 2, a computational analysis may be successfully used for rapid screening of protein sequences to predict the potential biological activity of selected fragments and to find a way to liberate them. As a result, seaweed sample *C. lentillifera* has great potential to supply functionally significant peptides. The findings of the study can be exploited in the development of foods with special health claims (e.g. treatment of hypertension) as well as in identifying new applications in food. In the future, seaweed-derived bioactive

peptides may be important components in foods sustaining health and the prevention of diseases such as cardiovascular diseases.

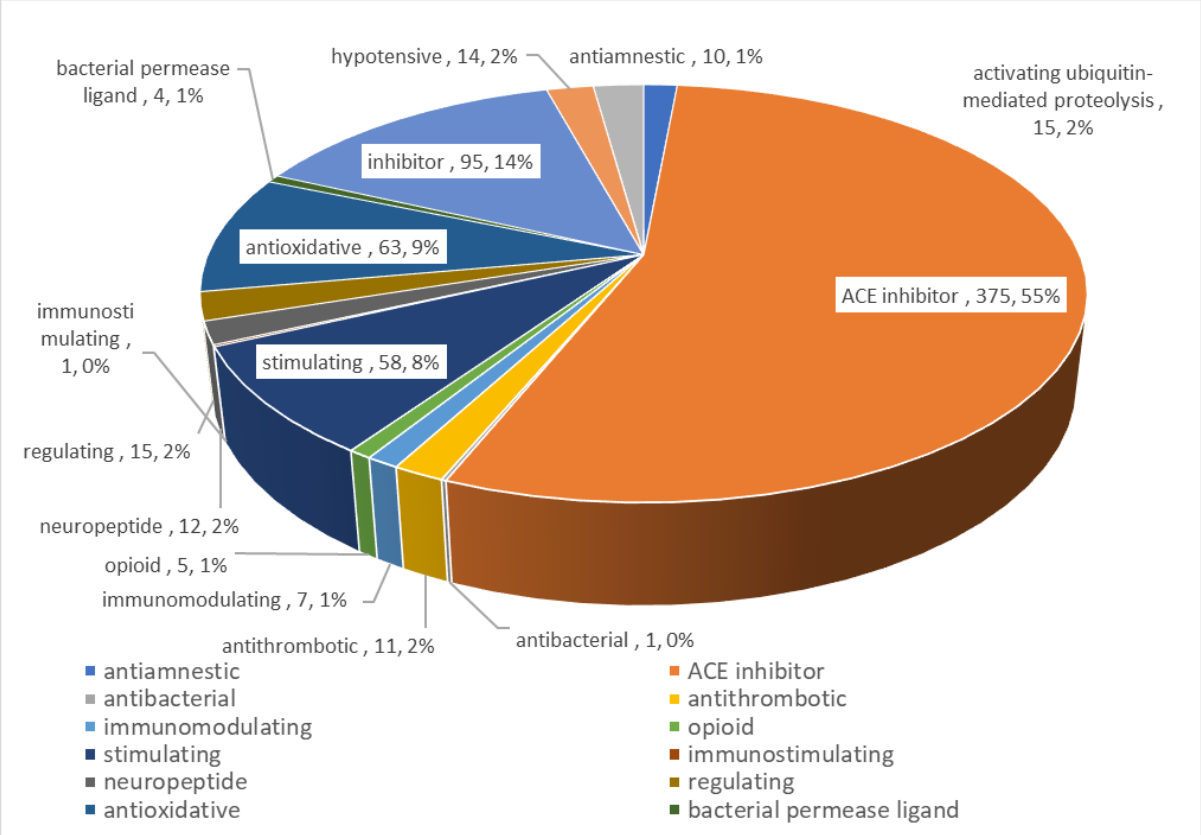


Figure 2. Distribution of bioactive peptides/motifs from *C. lentillifera* of *de novo* peptide according to their main activity (presented as a total number of motifs occurring and percentage).

Conclusion

In conclusion, this *in silico* study shows that this protein raw material (*C. Lentillifera*), which remains a relatively untapped reservoir, can act as a resource for generating bioactive peptides with potential health-promoting and disease-preventing properties. In general, various bioactive peptides can be found in these seaweed protein extracts, and the studies showed that these seaweed samples contain protein sequences that can be a potential source of ACE and inhibitory (dipeptidyl peptidase IV inhibitory) activity bioactive peptides. The study's findings can be exploited in the development of foods with special health claims (e.g. treatment of hypertension) as well as in identifying new applications in food. These investigations may significantly contribute as an initial step that can improve market value for seaweed produce and provide new insights into human nutrition.

Recommendations

Further studies are still required to better understand the possible adverse effects exerted by the respective peptides or their by-products, which would inevitably be contained in such foods. Safety requirements include the absence of toxicity, cytotoxicity, and allergenicity.

Scientific Ethics Declaration

The authors declare that the scientific, ethical, and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey, on May 02-05, 2024.

* We would like to express our gratitude to all those who have contributed to the completion of this research project and the preparation of this manuscript.

References

Awang, A., Karim, R. & Mitsui, T. (2010). Proteomic analysis of Theobroma cacao pod husk. *Journal of Applied Glycoscience, 57* (1), 245-264.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 7* (1)248-254.

Carpentier, S. C., Witters, E., Laukens, K., Deckers, P., Swennen, R. & Panis, B. (2005). Preparation of protein extracts from recalcitrant plant tissues: An evaluation of different methods for two-dimensional gel electrophoresis analysis. *Proteomics, 5* (1), 2497-2507.

Chen, C.H. (2008). Review of a current role of mass spectrometry for proteome research. *Analytical Chemical Acta, 624* (1), 16-36.

Cheung, I. W. Y., Nakayama, S., Hsu, M. N. K., Samaranayaka, A. G. P., & Li-Chan, E. C. Y. (2009). Angiotensin I converting enzyme inhibitory activity of hydrolysates from oat (avenasativa) proteins by in silico and in vitro analyses. *Journal of Agricultural and Food Chemistry, 57* (19), 9234-9242.

Darewicz, M. & Dziuba, J. (2000). Functional properties of proteins and peptides in the context of their molecular characteristics. *Natural Science, 6* (1), 8-23.

Dziuba, J., Minkiewicz, P., Naleucz, D., & Iwaniak, A. (1999). Database of biologically active peptide sequences. *Nahrung, 43* (1),190-195.

Fleurence, J. (2004). *Seaweed proteins*. R. Y. Yada (Ed.). *Proteins in food processing* (pp.197-213). Cambridge, UK: Woodhead Publishing Limited

Hartmann, R., & Meisel, H. (2007). Food-derived peptides with biological activity: from research to food applications. *Current Opinion in Biotechnology, 18(*2),163-169.

Hernández-Ledesma, B., del Mar Contreras, M. & Recio, I. (2011). Antihypertensive peptides: Production, bioavailability and incorporation into foods. *Advances in Colloid and Interface Science, 165* (1), 23-35.

Minkiewicz P., Iwaniak A., & Darewicz M., (2019). BIOPEP-UWM database of bioactive peptides: current opportunities. *International Journal of Molecular Sciences, 20*, 5978.

Minkiewicz, P., Dziuba, J., Darewicz, M., Iwaniak, A., Dziuba, M., & Nałęcz, D. (2008). Food peptidomics. *Food Technology and Biotechnology*, 46(1),1-10

Miyoshi, S., Ishikawa, H., Kaneko, T., Fukui, F., Tanaka, H., & Marutama, S. (1991). Structures and activity of angiotensin-converting enzyme inhibitors in-zein hydrolysate. *Agricultural and Biological Chemistry, 55* (1), 1313-1318.

Nogata, Y., Nagamine, T., Yanaka, M., & Ohta, H. (2009). Angiotensin I converting enzyme inhibitory peptides produced by autolysis reactions from wheat bran. *Journal of Agricultural and Food Chemistry, 57 (15)*,6618-6622.

Saravanan, R. S. & Rose, J.K.C. (2004). A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues. *Proteomics, 4* (1), 2522-2532.

Tonouchi, H., Suzuki, M., Uccida, M., & Oda, M. (2008). Antihypertensive effect of an angiotensin converting enzyme inhibitory peptide from enzyme modified cheese. *Journal of Dairy Research, 75* (1),284-290.

Udenigwe, C. C., & Aluko, R. E. (2012). Food protein-derived bioactive peptides: production, processing, and potential health benefits. *Journal of Food Science, 77*(1), R11-R24.

Udenigwe, C. C., & Howard, A. (2013). Meat proteome as source of functional biopeptides. *Food Research International, 54*(1), 1021-1032.

Vercruysse, L., Smagghe, G., Van der Bent, A., Van Amerongen, A., Ongenaert, M., & Van Camp, J. (2009). Critical evaluation of the use of bioinformatics as a theoretical tool to find high-potential sources of ACE inhibitory peptides. *Peptides, 30*(3), 575-582.

Wong, K.H. & Cheung, P.C.K. (2000). Nutritional evaluation of some subtropical red and green seaweeds - Part I - proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry, 71*(1), 475-482.

Wu, X., Edwards, N., & Tseng, C. W. (2006). Peptide identification via tandem mass spectrometry. *Advances in Computers, 68*, 253-278.

Zhang, J., Xin, L., Shan, B., Chen, W., Xie, M.,…& Yuen, D. (2012). PEAKS DB: De novo sequencing assisted database search for sensitive and accurate peptide identification. *Molecular and Cellular Proteomics, 11* (4), 1-8.

Author Information

Fisal Ahmad
Faculty of Fisheries and Food Science,
Universiti Malaysia Terengganu, 21030 Kuala Nerus,
Terengganu, Malaysia.
Functional Food RIG, Food Security in a Changing Climate
SIG, Food Security Research Cluster, Universiti Malaysia
Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
Contact e-mail: *fisal@umt.edu.my*

To cite this article:

Ahmad, F. (2024). Peptide analysis of protein extracts from *Caulerpa Lentillifera* by nano LC-ESI-Ms/Ms and their potential as precursor of biologically active peptides – *In silico* approach. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 67-75.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 76-81

ICGeHeS 2024: International Conference on General Health Sciences

XRCC2 Gene Study by Next Generation Sequencing and Establishing Its Relation with Breast Cancer

Naser Gilani
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract: Breast cancer (BC) is a leading cause of morbidity and mortality among women, with its development influenced by genetic factors such as mutations in the XRCC2 gene, a key player in DNA repair via homologous recombination. This study aimed to elucidate the role of XRCC2 in BC by utilizing Next Generation Sequencing (NGS) to identify genetic variants and assess their association with BC risk and progression. This study was done between 2021 and 2024. Specimens were meticulously collected from Zheen International Hospital, located in Erbil, Iraq. In total, 44 peripheral blood samples of 44 BC patients were included in this study. DNA extracted from these samples underwent NGS, revealing seven XRCC2 variants with varying predictions of pathogenicity. In terms of pathogenicity, 5 of these mutations were Uncertain Significance, including (c.134A>C, c.271C>T, c.283A>C, c.181C>A, c.-1G>A (5UTR variant)), 1 of them was Likely Pathogenic including (c.651_652del) and other (c.582G>T) was Likely benign. In conclusion, the XRCC2 could serve as a biomarker for BC, warranting further investigation for its inclusion in genetic screening programs.

Keywords: Breast cancer, Genomic analysis, Next generation sequencing

Introduction

Introduction to XRCC2 and Breast Cancer

Breast cancer (BC) is the most frequent malignancy among women worldwide, with approximately 2.3 million new cases identified annually (Arnold et al., 2022). BC is the most commonly diagnosed cancer among women worldwide, with a multifactorial etiology that encompasses genetic, environmental, and lifestyle factors (Obeagu and Obeagu, 2024). Among the myriad of genes implicated in the maintenance of genomic integrity, the X-ray repair cross-complementing group 2 (XRCC2) gene is a pivotal element in the homologous recombination repair (HRR) pathway (Liu et al., 2023, Yu & Wang, 2023).

The XRCC2 gene plays a pivotal role in the HRR pathway, a crucial mechanism for the repair of DNA double-strand breaks (DSBs) (Yu and Wang, 2023). DSBs are among the most lethal forms of DNA damage, and if not accurately repaired, they can lead to genomic instability, a hallmark of cancer development (Alhmoud et al., 2020, Berzsenyi et al., 2021). The XRCC2 gene provides a plugin for the formation of the RAD51 paralog complex that is vital for the processing of DSBs and the maintenance of chromosome stability Mutations or dysregulation in the components of the HRR pathway, including XRCC2, can compromise DNA repair and contribute to carcinogenesis (Ivy et al., 2021, Yu &Wang, 2023). The XRCC2 gene, by virtue of its role in HRR, has thus become a subject of intense research interest, particularly in the context of BC susceptibility (Liu et al., 2021).

Next Generation Sequencing (NGS) and Its Role in XRCC2 Analysis

The evolution of NGS technologies has tremendously changed the way genetic research is conducted as it allows full-scale analysis of genomic sequences with unmatched detail and extent. NGS offers a detailed and efficient approach to identifying genetic variations, including single nucleotide polymorphisms (SNPs), insertions, deletions, and complex rearrangements (Satam et al., 2023). These variations, when occurring within or in close proximity to genes like XRCC2, can potentially modulate gene function and, consequently, an individual's risk of developing BC (Yu &Wang, 2023). By employing NGS, researchers can dissect the intricate relationship between XRCC2 gene variants and BC predisposition with greater specificity and sensitivity than was previously possible with traditional sequencing methods (Qin, 2019).

Necessity and Aim of the Present Study

Despite the progress made in understanding the genetic landscape of BC, the role of XRCC2 in BC susceptibility remains incompletely characterized. Previous studies have provided conflicting results regarding the association of XRCC2 variants with BC risk, necessitating further investigation using large-scale, robust genomic methodologies like NGS.

A study conducted by Dastgheib et al. (2024) showed that XRCC2 may play a role in increasing the risk of BC (Dastgheib et al., 2024). On the other hand, a discovery by Decker et al. (2017), showed that rare, protein-truncating variants in ATM, CHEK2, or PALB2, but not XRCC2 were associated with increased BC risks (Decker et al., 2017). The present study is therefore essential to clarify the contribution of XRCC2 to BC and to establish whether it should be considered as part of genetic screening programs for at-risk populations.

Method

Specimen Collection and Ethical Considerations

In this observational analytical research study that utilizes a case-control design, we focused on the analysis of the XRCC2 gene and its association with BC. This study was done between 2021 and 2024. Specimens include 44 peripheral blood samples with BC were collected from Zheen International Hospital, located in Erbil, Iraq. These samples were stratified based on the type of BC, patient age, and clinical characteristics.

All subjects provided informed permission before sample collection, and the Local Ethics Committee accepted the study procedure (Approval number: 05.01.2020\17). To ensure the integrity of the RNA, tissues were preserved in RNALater (ThermoFisher, USA) until genomic isolation could be performed.

DNA Extraction and Quantification

Following the manufacturer's instructions, DNA was extracted from peripheral blood samples using the PureLinkTM genomic DNA micro kit (ThermoFisher, USA). Using a NanoDrop (Biometrica-Taiwan), the concentration and purity of DNA were measured.

Next Generation Sequencing (NGS)

Using the Twist Human Core Exome Enzymatic Fragmentation (EF) Multiplex Complete kit, libraries were prepared for next-generation sequencing (NGS). Next, we used the MGIEasy FS DNA Library Prep Kit to get this library ready for sequencing. A mean target coverage of 100X was attained by sequencing the prepared library using the MGI-DNBSEQ-G400 platform (China), which produced 150 bp paired-end reads. FastQC was used for quality control on the raw FASTQ files. Burrows-Wheeler Aligner (BWA) was used to align reads to the human reference genome (hg19), and the Genome Analysis Toolkit (GATK) program was used to call variants. The depiction of variants was done using the Integrative Genomic Viewer (IGV).

In Silico Analysis

To estimate the effect of mutations on protein function, in silico methods like Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping (PolyPhen-2) were used. Mutation Taster and Align GVGD were used to evaluate the effects of mutations on protein structure and function.

Primer Design and PCR Optimization

Primers targeting the XRCC2 gene were designed to span exon-exon junctions, ensuring specificity to mRNA. Primers for the XRCC2 gene and the housekeeping gene GAPDH were designed using an online primer design program (http://workbench.sdsc.edu). The PCR conditions were optimized using gradient thermocycling in an ABI Vertti PCR System (ABI, USA). The optimal annealing temperature was determined by examining the yield of PCR products on a 2% agarose gel (Table 1).

Table 1. Primer sequences, PCR product size of three targets region of XRCC2/Exp optimal annealing temperature.

Gene name		Primer sequence	Optimal annealing temperature	PCR Product size
XRCC2	F R	TGTTTGCTGATGAAGATTCAC TCGTGCTGTTAGGTGATAAAGC	59.2 °C	255 bp
GAPDH	F R	GGTCCACCACCCTGTTGCTGT AGACCACAGTCGATGCCATCAC	59,4 °C	456 bp

Results and Discussion

To elucidate the implications of germline variants in breast cancer, seven inherited mutations identified within the XRCC2 gene were subjected to in silico analysis using a suite of computational prediction tools. The functional consequences of these variants were evaluated using Polymorphism Phenotyping (PolyPhen), which assesses potential structural and functional changes due to amino acid substitutions. PolyPhen scores provide a gradient of functional impact, ranging from benign (score of 0.0), possibly damaging (score between 0.15 and 0.85), to likely damaging (score above 0.85). Additionally, the Sorting Intolerant from Tolerant (SIFT) algorithm was employed, which examines sequence homology and the conservation of amino acid residues to predict the phenotypic effects of substitutions, with scores below 0.05 indicating deleterious changes.

MutationTaster and Align-GVGD further supplemented the predictive landscape, with the former analyzing potential impacts on protein function, mRNA expression, or splicing, and categorizing mutations from benign polymorphisms to likely disease-causing alterations. Align-GVGD combines biochemical distance scores (Grantham difference) and conservation scores (Grantham variation) to stratify substitutions into seven classes, with C0 being the least likely to affect the function and C65 the most.

Table 2. XRCC2 mutations identified in BC patients.

SNP ID	Allele Change	Amino Acid Change	Molecular consequence	Interpretation	SIFT Prediction	PolyPhen Prediction
*	c.134A>C	p.Glu45Gly	missense	Uncertain significance	Deleterious	NA
rs730882043	c.271C>T	p.Arg91Trp	missense	Uncertain significance	Deleterious	Probably Damaging
rs140214637	c.283A>C	p.Ile95Val	missense	Uncertain significance	Tolerated	Benign
rs746142129	c.651_652 del	p.Cys217_Asp 218delinsTer	frameshift	Likely pathogenic	NA	NA
rs769829135	c.582G>T	p.Thr194=	synonymous	Likely pathogenic	NA	NA
rs569810249	c.181C>A	p.Leu61Ile	missense	Uncertain significance	Deleterious	Possibly Damaging
rs768232997	c.-1G>A	-	5 prime UTR	Uncertain significance	NA	NA

* This mutation was observed for the first time in this study in BC

The present study investigated the mutations created in the XRCC2 gene in 44 BC patients by the NGS method. After performing this method, 7 mutations were found in the mentioned gene. All these mutations were heterozygous. In terms of pathogenicity, 5 of these mutations were Uncertain Significance, including (c.134A>C, c.271C>T, c.283A>C, c.181C>A, c.-1G>A (5UTR variant)), 1 of them was Likely Pathogenic including (c.651_652del) and other (c.582G>T) was Likely benign (Table 2).

The XRCC2 gene that encodes a protein related to DNA repair has been examined in BC. The XRCC2 gene is one component of the RAD51 gene family that has seven primary copies maintained and is involved in homologous recombination as well as DNA repair (Wang et al., 2014, Kluźniak et al., 2019). High-throughput sequencing technology known as NGS has completely changed the fields of molecular biology and genomics. Large volumes of DNA or RNA can be quickly and accurately sequenced thanks to it, allowing researchers to examine intricate genetic and biological systems in previously unheard-of detail (Satam et al., 2023). Therefore, this study aimed to elucidate the role of XRCC2 in BC by utilizing NGS to identify genetic variants and assess their association with BC risk and progression.

The present study using Next-Generation Sequencing (NGS) identified 7 heterozygous mutations in the XRCC2 gene, with varying degrees of pathogenicity. Five mutations were classified as Variants of Uncertain Significance (VUS), one as Likely Pathogenic, and one as Likely Benign. The mutation c.134A>C, known as p.Glu45Gly, in the XRCC2 gene's coding exon 3 is of uncertain significance. This variant that was observed for the first time in this study in BC involves an A to C substitution at nucleotide position 134, leading to the replacement of glutamic acid with glycine, an amino acid with different properties. In-silico tests indicate that this missense mutation at a conserved and deleterious amino acid position is associated with hereditary cancer-predisposing syndromes. Due to the significant changes it induces in the protein's amino acid sequence, it is likely to disrupt the protein's function.

Another mutation, XRCC2 c.271C>T, causes the p.Arg91Trp (R91W) to change from Arginine to Tryptophan at the protein level. This alteration, observed in BC families, shows potential protein structure and function damage as per in silico analyses. It has been identified in Caucasian families with a strong BC history and has shown a moderate ability to restore XRCC2-DNA repair deficiencies in certain complementation assays (Hilbers et al., 2012, Park et al., 2012). While this alteration has been detected in a BC patient within a UK study, its clinical significance remains uncertain due to limited supporting evidence and inconclusive in-silico predictions (Kluźniak et al., 2019). The position of this altered amino acid is highly conserved in vertebrate species, adding to the complexity of determining its clinical impact.

The c.582G>T variant in ClinVar database is considered likely pathogenic despite being a synonymous mutation with a low population frequency (1 in 100,000). The XRCC2 protein's c.181C>A variant results in a conservative p.Leu61Ile substitution, with leucine being replaced by isoleucine (both neutral, non-polar amino acids). Despite its presence in population databases (rs569810249, gnomAD 0.02%) and association with certain cancers (BC and stomach cancer) (Park et al., 2012, Lu et al., 2015), experimental studies suggest minimal impact on XRCC2 function (Hilbers et al., 2016). The variation occurs at a conserved position but not within a recognized functional domain, leading to mixed predictions about its influence on protein structure and function. Finally, the c.-1G>A variant in the XRCC2 gene's 5' UTR shows a G to A substitution close to the translation starting site. Its occurrence in a small subset of early-onset BC patients versus controls, and conservation across vertebrates, suggests potential significance, yet clinical relevance remains uncertain due to limited evidence (Park et al., 2012).

Given the significant associations observed between XRCC2 variants and BC, this gene merits consideration for inclusion in genetic screening programs for at-risk populations. Such screening could potentially aid in early detection and personalized therapeutic strategies, improving patient outcomes.

Conclusion

Through Next-Generation Sequencing, we identified seven heterozygous mutations in the XRCC2 gene, with one being likely pathogenic, one likely benign, and five of uncertain significance. The potential impact of these mutations on protein function varies, with some showing a possible link to cancer predisposition. These findings highlight the XRCC2 gene's role in the DNA repair process and underscore the importance of including it in genetic screening for breast cancer, which could lead to enhanced early detection and more individualized treatments for patients at risk. Further research is needed to clarify the clinical significance of the uncertain variants and to determine their precise role in breast cancer etiology.

Recommendations

Future studies should look at increasing the size of the cohort so that the guided XRCC2 mutations can be clinically validated. Moreover, they should be explored as potential biomarkers for both the BC risk and prognosis. Presence or absence of these mutation may be asymmetrical, which may give rise to health conditions and that needs to be examined further. Development and implementation of the XRCC2 genetic screening could be thought of in the high-risk populations; it should be combined with the creation of individual treatment regimens depending on the status of this gene. Subsequently, research is needed to investigate the role of XRCC2 in therapy resistance and as a key point for new therapeutic methods.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral/poster presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

* Our sincere gratitude goes out to everyone who contributed their time, effort, and expertise to make this study a success.

References

Alhmoud, J. F., Woolley, J. F., Al Moustafa, A. E., & Malki, M. I. (2020). DNA damage/repair management in cancers. *Cancers (Basel), 12*(4), 1050.

Arnold, M., Morgan, E., Rumgay, H., Mafra, A., Singh, D., Laversanne, M., Vignat, J., Gralow, J. R., Cardoso, F., Siesling, S., & Soerjomataram, I. (2022). Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast, 66*(1), 15-23.

Berzsenyi, I., Pantazi, V., Borsos, B. N., & Pankotai, T. (2021). Systematic overview on the most widespread techniques for inducing and visualizing the DNA double-strand breaks. *Mutation Research/Reviews in Mutation Research, 788*,108397.

Dastgheib, S. A., Sayad, S., Azizi, S., Hajizadeh, N., Asadian, F., Karimi-Zarchi, M., Barahman, M., Shiri, A., Manzourolhojeh, M., Aghili, K., & Neamatzadeh, H. (2024). Association between XRCC2 Arg188His polymorphism and breast cancer susceptibility: a systematic review and meta-analysis. *Asian Pac J Cancer Prev., 25*(1), 43-55

Decker, B., Allen, J., Luccarini, C., Pooley, K. A., Shah, M., Bolla, M. K., Wang, Q., Ahmed, S., Baynes, C., Conroy, D. M., Brown, J., Luben, R., Ostrander, E. A., Pharoah, P. D., Dunning, A. M., & Easton, D. F. (2017). Rare, protein-truncating variants in ATM, CHEK2 and PALB2, but not XRCC2, are associated with increased breast cancer risks. *Journal of Med Genetics, 54*(11), 732-741.

Hilbers, F. S., Wijnen, J. T., Hoogerbrugge, N., Oosterwijk, J. C., Collee, M. J., Peterlongo, P., Radice, P., Manoukian, S., Feroce, I., Capra, F., Couch, F. J., Wang, X., Guidugli, L., Offit, K., Shah, S., Campbell, I. G., Thompson, E. R., James, P. A., Trainer, A. H., Gracia, J., Benitez, J., Van Asperen, C. J., & Devilee, P. (2012). Rare variants in XRCC2 as breast cancer susceptibility alleles. *Journal of Med Genetics, 49*(10), 618-20.

Hilbers, F. S., Luijsterburg, M. S., Wiegant, W. W., Meijers, C. M., Volker-Albert, M., Boonen, R. A., Van Asperen, C. J., Devilee, P., & Van Attikum, H. (2016). Functional analysis of missense variants in the Putative Breast Cancer Susceptibility Gene XRCC2. *Hum Mutat, 37*(9), 914-25

Ivy, S. C., Shabnaz, S., Shahriar, M., Jafrin, S., Aka, T. D., Aziz, M. A., & Islam, M. S. (2021). Association of RAD51 and XRCC2 gene polymorphisms with cervical cancer risk in the Bangladeshi women. *Asian Pac J Cancer Prev., 22*(7)**,** 2099-2107. putative breast cancer susceptibility gene XRCC2. *Hum Mutat., 37*(9)**,** 914-25.

Kluźniak, W., Wokołorczyk, D., Rusak, B., Huzarski, T., Gronwald, J., Stempa, K., Rudnicka, H., Kashyap, A., Dębniak, T., Jakubowska, A., Lener, M., Szwiec, M., Tomiczek-Szwiec, J., Jarkiewicz-Tretyn, J., Cechowska, M., Domagała, P., Szymiczek, A., Bagherzadeh, M., Lubiński, J., Narod, S. A., Akbari, M.

R., & Cybulski, C. (2019). Inherited variants in XRCC2 and the risk of breast cancer. *Breast Cancer Research and Treatment, 178*(3), 657-663

Liu, Z., Zhang, W., Cheng, X., Wang, H., Bian, L., Wang, J., Han, Z., Wang, Y., Lian, X., Liu, B., Ren, Z., Zhang, B., Jiang, Z., Lin, Z., & Gao, Y. (2021). Overexpressed XRCC2 as an independent risk factor for poor prognosis in *glioma* patients. *Molecular Medicine, 27*(1)**,** 52.

Liu, Q., Peng, Q., Zhang, B., & Tan, Y. (2023). X-ray cross-complementing family: the bridge linking DNA damage repair and cancer. *Journal of Translational Medicine, 21*(1)**,** 602.

Lu, C., Xie, M., Wendl, M. C., Wang, J., Mclellan, M. D., Leiserson, M. D., Huang, K. L., Wyczalkowski, M. A., Jayasinghe, R., Banerjee, T., Ning, J., Tripathi, P., Zhang, Q., Niu, B., Ye, K., Schmidt, H. K., Fulton, R. S., Mcmichael, J. F., Batra, P., Kandoth, C., Bharadwaj, M., Koboldt, D. C., Miller, C. A., Kanchi, K. L., Eldred, J. M., Larson, D. E., Welch, J. S., You, M., Ozenberger, B. A., Govindan, R., Walter, M. J., Ellis, M. J., Mardis, E. R., Graubert, T. A., Dipersio, J. F., Ley, T. J., Wilson, R. K., Goodfellow, P. J., Raphael, B. J., Chen, F., Johnson, K. J., Parvin, J. D. & Ding, L. (2015). Patterns and functional implications of rare germline variants across 12 cancer types. *Nature Communications, 6*, 10086

Obeagu, E. I., & Obeagu, G. U. (2024). Breast cancer: A review of risk factors and diagnosis. *Medicine (Baltimore), 103*(3), e36905.Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R. P., Banday, S., Mishra, A. K., Das, G. & Malonia, S. K. (2023). Next-generation sequencing technology: Current trends and advancements. *Biology (Basel), 12*(7), 997.

Park, D. J., Lesueur, F., Nguyen-Dumont, T., Pertesi, M., Odefrey, F., Hammet, F., Neuhausen, S. L., John, E. M., Andrulis, I. L., Terry, M. B., Daly, M., Buys, S., Le Calvez-Kelm, F., Lonie, A., Pope, B. J., Tsimiklis, H., Voegele, C., Hilbers, F. M., Hoogerbrugge, N., Barroso, A., Osorio, A., Giles, G. G., Devilee, P., Benitez, J., Hopper, J. L., Tavtigian, S. V., Goldgar, D. E., & Southey, M. C. (2012). Rare mutations in XRCC2 increase the risk of breast cancer. *American Journal of Human Genetics, 90*(4), 734-739.

Qin, D. (2019). Next-generation sequencing and its clinical application. *Cancer Biol Med., 16*(1), 4-10.

Wang, Y., Xiao, R., Wang, H., Cheng, Z., Li, W., Zhu, G., Wang, Y., & Ma, H. (2014). The Arabidopsis RAD51 paralogs RAD51B, RAD51D and XRCC2 play partially redundant roles in somatic DNA repair and gene regulation. *New Phytologist, 201*(1), 292-304.

Yu, J., & Wang, C. G. (2023). Relationship between polymorphisms in homologous recombination repair genes RAD51 G172T、XRCC2 & XRCC3 and risk of breast cancer: A meta-analysis. *Front Oncology, 13*, 1047336.

Author Information

Naser Gilani
Department of Biology, Gaziantep University
Gaziantep, Turkiye

Mehmet Ozaslan
Department of Biology, Gaziantep University
Gaziantep, Turkiye
ozaslanmd@gantep.edu.tr

To cite this article:

Gilani, G., & Ozaslan, M. (2024). XRCC2 gene study by next generation sequencing and establishing its relation with breast cancer. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 76-81.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 82-87

ICGeHeS 2024: International Conference on General Health Sciences

SLC5A6 Gene Mutations Associated with Developmental Delay in a Child: Case Report

Victoria Kononets
West Kazakhstan Marat Ospanov Medical University

Gulmira Zharmakhanova
West Kazakhstan Marat Ospanov Medical University

Lyazzat Syrlybayeva
West Kazakhstan Marat Ospanov Medical University

Eleonora Nurbaulina
West Kazakhstan Marat Ospanov Medical University

Abstract: Sodium-dependent multivitamin transporter (SMVT) deficiency is a recently described multivitamin-dependent inherited metabolic disorder (IMD). SMVT is encoded by the SLC5A6 gene located on chromosome 2p23.3. We describe a clinical case of SMVT deficiency in a child with developmental delay, microcephaly, persistent neurological symptoms, skin lesions and frequent upper respiratory tract infections. A tandem mass spectrometry study of the metabolic profile of amino acids and acylcarnitines, carried out twice, showed increased blood levels of methylmalonylcarnitine + 3-hydroxyisovalerylcarnitine (C4DC/C5OH). Urine analysis by gas chromatography-mass spectrometry showed persistently increased excretion of 3-OH-isovaleric acid. The combination of increased blood concentrations of C4DC/C5OH and increased urinary excretion of 3-OH-isovaleric acid suggested a metabolic disorder associated with impaired biotin metabolism. Determination of the level of biotinidase in the blood did not reveal deviations from the reference values. Biotin was prescribed. As a result of clinical exome sequencing, complex heterozygous variants of the SLC5A6 gene encoding SMVT were identified. The patient was treated with increased doses of oral biotin, pantothenic acid, and lipoic acid, which resulted in significant clinical improvement.

Keywords: SLC5A6, SMVT, Biotin, Pantothenic acid, Lipoic acid

Introduction

The sodium-dependent multivitamin transporter, which facilitates the absorption of the water-soluble vitamins biotin, pantothenic acid, and the vitamin-like substance lipoate, is encoded by the SLC5A6 gene. Mutations in this gene cause an extremely rare and poorly understood metabolic disorder (Schwantje et al., 2019; Hauth et al., 2022). The Na(+)/multivitamin transporter (SMVT) is a member of the sodium solute symporter family that catalyzes the Na(+)-dependent absorption of the structurally diverse water-soluble vitamins, pantothenic acid (vitamin B5) and biotin (vitamin H).), α-lipoic acid - a vitamin-like substance with strong antioxidant properties and iodide (Quick & Shi, 2015).

The transporter-mediated transport process is activated by the transmembrane sodium ion gradient as well as by the membrane potential. The transporter belongs to the sodium-coupled glucose transporter family (Figure 1). The ubiquitous expression of this transporter in mammalian tissues and the fact that it is highly conserved across species indicate the significance and importance of this transporter (Prasad & Ganapathy, 2000).

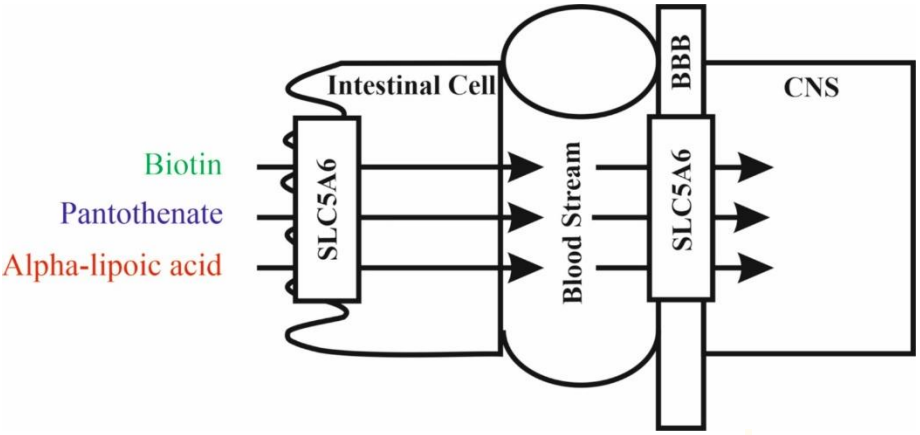


Figure 1. SCL5A6 function. *BBB blood–brain barrier.*

SMVT has been found in many types of tissues, including liver, kidney, and placenta tissues (Quick & Shi, 2015). Organic SMVT substrates play a central role in cellular metabolism and are therefore essential for normal human health and development. SMVT is the only biotin transport system active in the intestine, and its knockout results in biotin deficiency (Ghosalet et al., 2013; Sabui et al., 2018).

Biotin (vitamin B7) serves as an activating cofactor for five carboxylases involved in various metabolic reactions, including fatty acid synthesis, gluconeogenesis, and amino acid catabolism (Figure 2) (Baumgartner & Suormala, 2016). Biotin deficiency leads to stunting, dermatological disorders and neurological disorders (Quick & Shi, 2015; de Carvalho & Quick, 2011; Holling et al., 2022).

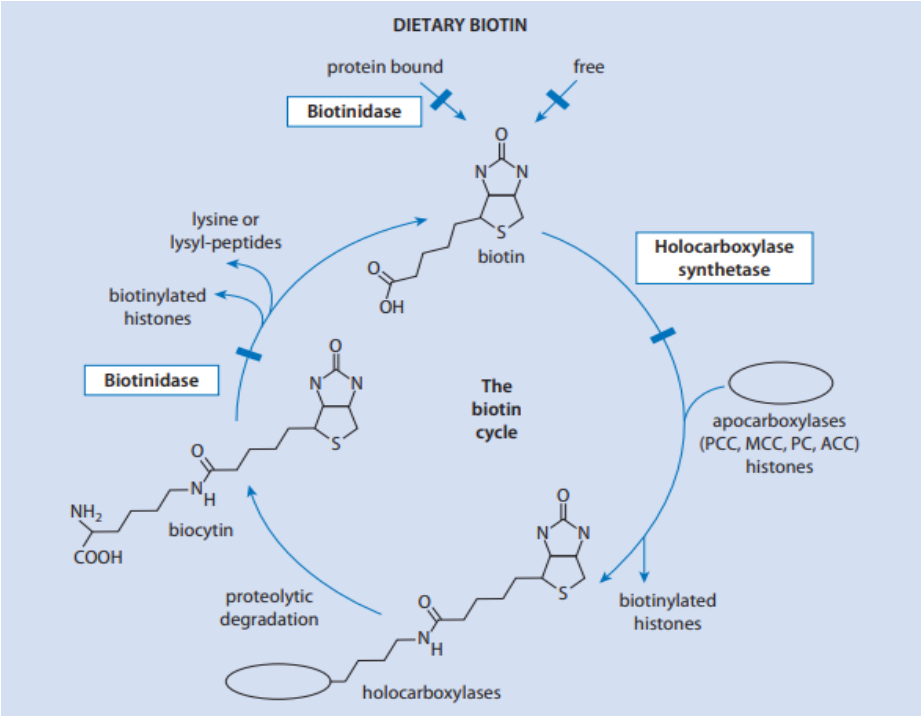


Figure 2. The biotin cycle. Sites of the enzyme and transport defects are indicated by solid bars. *ACC, acetyl-CoA carboxylases (ACC-1, cytosolic; ACC-2, outer mitochondrial membrane); MCC, 3-methylcrotonyl-CoA carboxylase; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase.* (Baumgartner & Suormala, 2016).

Pantothenic acid is a precursor of coenzyme A, therefore, a deficiency of pantothenic acid can lead to limited availability of coenzyme A. Coenzyme A acts as a cofactor of enzymes involved in the tricarboxylic acid cycle and fatty acid metabolism (Miallot et al., 2023). Lipoate is one of the cofactors in the glycine cleavage system and pyruvate dehydrogenase, branched chain ketoacid dehydrogenase and ketoglutarate dehydrogenase complexes. These enzymes catalyze redox reactions in the production of mitochondrial energy and provide oxidative decarboxylation reactions of amino acids and ketoacids (Van Hove, Hennerman & Coughlin, 2016). In addition to its function as a cofactor, lipoate may also have antioxidant and anti-inflammatory effects. In

addition to facilitating the absorption of biotin, pantothenic acid and lipoate, SMVT is capable of transporting iodide and therefore possibly also influences iodide homeostasis in the body (de Carvalho & Quick, 2011) (Figure 3).

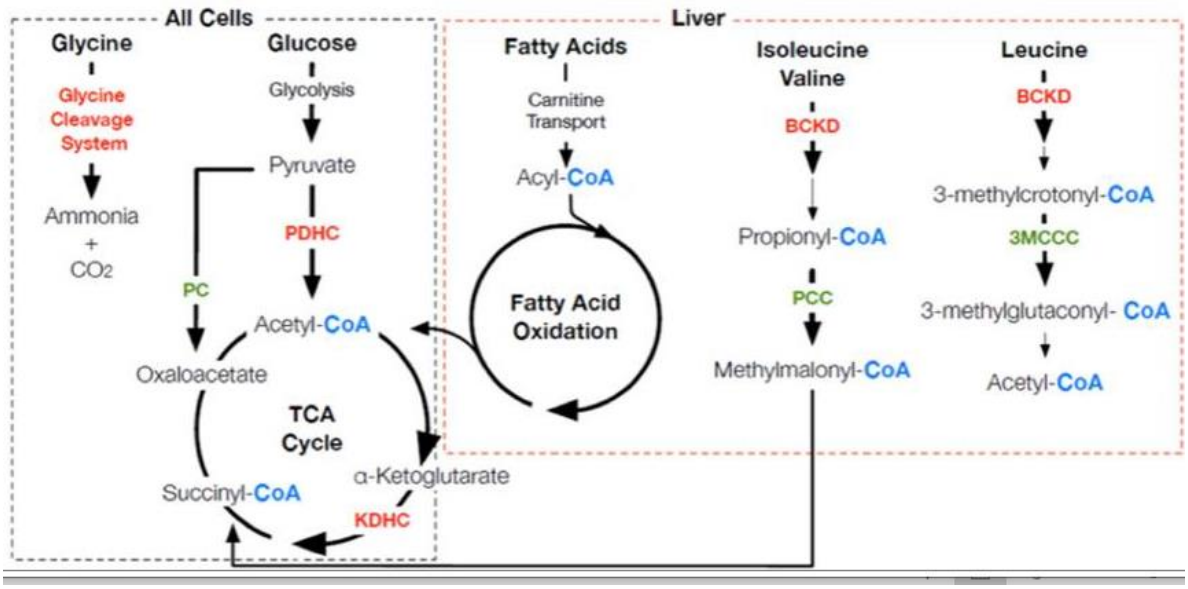


Figure 3. Biotin, pantothenate and lipoate-dependent metabolic pathways and the effect of identified variants in SLC5A6. *Enzymes for which the vitamins Biotin (green), Pantothenate (blue) and Alpha-lipoic acid (red) play a role as important cofactors in: the degradation pathways of the amino acids leucine, isoleucine, valine and glycine; glucose energy metabolism; the TCA cycle; and fatty acid oxidation metabolism. BCKD Branch chain ketoacid dehydrogenase, CNS central nervous system, KDHC ketoglutarate dehydrogenase complex, PC pyruvate carboxylase, PCC propionyl-CoA carboxylase, PDHC pyruvate dehdrogenase complex, 3MCCC 3-methyl crotonyl-CoA carboxylase* (Byrne et al., 2019).

The gene encoding SMVT, SLC5A6, is located on chromosome 2. Variants leading to disruption of SMVT protein function may result in intracellular deficiency of its organic substrates, as it is the only known combined transporter of biotin, pantothenic acid and lipoates (Quick & Shi, 2015). Given their metabolic functions, intracellular deficiencies of biotin, pantothenic acid and lipoate can cause a wide range of signs and symptoms.

Holling et al. (2022) reported complex heterozygous variants of SLC5A6 in people with various multisystem diseases, including developmental delay, developmental delay, seizures, cerebral palsy, brain atrophy, gastrointestinal problems, immunodeficiency, and/or osteopenia. Holling et al. (2022) identified the homozygous variant c.1285 A > G [p.(Ser429Gly)] in three affected siblings and a simplex patient, as well as the maternally inherited variant c.280 C > T [p.(Arg94*)] and inherited paternal line option c.485 option A > G [p.(Tyr162Cys)] in a simplex patient of the third family. Subramanian et al. (2017) reported the identification of mutations R94X (premature termination) and R123L (dysfunctional amino acid change) in exon 3 of the SLC5A6 gene in a child using a genome-wide scan. Variants of the SLC5A6 gene can lead to metabolic disorders that mimic biotinidase deficiency, which can be treated with supplements of the vitamins biotin and pantothenic acid (Schwantje et al., 2019; Sabui et al., 2018).

Case report

The patient, a 2-year-old girl, is the first child of healthy, unrelated parents. She was born full term, at 39 weeks, after an uncomplicated pregnancy. The Apgar score was 7 at 1 minute and 8 at 5 minutes. Her weight at birth was 3.4 kg, height 54 cm. She screamed immediately after birth and attached to the breast on the 1st day. The breast sucked actively. There were no dysmorphic features at birth. The child's nutrition throughout the year is breastfeeding.

Growth and development were normal during the neonatal period. However, in the first year of the child's life, frequent colds with symptoms of respiratory failure and delayed physical, motor and psycho-speech development were noted. Also, during the first year of life, low hemoglobin levels, lesions of the facial skin, conjunctiva, cheilitis, cracks in the corners of the mouth, and hair loss were noted. She was repeatedly

hospitalized in infectious diseases and multidisciplinary hospitals. She suffered from bronchitis, pneumonia and was admitted to the intensive care unit several times with signs of respiratory failure.

At the age of 12 months after the third hospitalization, she was examined by a hospital neurologist. Neurological status: Level of consciousness - clear. Round head, microcephaly. The palpebral fissures are symmetrical D=S, the pupils are narrow D=S. Full movement of the eyeballs. The pupils react to light. The face is symmetrical, the tongue is in the midline. Swallowing and phonation were preserved. Muscle tone is normal. Tendon reflexes: increased, expansion of reflexogenic zones. Babinski's sign is positive on both sides. Pathological foot signs are positive. Meningeal signs are negative. Psychomotor and physical development: holds his head from 3 months, turns from side to side from 4 months, sits from 6 months, does not crawl, does not walk. A CT scan of the brain at the age of 12 months revealed cysts of the septum pellucida, periventricular leukomalacia of both cerebral hemispheres, non-occlusive internal hydrocephalus, and signs of bilateral mesotympanitis.

The audiologist's examination revealed no pathology. An examination by an optometrist at the age of 20 months revealed bilateral optic nerve atrophy and keratopathy. Heredity, according to my mother, is burdened by the birth of children with Down syndrome from paternal and maternal great-aunts. Due to constant recurrent infectious diseases, inflammation around natural orifices, developmental delays, low weight gain, anemia (hemoglobin 79 g/l), microcephaly, and non-growing hair, a suspicion of hereditary metabolic diseases arose. The child was referred for consultation to a geneticist; if hereditary metabolic diseases were suspected, it was recommended to undergo examination using tandem mass spectrometry (MS/MS) and karyotyping. At the age of 20 months, she had developmental delay with inability to move independently and impaired intellectual development with severe speech delay. Karyotyping revealed a karyotype of 46 *XX;* no chromosomal pathology was detected.

The results of determining the amino acid and acylcarnitine profile by MS/MS at the age of 14 months revealed an increase in the blood concentration of methylmalonylcarnitine + 3-hydroxyisovalerylcarnitine (C4DC/C5OH) to 2.7 μmol/L (upper limit: 0.9 μmol/L). Urine analysis showed elevated excretion of 3-OH-isovaleric acid (604 mmol/mol creatinine; upper limit: 67 mmol/mol creatinine). A repeat MS/MS analysis performed at the age of 15 months also revealed an increase in methylmalonylcarnitine+3-hydroxyisovalerylcarnitine (C4DC/C5OH) in the blood to 3.22 μmol/L. The combination of increased blood concentrations of C4DC/C5OH and increased urinary excretion of 3-OH-isovaleric acid suggested a metabolic disorder associated with impaired biotin metabolism (beta ketothiolase deficiency, multiple carboxylase deficiency, and biotinidase deficiency). Determination of the level of biotinidase in the blood (5.80 nmol/min/ml) did not reveal deviations from the reference values (4.40 - 12.00 nmol/min/ml). However, clinical symptoms and biochemical analysis indicated biotin deficiency. Therefore, from December 2022, pathogenetic treatment was prescribed - taking biotin in a daily dose of 10 mg per day. While taking biotin, positive dynamics were noted - hair began to grow, dry skin disappeared, signs of severe conjunctivitis disappeared, cheilitis disappeared, the concentration of hemoglobin in the blood increased to 119 g/l.

The child's blood samples were sent for confirmatory molecular genetic analysis using Clinical exome sequencing (CES). DNA analysis was carried out using next-generation sequencing technology using the paired-end reading method. For sample preparation, a technique was used to selectively capture DNA sections related to the coding regions of genes with known clinical significance (clinical exome) or genes associated with a group of diseases and described in the curated OMIM database. The method allows you to identify inherited or newly emerged (de novo) variants of the nucleotide sequence (single-nucleotide substitutions, small insertions and deletions - up to 10 base pairs), which can cause a genetic disease. As a result of the sequencing of the clinical exome, complex heterozygous variants of the gene encoding SMVT were identified (Table 1).

Table 1. Variants of the SLC5A6 gene encoding SMVT and having significant signs of pathogenicity

Gene	Chromosome	Genome assembly GRCh 38 (hg38)	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	Genotype	ClinVar ID
SLC5A6	2	chr2:27207441 CG>C	c.209delC	p.Pro70fs 206	Deletion	Deletion	Heterozygous	ENST00000310 574
SLC5A6	2	chr2:27207618 AAG>A	c.31_32delCT	p.Leu11fs 196	Deletion	Deletion	Heterozygous	ENST00000310574

The SLC5A6 gene variants listed in Table 1 have been described as pathogenic, leading to frameshifts. These variants are not available in the EXAC, GNOMAD, and GENOMED population databases. The detected heterozygous variants in trans position are described as a possible cause of the disease.

Thus, mutations in the SLC5A6 gene, encoding SMVT, led to the formation of clinical signs of biotin deficiency, and the insufficient effectiveness of biotin therapy was explained by the involvement in the process, in addition to biotin, of pantothenic acid, lipoic acid and iodide. Based on this diagnosis, our patient was started on increased oral doses of biotin, pantothenic acid, and lipoic acid at dosage levels of 15, 300, and 200 mg per day, respectively. Doses were then increased at 24 months of age to 30 mg/day, 500 mg/day, and 300 mg/day, respectively. The child's motor skills have improved. Verbal skills have also improved when pronouncing a few simple words. Weight and height reached normal levels, manifestations of microcephaly became less pronounced. The condition of the skin and mucous membranes has improved significantly, hair growth has been restored.

The child's positive response to high pharmacological doses of these vitamins confirms that dysfunction of SMVT is the cause of the observed clinical picture. Uptake of biotin, pantothenic acid and lipoic acid into various cells at high pharmacological concentrations also occurs through simple diffusion and may, at least partially, reduce the need for a functional SMVT system (Subramanian et al., 2017; Holling et al., 2022), and triple vitamin replacement therapy, likely through a simple diffusion mechanism, had a beneficial effect in patients (Subramanian et al., 2017; Byrne et al., 2019; Schwantje et al., 2019; Holling et al., 2022).

Conclusion

Therefore, the general condition, growth, physical and mental development of the patient improved. A positive response to biotin, pantothenic acid and lipoic acid confirms the clinical and genetic diagnosis. The practical aspect of identifying mutations in the SLC5A6 gene encoding SMVT in our patient is to confirm the possibility of pharmacological correction of the deficiency of biotin, pantothenic acid and lipoates that occurs in this condition. However, it must be taken into account that treatment should be started as early as possible, before irreversible damage to the nervous system and brain occurs.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, grant No. AP14869996.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as a poster presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

References

Baumgartner, M.R., Suormala, T. (2016). Biotin-responsive disorders. In J. M. Saudubray, M. R.Baumgartner, J. Walter (Eds.). *Inborn metabolic diseases* (pp.375-383). Berlin: Springer.

Byrne, A. B., Arts, P., Polyak, S. W., Feng, J., Schreiber, A. W., Kassahn, K. S., Hahn, C. N., Mordaunt, D. A., Fletcher, J. M., Lipsett, J., Bratkovic, D., Booker, G. W., Smith, N. J., & Scott, H. S. (2019). Identification and targeted management of a neurodegenerative disorder caused by biallelic mutations in SLC5A6. *NPJ Genomic Medicine, 4*, 28.

de Carvalho, F. D., & Quick, M. (2011). Surprising substrate versatility in SLC5A6: Na+-coupled I- transport by the human Na+/multivitamin transporter (hSMVT). *The Journal of Biological Chemistry*, *286*(1), 131–137.

Ghosal, A., Lambrecht, N., Subramanya, S. B., Kapadia, R., & Said, H. M. (2013). Conditional knockout of the Slc5a6 gene in mouse intestine impairs biotin absorption. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *304*(1), G64–G71.

Hauth, I., Waterham, H. R., Wanders, R. J. A., van der Crabben, S. N., & van Karnebeek, C. D. M. (2022). A mild case of sodium-dependent multivitamin transporter (SMVT) deficiency illustrating the importance of treatment response in variant classification. *Cold Spring Harbor Molecular Case Studies*, *8*(2), a006185.

Holling, T., Nampoothiri, S., Tarhan, B., Schneeberger, P. E., Vinayan, K. P., Yesodharan, D., Roy, A. G., Radhakrishnan, P., Alawi, M., Rhodes, L., Girisha, K. M., Kang, P. B., & Kutsche, K. (2022). Novel biallelic variants expand the SLC5A6-related phenotypic spectrum. *European Journal of Human Genetics : EJHG*, *30*(4), 439–449.

Mayr, J. A., Feichtinger, R. G., Tort, F., Ribes, A., & Sperl, W. (2014). Lipoic acid biosynthesis defects. *Journal of Inherited Metabolic Disease*, *37*(4), 553–563.

Miallot, R., Millet, V., Galland, F., & Naquet, P. (2023). The vitamin B5/coenzyme A axis: A target for immunomodulation?. *European Journal of Immunology*, *53*(10), e2350435.

Prasad, P. D., & Ganapathy, V. (2000). Structure and function of mammalian sodium-dependent multivitamin transporter. *Current Opinion in Clinical Nutrition and Metabolic Care*, *3*(4), 263–266.

Quick, M., & Shi, L. (2015). The sodium/multivitamin transporter: a multipotent system with therapeutic implications. *Vitamins and Hormones*, *98*, 63–100.

Sabui, S., Kapadia, R., Ghosal, A., Schneider, M., Lambrecht, N. W. G., & Said, H. M. (2018). Biotin and pantothenic acid oversupplementation to conditional SLC5A6 KO mice prevents the development of intestinal mucosal abnormalities and growth defects. *American Journal of Physiology. Cell Physiology*, *315*(1), C73–C79.

Schwantje, M., de Sain-van der Velden, M., Jans, J., van Gassen, K., Dorrepaal, C., Koop, K., & Visser, G. (2019). Genetic defect of the sodium-dependent multivitamin transporter: A treatable disease, mimicking biotinidase deficiency. *JIMD Reports*, *48*(1), 11–14.

Subramanian, V. S., Constantinescu, A. R., Benke, P. J., & Said, H. M. (2017). Mutations in SLC5A6 associated with brain, immune, bone, and intestinal dysfunction in a young child. *Human Genetics*, *136*(2), 253–261.

Van Hove, J.L.K., Hennerman, J.B., & Coughlin, IIC.R. (2016). Nonketotic hyperglycinemia (glycine encephalopathy) and lipoate deficiency disorders. In J. M. Saudubray, M. R. Baumgartner, J. Walter (Eds*.). Inborn metabolic diseases* (pp.349-356). Berlin: Springer.

Zehnpfennig, B., Wiriyasermkul, P., Carlson, D. A., & Quick, M. (2015). Interaction of α-lipoic acid with the human Na+/multivitamin transporter (hSMVT). *The Journal of Biological Chemistry*, *290*(26), 16372–16382.

Author Information

Victoria Kononets
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan
Contact e-mail: *micropaleontolog@yandex.kz*

Gulmira Zharmakhanova
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

Lyazzat Syrlybayeva
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

Eleonora Nurbaulina
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

To cite this article:

Kononets, V., Zharmakhanova, G., Syrlybayeva, L., & Nurbaulina, E. (2024). SLC5A6 gene mutations associated with developmental delay in a child: Case report. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13,* 82-87.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 88-97

ICGeHeS 2024: International Conference on General Health Sciences

Building Public Awareness: Education and Campaigns to Prevent Stunting in the Next Generation

Debi S Fuadi
Universitas Pendidikan Indonesia

Achmad Hufad
Universitas Pendidikan Indonesia

Dwi Ismawati
Universitas Bengkulu

Amal Jaya
Universitas Pendidikan Indonesia

Andika Pratama
Universitas Pendidikan Indonesia

Haryanto Haryanto
Universitas Pendidikan Indonesia

Toni Hidayat
Universitas Pendidikan Indonesia

Abstract: This study aims to explore the effectiveness of education and campaign approaches in building public awareness about the importance of preventing stunting in future generations. Employing a qualitative descriptive study approach, the researcher engaged three informants with relevant experience and knowledge in the field of child health and stunting prevention. Through in-depth interviews and content analysis, this study describes the perceptions, understandings, and experiences of informants regarding the education and campaign efforts conducted to prevent stunting. The results reveal a variety of strategies employed, including direct counselling, provision of educational materials, social media campaigns, and community activities. Data analysis underscores challenges encountered in implementing the program, such as low levels of community participation, limited access to health services, and financial constraints. Nonetheless, the findings also identify opportunities to enhance the program's effectiveness, such as collaboration with community institutions and a culturally nuanced approach. This research offers valuable insights for the development of more effective strategies to cultivate community awareness about stunting and foster positive behavior change to prevent stunting in future generations. In conclusion, education and campaigns play a pivotal role in stunting prevention efforts, but sustained and coordinated endeavors are requisite to achieve substantial impact.

Keywords: Education and campaign approaches, Public awareness, Preventing stunting.

Introduction

The approach to addressing stunting in Indonesia involves a comprehensive strategy that includes targeted initiatives, capacity building, community engagement, education, policy interventions, and the involvement of

various stakeholders. The government has prioritized specific regions for combating stunting, as evidenced by the implementation of targeted initiatives in 160 priority districts for reducing stunting Rahmadhita (2020). These efforts include the formation of priority areas at the district and city levels to address the issue of stunting (Herbawani et al., 2022). Capacity building for community health workers, particularly those in integrated health posts (Posyandu), has been emphasized as a crucial component in detecting and preventing stunting (Megawati & Wiramihardja, 2019). The government's policies and interventions play a significant role in reducing the prevalence of stunting in Indonesia (Binuko, 2023). Education and community empowerment are essential components of stunting prevention. Community engagement and education programs aimed at increasing awareness of healthy living behaviors have been identified as crucial for preventing stunting (Sarifudin, 2023).

The implementation of programs such as the Infant and Child Feeding Program (PMBA) has been highlighted as a key strategy for accelerating the mitigation of stunting (Sari et al., 2022). Strengthening internal resources and data related to stunting is also crucial for the success of these programs (Sari et al., 2022). Moreover, the involvement of various stakeholders, including midwives, in specific interventions and the implementation of national and local programs, is essential for addressing stunting effectively (Aisyah & Suparni, 2022). The use of technology, such as web applications for classifying stunting cases, has been identified as a supportive tool for expediting the handling of stunting cases at the community level (Sholikhin & Atmojo, 2022). The government's role in socializing and implementing stunting prevention programs at the community level is crucial. The dissemination of information and socialization of stunting prevention in various communities, such as urban areas and fishing communities, is essential for addressing the issue comprehensively (Mahrus et al., 2022; Rasmaniar et al., 2022).

Additionally, the mapping of relative risk for stunting in different provinces provides valuable data for targeted interventions and resource allocation (Aswi et al., 2022). In conclusion, the handling of stunting in Indonesia involves a multi-faceted approach, including targeted initiatives, capacity building, community engagement, education, policy interventions, and the involvement of various stakeholders. These efforts are crucial for addressing the prevalence of stunting and mitigating its impact on child health and development in Indonesia.

The methods for addressing stunting in Indonesia encompass a range of interventions and strategies aimed at prevention, detection, and mitigation of stunting cases. These methods include targeted initiatives, community empowerment, policy interventions, capacity building, and the involvement of various stakeholders. The government has prioritized specific regions for combating stunting, indicating a targeted approach to addressing the issue Herbawani et al. (2022). Additionally, the implementation of the Village Fund (Dana Desa) has been identified as an intervention to support the reduction of stunting in specific areas (Raikhani et al., 2022).

Furthermore, the utilization of the Maternal and Child Health Book (KIA) as a tool for early detection of stunting has been highlighted as a method for independent stunting detection (Ambarwati et al., 2022). Water and sanitation have been recognized as factors associated with stunting in children, emphasizing the importance of environmental health interventions in addressing stunting (Olo et al., 2020). Moreover, the role of community health workers, such as midwives, in stunting prevention has been emphasized, highlighting the significance of capacity building and the involvement of healthcare professionals in addressing stunting (Sholikhin & Atmojo, 2022). The use of web applications for classifying stunting cases has been identified as a supportive tool for expediting the handling of stunting cases at the community level (Sholikhin & Atmojo, 2022). Socialization across sectors and the involvement of various stakeholders have been recognized as crucial components of stunting prevention, indicating the importance of collaborative efforts and community engagement in addressing stunting (Khobibah et al., 2022).

Additionally, the implementation of convergence programs for stunting prevention has been highlighted as a strategy to address the multifaceted nature of stunting (Permanasari et al., 2020). The role of education and awareness programs, such as the Emo-Demo approach and the utilization of social media for health communication, has been identified as effective methods for increasing knowledge and understanding of stunting prevention among different segments of the population (Yustiyani & Nurmansyah, 2022; Prasanti & Indriani, 2022).

Furthermore, the use of local resources, such as marine food sources, has been recognized as a potential strategy for optimizing nutritional intake in vulnerable groups, indicating the importance of context-specific interventions in addressing stunting (Nirmala & Octavia, 2022). In conclusion, the methods for addressing stunting in Indonesia encompass a multifaceted approach, including targeted initiatives, community empowerment, policy interventions, capacity building, involvement of various stakeholders, environmental

health interventions, and context-specific strategies. These methods reflect the comprehensive efforts being made to combat stunting and mitigate its impact on child health and development in Indonesia.

This study aims to enhance community awareness through a stunting counseling program conducted in RW 07 Cibunut, Kebon Pisang, Sumur Bandung Sub-district, Bandung City. Stunting represents a significant public health concern, particularly in urban areas like Bandung. The outreach program, targeted at neighborhood communities, seeks to augment comprehension regarding the significance of balanced nutrition and healthy dietary practices in averting stunting among children. This research will assess the program's efficacy in achieving its objectives, alongside the factors influencing its success or failure. Consequently, the findings of this study are anticipated to furnish valuable insights for policymakers, healthcare professionals, and other stakeholders involved in stunting prevention and control at the community level. targeting stunting prevention programmes for children aged 0-5 years is a very appropriate step. This age is a critical period in a child's growth and development, where adequate nutrition is essential to prevent stunting. By focusing on this age group, the programme can provide appropriate and effective interventions to reduce the risk of stunting early on.

Method

The research design employed in this study is qualitative. Qualitative research is an investigative approach used to explore issues and develop central phenomena to examine in detail how facts and reality unfold. Researchers are actively involved and even establish relationships with participants [Creswell, 2015]. Additionally, the descriptive approach involves investigative methods aimed at providing a comprehensive summary of a phenomenon or problem. This approach focuses on capturing the richness and complexity of the subjects under study through detailed description and interpretation of collected data.

Qualitative descriptive approaches are highly valuable in exploring and understanding social and human phenomena, as well as providing insights into individual experiences, perceptions, and behaviours. In the context of educational research, qualitative descriptive approaches are utilised to gain in-depth understanding of various educational phenomena. In summary, the qualitative descriptive approach in educational research entails thorough and comprehensive exploration of educational phenomena, providing rich descriptions and interpretations of the subjects under study. The subjects in this research are posyandu cadres and parents.

Results and Discussion

Stunting presents a significant health development challenge in Indonesia. The RW 07 Cibunut area, Kebon Pisang Village, Sumur Bandung District, Bandung City, is not immune to this issue. Counselling emerges as a crucial strategy in tackling stunting at the community level. This research seeks to investigate the factors contributing to stunting and formulate appropriate counselling strategies to address this issue.

A stunting programme organised in RW 07 Kebon Pisang Cibunut Village, known as "Banting Pintu" (Help Stunting Innovation Posyandu), has successfully implemented compost planting media to grow vegetables and raise catfish. The harvest from this programme is done once a month, and the proceeds are distributed to families at risk of stunting. According to the head of the neighbourhood, there are around 20 individuals in RW 07 Cibunut who are at risk of stunting. Families at risk of stunting receive assistance in preparing healthy meals based on guidelines from the community health centre

The causes of stunting in a locality can portray a complex interplay of social, economic, and environmental factors impacting children's growth and development. One primary factor is the limited access to adequate nutrition. In regions with high poverty rates, families may encounter difficulties in fulfilling their children's nutritional requirements. Restricted access to nutritious food and quality healthcare services can result in sustained malnutrition, thereby impeding children's linear growth. Moreover, inadequate sanitation and hygiene practices can also contribute to stunting. The absence of proper sanitation facilities heightens the risk of infections and diseases that disrupt nutrient absorption in children's bodies. Social factors also wield significant influence; low levels of education, economic instability, and a lack of awareness regarding the importance of nutrition and health can exacerbate stunting issues. Hence, stunting intervention strategies in a locality must adopt a holistic approach and consider various factors affecting children's health conditions, including nutritional interventions, access to healthcare services, and enhancements in sanitation and community education.

The identified stunting risk factors in the research location mirror the social, economic, and environmental conditions shaping overall children's growth and development. Limited access to adequate nutrition, particularly in areas with high poverty rates, stands out as a major risk factor for stunting. Additionally, chronic infections such as diarrhea and respiratory infections, compounded by poor sanitation and hygiene practices, can also precipitate stunting by disrupting nutrient absorption in children's bodies. Low education and awareness levels regarding the significance of children's nutrition and health, coupled with family poverty and economic instability, contribute to heightened stunting risk in a locality. Unhealthy dietary patterns and exposure to environmental pollutants further exacerbate the risk. Therefore, in devising stunting prevention and intervention programmes, it is imperative to comprehensively consider these risk factors. Collaborative efforts involving the government, NGOs, health organizations, and civil society are pivotal in effectively and sustainably addressing the stunting issue.

Consequently, these programmes can concentrate on enhancing access to nutrition and healthcare services, heightening community awareness of children's nutrition and health, and implementing sanitation and environmental enhancements. Through this integrated and collaborative approach, it is envisaged that stunting incidence can be diminished and the health and quality of life of children in the area can be enhanced.

Strategy for Handling Stunting

The prevalence of stunting in Indonesia decreased from 24.4% in 2021 to 21.6% in 2022 (https://sehatnegeriku.kemkes.go.id). Based on this data, the decrease in stunting rates is attributed to the participation of both the government and the community in stunting control. Strategies involving direct counselling, provision of educational materials, social media campaigns, and community activities constitute a comprehensive approach to stunting management in a region. Below are detailed strategies that can be implemented:

Direct Counselling

Direct counselling has proven to be an effective strategy in providing information and personal support to individuals or families affected by stunting. Through this approach, the posyandu cadre team can provide in-depth knowledge about healthy eating patterns, the importance of balanced nutrition, and effective ways to overcome nutritional problems in children.The role of the posyandu cadre team is vital in this counselling process. They not only provide information, but also help motivate parents to be more aware of the negative impact of stunting on their children's future development. This counselling serves as a means to increase parents' awareness and knowledge of the importance of stunting prevention through a healthy diet and balanced nutrition.

In addition, direct counselling has also proven to be effective in increasing parents' participation in stunting intervention programs in the RW 07 Cibinut area. By providing comprehensive information and personalised support, parents become more motivated and eager to be actively involved in programmes designed to address stunting. Their awareness of the dangers of stunting and the importance of prevention has increased, making them more committed to ensuring their children receive adequate and balanced nutrition. The work of posyandu cadres does not stop there. They also ensure that families at high risk of stunting receive special assistance from the health centre. This assistance includes providing detailed information on balanced nutrition and how to apply it in their daily lives. With this assistance, families at risk can receive more intensive attention and support that is more specific to their needs. Overall, the direct counselling conducted by the posyandu cadre team in RW 07 Cibinut showed very positive results. This strategy not only increases parents' awareness and participation in stunting prevention efforts, but also ensures that at-risk families get the support and information they need. By continuing to optimise this approach, it is hoped that the stunting rate in the area can be significantly reduced, so that children can grow and develop healthily and optimally.

Provision of Educational Materials

The provision of educational materials such as brochures, leaflets, and pamphlets on stunting and balanced nutrition is a strategic step that can provide an easily accessible source of information for the RW 07 Cibinut community. These educational materials can be distributed in various locations frequented by the community, such as health centres, posyandu, and other public places. Thus, important information about stunting and

balanced nutrition can reach more people and increase public awareness of this issue. The content of the educational materials needs to be designed in a way that is easily understood by all.

The information conveyed can cover various important aspects of stunting, such as the main causes of stunting, the negative impact of stunting on children's growth and development, and concrete steps that parents and families can take to prevent and overcome stunting. With the provision and distribution of good educational materials, it is hoped that the community of RW 07 Cibinut can be more aware and informed about the importance of balanced nutrition and stunting prevention measures. This will ultimately contribute to reducing the stunting rate in the area and improving the quality of children's health.

Social Media Campaigns

Social media campaigns are an effective way to disseminate information about stunting to the wider community. Through social media platforms such as Facebook and Instagram, information about balanced nutrition, healthy eating, and the importance of stunting prevention can be communicated quickly and widely. By utilising the speed and wide reach of social media, these important messages can reach a wide range of people in a short period of time.Creative content such as videos, infographics and images can be used to attract attention and raise awareness about stunting. Videos depicting the impact of stunting and prevention measures can provide a deeper and more emotional understanding. Simple and informative infographics can help convey important data and facts in a way that is easy to understand. Interesting images and illustrations can also increase engagement and spread the message more effectively.

Social media plays an important role in building community awareness in RW 07 Cibinut. With this platform, information about posyandu activities, programmes to use the yard as a food source, and various other local initiatives can be posted and shared with the community. This not only helps increase community knowledge, but also allows them to see and understand the efforts being made to address stunting in their area.

By posting posyandu activities, the community can see first-hand the efforts made to monitor and improve children's health. Information on the utilisation of yard land as a source of healthy food can also inspire people to follow similar steps in their own homes. In addition, documentation of programmes and activities also helps stakeholders to monitor and support stunting prevention programmes in the area.

Overall, the social media campaign can strengthen the stunting response in RW 07 Cibinut in an efficient and effective way. With the right communication strategy, creative and informative content, and active community involvement, it is expected that awareness of the importance of balanced nutrition and stunting prevention can increase significantly. This will ultimately help reduce stunting rates and improve the quality of life of children in the area.

Community Activities

Community activities such as workshops, training sessions and group meetings are effective ways to build awareness and strengthen community knowledge about stunting. These activities can be facilitated by the health centre or conducted independently by the community itself, providing space for the community to actively participate in stunting prevention efforts.Regularly organised training sessions can help strengthen community knowledge and skills on child nutrition and health. These trainings can cover a range of topics, from the importance of exclusive breastfeeding, to healthy complementary feeding, to how to detect early signs of stunting. With ongoing training, communities can continue to update their knowledge and implement better practices in their daily lives. Overall, structured and sustainable community activities can have a significant impact in the effort to tackle stunting. By involving various elements of the community and utilising local resources, it is expected that awareness and knowledge about the importance of stunting prevention can increase. This will ultimately contribute to reducing stunting rates and improving the quality of life of children in RW 07 Cibinut.

Household Farming

In an effort to address stunting, a household farming programme conducted by posyandu cadres can be an effective and sustainable strategy. Through this programme, posyandu cadres cultivate vegetables and fish

farming. This activity aims to increase community access to nutritious food sources, which can help address the problem of families at risk of stunting.

Household farming provides multiple benefits. First, by growing vegetables such as kale, spinach, or long beans, residents can obtain a source of vitamins, minerals, and fibre that are important for the health of the body, especially in children who are growing. Secondly, raising catfish provides additional animal protein needed to fulfil daily nutritional needs. The harvest from this activity is then distributed evenly to families at risk of stunting in the neighbourhood.

In addition to providing nutritious food sources, the home farming programme also plays a role in nutrition and hygiene education. Posyandu cadres can provide information on the importance of a balanced diet and healthy nutrition to residents, as well as provide practical examples of preparing and cooking nutritious meals with ingredients from the home farm. In addition, this activity also encourages awareness of the importance of hygiene in managing household farms, including in the handling and storage of crops.

The integration of household agriculture in the stunting prevention programme not only provides a short-term solution in terms of meeting nutritional needs, but also supports the formation of sustainable healthy living habits in the community. With good collaboration between posyandu cadres, the community, and related parties, this programme can have a positive impact in reducing stunting rates and improving family health and welfare.

Nutrition Mentoring

Nutrition assistance is a key aspect of addressing stunting in the community. By providing assistance to families at risk of stunting in cooking food according to the nutritional standards recommended by the health centre, it can help increase awareness and knowledge about a healthy and nutritious diet. Through this assistance, families will learn how to select and process food ingredients that are rich in essential nutrients for children's growth and development. Nutrition assistance is provided by trained health workers, such as posyandu cadres or community health centre workers, who educate families directly. They teach proper cooking techniques, including how to combine different ingredients to create balanced meals that contain the necessary proteins, carbohydrates, fats, vitamins and minerals.

In addition, nutrition mentoring also involves a personalised and interactive approach, where health workers provide practical examples of cooking and serving healthy meals. They also provide information on appropriate portions and frequency of consumption of healthy foods according to the age and needs of family members.
Nutrition assistance not only provides direct benefits in improving the nutritional quality of families at risk of stunting, but also helps to create a supportive environment for behaviour change towards a healthier and more regular diet. Through this approach, it is hoped that positive changes in people's consumption patterns can ultimately help reduce the incidence of stunting and improve the overall health and quality of life of children and families.

Collaboration with various parties, including local governments, NGOs and universities, is a very important strategy in addressing stunting. Through this collaboration, various resources and expertise can be combined to design and implement effective and sustainable programmes to reduce stunting in an area.Local governments have a key role in providing policy support and resource allocation to support stunting reduction programmes. Collaboration with local government makes it possible to integrate stunting prevention efforts into broader community development and welfare programmes. Non-governmental organisations (NGOs) can also play a role in providing human resources and logistics to support programme implementation, as well as helping to advocate health and nutrition issues to communities and other stakeholders. Universities bring research and innovation to the development of approaches and interventions to address stunting. Collaboration with universities makes it possible to utilise the latest scientific knowledge in designing effective and scalable strategies.

By combining the above strategies, it is hoped that a supportive environment for stunting management can be created in a region. Direct counselling provides a personal approach to help individuals and families address nutritional issues directly. The provision of educational materials ensures that information about stunting can be accessed independently by the wider community. Social media campaigns leverage technology to disseminate information massively and rapidly to many people. Meanwhile, community activities build social support and collaboration among the community in stunting prevention and management efforts. With these integrated

strategies, it is hoped that stunting rates can be reduced and the health and quality of life of children in a region can be improved.

Raising public awareness about tackling stunting is a key step in preventing and addressing this issue. Several approaches have been identified to achieve this goal, firstly, through education and outreach at various strategic locations such as integrated health posts, community health centres, schools, and places of worship. Educational materials provided should include information about the causes, impacts, and methods of preventing and managing stunting. This approach may involve lectures, group discussions, and the provision of educational materials such as brochures, posters, and leaflets.

Furthermore, leveraging social media is one effective way to disseminate information about stunting to the wider community. Creative content such as short videos, infographics, and images can be used to attract attention and raise awareness about this issue. Through social media campaigns, information about stunting can be accessed quickly and easily by many people.

Collaboration with community leaders, religious leaders, and local figures is also a crucial strategy in building public awareness about stunting. They can serve as effective agents of change in conveying important messages about the importance of balanced nutrition and healthy eating habits in preventing stunting. Additionally, organizing community activities such as workshops, group meetings, or other social events can provide a platform for people to share experiences, ideas, and knowledge about preventing and addressing stunting. Through these activities, strong social support can be created in the efforts to tackle stunting.

Finally, establishing support groups for parents and families with children experiencing stunting can provide emotional support, information, and practical advice to members in addressing child nutrition issues. Through sharing experiences and knowledge, group members can support each other in efforts to improve the health of their children. By combining these various strategies, it is hoped that an environment supportive of raising awareness about stunting can be created, as well as increasing understanding about the importance of balanced nutrition and healthy eating habits in preventing stunting.

Discussion

Stunting is a pressing health issue in Indonesia, including in the RW 07 Cibunut area of Kebon Pisang Village, Sumur Bandung District, Bandung City. The complexity of factors contributing to stunting underscores the need for multifaceted approaches, with counselling emerging as a crucial strategy at the community level. Factors such as limited access to adequate nutrition, poor sanitation, low education levels, and economic instability exacerbate the risk of stunting. Thus, intervention strategies must encompass various aspects, including nutritional interventions, access to healthcare services, and community education.

The identified risk factors in the research location underscore the intricate interplay of social, economic, and environmental conditions in shaping children's growth and development. Addressing stunting requires a comprehensive approach that considers these factors. Collaborative efforts involving government, NGOs, health organizations, and civil society are essential for effective and sustainable stunting prevention and intervention programs. These programs should focus on improving access to nutrition and healthcare services, raising community awareness, and enhancing sanitation and environmental conditions.

In Indonesia, the prevalence of stunting has decreased from 24.4% in 2021 to 21.6% in 2022, attributed to collaborative efforts between the government and the community. Effective strategies for stunting management include direct counselling, provision of educational materials, social media campaigns, and community activities. Direct counselling offers personalized support and information to affected individuals and families, while educational materials ensure widespread access to information. Social media campaigns leverage technology for mass dissemination of information, and community activities foster collaboration and support among community members.

Combining these strategies creates a conducive environment for stunting management. Direct counselling provides personalized support, educational materials ensure widespread access to information, social media campaigns reach a broad audience, and community activities foster collaboration. By integrating these strategies, it is hoped that stunting rates can be further reduced, and the health and quality of life of children in the region can be improved. Ultimately, raising public awareness and implementing comprehensive strategies are crucial steps in addressing the challenge of stunting in Indonesia.

The strategic steps outlined in the field findings provide a comprehensive overview of the efforts needed to reduce stunting rates in Indonesia. Discussion and deliberation on these steps can lead to a deeper understanding of the challenges faced and the efforts required to effectively address the issue of stunting. First and foremost, community outreach and education are crucial steps in raising awareness about the importance of balanced nutrition and child healthcare.

Through the delivery of clear and easily understandable information, parents and the general public can comprehend the negative impact of stunting and the measures that can be taken to prevent it. However, outreach alone is insufficient without the support of a strong healthcare system. Strengthening the healthcare system, especially in rural and remote areas, is a crucial step. Ensuring accessibility and quality of comprehensive healthcare services, including monitoring children's growth and early stunting intervention, can help detect and address stunting issues early on before they worsen.

Additionally, supplementary feeding programs and educating parents about healthy eating patterns are efforts that must be supported. Providing access to nutritious supplementary foods for children at risk of stunting can help improve their nutritional status. Furthermore, improvements in sanitation and environmental health are also crucial in reducing the risk of infections and diseases that contribute to stunting.

Empowering women and families is crucial in ensuring that family nutrition and child health are prioritized. By empowering women in decision-making regarding family nutrition and providing them access to reproductive health education and services, an environment conducive to optimal growth and development of children can be created.

Furthermore, child-centric public policies need to be comprehensively implemented. This includes nutrition policies, child protection, and poverty alleviation measures. Full support from the government in allocating adequate budgets and monitoring the implementation of these policies will be key to successfully addressing stunting.

Finally, cross-sector collaboration is essential in reducing stunting rates. Only through synergy among the government, private sector, NGOs, international organizations, and civil society can efforts to reduce stunting rates become more effective and sustainable. Overall, these strategic steps indicate that addressing stunting requires an integrated and comprehensive approach from various sectors and levels. Only with strong collaboration and commitment from all parties can stunting rates in Indonesia continue to decline and the health and quality of life of children significantly improve.

Conclusion

In conclusion, addressing the issue of stunting in Indonesia requires a multifaceted and coordinated approach involving various stakeholders. Stunting poses a significant health challenge, not only in Indonesia but also in specific areas like the RW 07 Cibunut area of Kebon Pisang Village, Sumur Bandung District, Bandung City. Counselling emerges as a vital strategy at the community level, given the complex interplay of social, economic, and environmental factors contributing to stunting. Key factors such as limited access to adequate nutrition, poor sanitation, low education levels, and economic instability exacerbate the risk of stunting. Therefore, intervention strategies need to encompass nutritional interventions, improved access to healthcare services, and community education. Collaborative efforts involving government agencies, NGOs, health organizations, and civil society are crucial for implementing effective and sustainable stunting prevention and intervention programs.

Significant progress has been made, as evidenced by the decrease in stunting prevalence from 24.4% in 2021 to 21.6% in 2022. This decline can be attributed to the joint efforts of the government and the community. Effective strategies include direct counselling, provision of educational materials, social media campaigns, and community activities. These strategies aim to provide personalized support, disseminate information widely, and foster community collaboration. Overall, the strategic steps outlined in the data underscore the comprehensive nature of efforts needed to reduce stunting rates in Indonesia. By combining various approaches and fostering collaboration among stakeholders, it is hoped that stunting rates will continue to decrease, leading to improved health and quality of life for children across the country.

Acknowledgements

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

*The author would like to express their gratitude to the Education Fund Management Institute (LPDP) under the Ministry of Finance of the Republic of Indonesia for supporting my doctoral studies and the publication of this article.

References

Aisyah, R. D., & Suparni, S. (2022). Peran kolaboratif bidan dalam pencegahan stunting di era adaptasi baru. *Window of Health: Jurnal Kesehatan*, 642-652.

Ambarwati, D., Kusuma, I. R., Riani, E. N., & Safitri, M. D. (2022). Pemanfaatan buku kia sebagai sarana deteksi dini stunting secara mandiri. *Jurnal Berdaya Mandiri, 4*(1), 852-859.

Aswi, A., Sukarna, S., & Nurhilaliyah, N. (2022). Pemetaan risiko relatif kasus stunting di provinsi sulawesi selatan. *Sainsmat : Jurnal Ilmiah Ilmu Pengetahuan Alam*, *11*(1), 11.

Binuko, R. S. (2023). Hubungan antara jumlah kelahiran balita dengan jumlah balita stunting. *Jurnal Teras Kesehatan, 6*(1), 22-28.

Cresswell, J. (2015). *Planning, conducting, and evaluating quantitative and qualitative research.* Retrieved from http://repository.unmas.ac.id/medias/journal/EBK-00121.pdf

Herbawani, C. K., Karima, U. Q., Syah, M. N. H., Hidayati, A. N., & Aprianto, B. (2022). Analisis determinan kejadian stunting di wilayah kerja puskesmas cinangka, kota depok. *Ghidza: Jurnal Gizi Dan Kesehatan, 6(*1), 64-79..

Herbawani, C. K., Karima, U. Q., Syah, M. N. H., Hidayati, A. N., & Aprianto, B. (2022). Analisis determinan kejadian stunting di wilayah kerja puskesmas cinangka, kota depok. *Ghidza: Jurnal Gizi Dan Kesehatan, 6*(1), 64-79.

Khobibah, K., Nurhidayati, T., Ruspita, M., Sapartinah, T., Hidayat, W., & Fathoni, A. (2022). Pelatihan aplikasi ehdw bagi kader dalam program konvergensi percepatan dan pencegahan stunting. *Link, 18*(2), 119-125.

Megawati, G., & Wiramihardja, S. (2019). Peningkatan kapasitas kader posyandu dalam mendeteksi dan mencegah stunting. *Dharmakarya, 8*(3), 154.

Nirmala, I. R., & Octavia, L. (2022). Peran makanan laut sumber protein dan anak stunting di wilayah pesisir. *Jurnal Stunting Pesisir Dan Aplikasinya, 1*(2),

Olo, A., Mediani, H. S., & Rakhmawati, W. (2020). Hubungan faktor air dan sanitasi dengan kejadian stunting pada balita di Indonesia. *Jurnal Obsesi : Jurnal Pendidikan Anak Usia Dini, 5*(2), 1113-1126.

Permanasari, Y., Permana, M., Pambudi, J., Rosha, B. C., Susilawati, M., Rahajeng, E., … & Prasodjo, R. (2020). Tantangan implementasi konvergensi pada program pencegahan stunting di kabupaten prioritas. *Media Penelitian Dan Pengembangan Kesehatan, 30*(4),315-328.

Prasanti, D. and Indriani, S. S. (2022). Strategi komunikasi kesehatan pencegahan lonjakan kasus covid-19 dalam youtube kemenkes ri. *Jurnal Ilmu Komunikasi, 20*(3), 398.

Rahmadhita, K. (2020). Permasalahan stunting dan pencegahannya. *Jurnal Ilmiah Kesehatan Sandi Husada,* 11(1), 225-229..

Raikhani, A., Masluchah, L., Widati Fatmaningrum, W., & Utomo, B. (2022). Implementasi dana desa sebagai upaya intervensi penurunan stunting desa pandan wangi kecamatan diwek kabupaten jombang implementation of village fund as stunting reduction intervention in Pandan Wangi village, Diwek district, Jombang regency, Indonesia. *Media Gizi Indonesia (National Nutrition Journal).*, *17*(1), 250-256.

Rasmaniar, R., Nurlaela, E., Ahmad, A., & Nurbaya, N. (2022). Pendidikan teman sebaya melalui pemberdayaan kader posyandu remaja terhadap pengetahuan, sikap, dan perilaku remaja tentang manfaat gizi dalam pencegahan stunting: Penelitian kuasi eksperimen. *Health Information : Jurnal Penelitian, 14*(1), 76-88.

Sari, N. M. W., Fadhila, F. R., Karomah, U., Isaura, E. R., & Adi, A. C. (2022). Program dan intervensi pemberian makan bayi dan anak (pmba) dalam percepatan penanggulangan stunting. *Media Gizi Indonesia*, *17*(1), 22-30.

Sarifudin, B. A. (2023). Edukasi pencegahan stunting melalui pendidikan keluarga guna menciptakan generasi sehat dan cerdas. *Jurnal Pengabdian Masyarakat Indonesia*, *3*(1), 25-29.

Sholikhin, N. A. and Atmojo, S. (2022). Aplikasi web untuk klasifikasi stunting pada balita dengan menggunakan metode k-nearest neighbours (studi kasus posyandu jawu kidul). *Journal of System Engineering and Technological Innovation (JISTI), 1*(2), 44-47.

Sholikhin, N. A. and Atmojo, S. (2022). Aplikasi web untuk klasifikasi stunting pada balita dengan menggunakan metode k-nearest neighbours (studi kasus posyandu jawu kidul). *Journal of System Engineering and Technological Innovation (JISTI), 1*(2), 44-47.

Author Information

Debi S Fuadi
Universitas Pendidikan Indonesia
Indonesia
Contact e. mail: *debisfuadi@upi.edu*

Achmad Hufad
Universitas Pendidikan Indonesia
Indonesia

Dwi Ismawati
Universitas Bengkulu
Indonesia

Amal Jaya
Universitas Pendidikan Indonesia
Indonesia

Andika Pratama
Universitas Pendidikan Indonesia
Indonesia

Haryanto Haryanto
Universitas Pendidikan Indonesia
Indonesia

Toni Hidayat
Universitas Pendidikan Indonesia
Indonesia

To cite this article:

Fuadi, D. S., Hufad, A., Ismiwat, D., Jaya, A., Pratama, A., Haryanto, H. & Hidayat, T. (2024). Building public awareness: Education and campaigns to prevent stunting in the next generation. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13,* 88-97.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 98-102

ICGeHeS 2024: International Conference on General Health Sciences

Adiwiyata Certification: Introducing Sustainability through Green School and Education

Nur Hamidah
Education University of Indonesia

Topik Hidayat
Education University of Indonesia

Siti Sriyati
Education University of Indonesia

Utari Akhir Gusti
Education University of Indonesia

Abstract: Education for Sustainable Development (ESD) gives learners the knowledge, skills, values and behavior to face the global challenges. ESD offers countries to develop and expand educational activities that focus on sustainability issues. Green school is one of the initiatives that introduce global challenges to schools such as climate change, water, biodiversity, the oceans, sustainable urbanization, disaster risk reduction, and sustainable lifestyles through education. The research aims to describe the implementation of integrated green school in Bandung public school as an initiative to introduce sustainability through education. Descriptive qualitative method is used in this research. Observations and interviews were conducted to principal, teachers, students, and other parts of the school community. Literature study was also conducted to compare the implementation of Bandung green school to other schools in sustainability. The result showed that Bandung integrated green school has the whole-school approach to sustainability than non- green school. There are 20 programs implemented by Bandung green school by integrating them to the whole school community. Integrated green school initiative led the school to introduce sustainability through education.

Keywords: Adiwiyata certification, Sustainability, Green school, Education for sustainable development

Introduction

One of the serious challenges that society will face in the future is environmental problems. No longer within the scope of each country or region, environmental issues have become one of the main focuses of global problems and challenges (Dunlap & Jorgenson, 2012). Therefore, environmental education plays a very important role in sustainable development, preparing an environmentally conscious and healthy society for future generations. Furthermore, the aim of providing environmental education is to ensure that all individuals grow as environmentally literate people (Scannella & McCarthy, 2014). Aspects of environmental awareness that can be taught in educational institutions according to Radwan and Khalil (2021) include three main contexts, namely teaching/education principles, activity programs and school policies that are in line with the concept of sustainability or Education for Sustainable Development (ESD). One of the programs and policies promoted by Indonesia to introduce and integrate ESD is through

Adiwiyata Certification. The term "green school" or "adiwiyata" (for Indonesian schools) refers to an ideal school that provides students with access to varied knowledge as well as standards and ethics that serve as the

foundation of their life to achieve goals in sustainable development initiatives (Wardani, 2020; Fathurrahman et al., 2022).

The school in this study was also chosen because it promoted a green environment through a variety of sustainable initiatives. Green schools were also established as a formal educational institution's response to achieving sustainable development goals and encouraging society's transition to sustainability. As a result, students can engage the knowledge, skill and atituted of sustainability through school education. In Indonesia, the green school program is called Adiwiyata (Wardani, 2020; Fathurrahman et al., 2022). The subject school has been recognized as a national green school since 2014 (Pusat PGLHK, 2014). This paper will describe praktik program yang dilaksanakan oleh Adiwiyata Public high school in Bandung dan bagaimana hal tersebut berpengaruh terhadap pengetahuan, sikap dan keterampilan keberlanjutan siswa.

Method

Descriptive qualitative method is used in this research. Observations and interviews were conducted to principal, teachers, students, and other parts of the school community. Literature study was also conducted to compare the implementation of Bandung green school to other schools in sustainability.

Result and Discussion

Adiwiyata or green school certification create a sustainable learning environment. This participatory action involves teachers, students, corporations and the community around the school to get Adiwiyata certification. The participatory actions that introduce sustainability are plant cultivation, waste management, independent food production and awareness campaign. This is supported by the presence of green house, green space, waste banks, biodigesters, hydroponics and fish cultivation in drums.

The school invites residents' participation in collecting green waste from canteen to be processed by a biodigester; the gas produced can be used by all canteen sellers. Meanwhile, the liquid residue can be used for hydroponic fertilizer. Lastly, cultivating fish in drums can also be done to provide a source of protein. Above the drum, some hydroponics may be planted. All the food produced by school members can finally be shared to the member or sell it at an affordable price.

Figure 1. Biodigester from CSR (left), classified trash bin in waste bank (right)

Similar green school initiatives are undertaken in other countries with different names, such as the Eco-Schools International Program in Europe, the Australian Sustainable Schools Initiative, New Zealand's 'EnviroSchools,' Israel Green School Certification, Swedish Schools for Sustainable Development, and UK Sustainable Schools

(Goldman et al., 2018). Despite variations in program execution in each school and hosting country, these programs generally share a common goal. Green schools embody the ideal concept of a school providing access to diverse knowledge, norms, and ethics, forming the foundation of students' lives to achieve goals in sustainable development.

Figure 2. Hydroponic and fish cultivation

The subject school has been recognized as a national green school since 2014. Several initiated programs related to flowering plant diversity include plant procurement in green space, vertical gardens, hydroponics, and the establishment of a greenhouse. It is documented that there are 8 (eight) green spaces filled with diverse plants in the school. 78 diverse species of flowering plants present at the Public Green School Bandung, comprising 23 orders and 37 families.

Figure 3. Green space inside the school

The subject green school initiated the green school (Adiwiyata) program in 2014, leading to diversity, as not all schools can attain the Adiwiyata certification. A school can achieve the Adiwiyata certification by fulfilling four aspects: (1) environmentally oriented policies; (2) an environmentally based school curriculum; (3) participatory-based activities; and (4) environmentally friendly facility management (Diyan Nurvika Kusuma

Wardani, 2020; Fathurrahman et al., 2022). The subject green school, has commendably fulfilled all the mentioned aspects. Detail initiatives are presented in Table 1.

Table 1. Subject green school initiatives

No.	Initiative action	No.	Initiative action
1.	Creating 8 green space	11.	Socialization of zero waste (recycling)
2.	Creating healthy canteen	12.	Paper recycling
3.	Creating waste bank	13.	Identification and tagging plant species
4.	Creating green house	14.	Plant cultivation
5.	Creating biopori	15.	Trash festival
6.	Creating vertical garden	16.	Making compost
7.	Creating hydroponic	17.	Using biodigester
8.	Bringing tumbler and lunchbox	18.	Fish cultivation
9.	Creating gazebos for outside learning	19.	Creating Alga (eco- friendly) community
10.	Socialization of waste sorting	20.	Clean and sustain classroom

Conclusion

Green school is one of the initiatives that introduce global challenges to schools such as climate change, water, biodiversity, the oceans, sustainable urbanization, disaster risk reduction, and sustainable lifestyles through education. Green school in Bandung public school as an initiative to introduce sustainability through education has the whole-school approach to sustainability than non-green school. There are 20 programs implemented by Bandung green school by integrating them to the whole school community. Integrated green school initiative led the school to introduce sustainability through education.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgement

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

* Researchers express their deepest gratitude to Lembaga Pengelolaan Dana Pendidikan (LPDP)/Indonesia Endowment Fund for Education Agency for providing fund for this research. The subject green school in Bandung for providing research place and to the biology teacher: Dra. Nenden Komara for helping in this research.

References

Dunlap, R. E., & Jorgenson, A. K. (2012). *Environmental problems*. Wiley Online Library Fathurrahman,

Fathurrahman, F., Kumasalari, D., Susanto, H., Nurholipah, N., & Saliman, S. (2022). Implementasi pembentukan karakter peduli lingkungan melalui program adiwiyata. *Jurnal Pendidikan dan Konseling (JPDK), 4*(6), 13038-13044.

Goldman, D., Ayalon, O., Baum, D., & Weiss, B. (2018). Influence of 'green school certification' on students' environmental literacy and adoption of sustainable practice by schools. *Journal of Cleaner Production, 183*, 1300–1313

Pusat Pengembangan Generasi (2014). *Daftar sekolah adiwiyata mandiri dan nasional*. Retrieved from https://Pusatpglhk.Bp2sdm.Menlhk.Go.Id/Public_area/Daftar_san_sam.

Radwan, A. F., & Khalil, E. M. A. S. (2021). Knowledge, attitude and practice toward sustainability among university students in UAE. *International Journal of Sustainability in Higher Education, 22*(5), 964–981.

Scannella, A., & McCarthy, S. (2014). Teacher evaluation adversity or opportunity? *Principal Leadership, 14(*5), 52-55.

Wardani, D. N. K. (2020). Analisis Implementasi Program Adiwiyata dalam Membangun Karakter Peduli Lingkungan. *Southeast Asian Journal of Islamic Education Management*, *1*(1), 60-73.

Author Information

Nur Hamidah
Biology Education, Education University of Indonesia, Indonesia
Contact Email: izunhamidah@upi.edu

Topik Hidayat
Biology Education, Education University of Indonesia, Indonesia

Siti Sriyati
Biology Education, Education University of Indonesia, Indonesia

Utari Akhir Gusti
Biology Education, Education University of Indonesia, Indonesia

To cite this article:

Hamidah, N., Hidayat, T., Sriyati, S., & Gusti, U. A. (2024). Adiwiyata certification: Introducing sustainability through green school and education. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 98-102.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 103-110

ICGeHeS 2024: International Conference on General Health Sciences

Age-Specific Reference Values for Free Carnitine and Short Chain Acylcarnitines Content in Dried Blood Spots in Newborns in Western Kazakhstan: A Tandem Mass Spectrometry Measurement

Lyazzat Syrlybayeva
West Kazakhstan Marat Ospanov Medical University

Gulmira Zharmakhanova
West Kazakhstan Marat Ospanov Medical University

Victoria Kononets
West Kazakhstan Marat Ospanov Medical University

Zhanylsyn Gaisiyeva
West Kazakhstan Marat Ospanov Medical University

Abstract: Measuring the level of acylcarnitines and free carnitine in the blood is one of the stages of early diagnosis of inborn errors of metabolism, including disorders of fatty acid oxidation, and ensures timely initiation of therapeutic measures. Currently, tandem mass spectrometry (MS/MS) is successfully used for these purposes. Dried blood spot acylcarnitine reference values developed for neonates are critical for interpreting test results and diagnosing fatty acid metabolic disorders. Objectives: To establish reference values for the concentrations of free carnitine and short-chain acylcarnitines in samples of dried blood spots of newborns in Western Kazakhstan using LC-MS/MS technology (liquid chromatography-tandem mass spectrometry). Methods: The cross-sectional study included 250 healthy newborns from Western Kazakhstan aged 1-3 days, born at term and breastfed, 49.2% boys and 50.2% girls. To establish age reference values for C0 and short-chain acylcarnitines, newborns were divided into three groups (1) 1-day, (2) 2-day and (3) 3-day. Guthrie blood samples were collected on days 1–3 of life and quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Nonparametric statistical approaches were used to obtain percentile distributions for newborns ranging from 2.5 to 97.5. Results: A statistically significant difference was found in the mean levels of acetylcarnitine (C2), butyrylcarnitine (C4) and tiglylcarnitine (C5:1) in men and women. The highest values were determined in the female group. Age-related differences were observed in the concentration levels of malonylcarnitine (C3DC), butyrylcarnitine (C4), isovalerylcarnitine (C5) and glutarylcarnitine (C5DC). No significant correlations were found between the content of C0 and 10 short-chain acetylcarnitines in dried blood spots and the body weight of newborns. Conclusion: The present study established concentrations of acylcarnitines and free carnitine that can be used as reference standards in a newborn screening program for inherited metabolic diseases in Kazakhstan.

Keywords: Newborn screening, Acylcarnitines, Free carnitine, Dried blood spots, Tandem mass spectrometry

Introduction

Inborn errors of metabolism (IED) belong to the group of so-called rare or orphan diseases. Their total frequency is low, which makes these diseases little studied and creates difficulties in diagnosis and treatment (Céspedes et al., 2017; Sarker et al., 2019). A major problem with these diseases is delay in diagnosis or

misdiagnosis due to lack of specialized laboratories, resulting in delayed or missing treatment. Early diagnosis and adequate treatment allow patients to lead almost normal lives, reducing the consequences or at least significantly reducing organ damage (Scolamieroet al., 2015).

Tandem mass spectrometry (MS/MS) is a technology that allows the simultaneous detection and identification of multiple analytes with high sensitivity, precision and accuracy, with high specificity (Mak et al., 2013). In plasma and whole blood, total carnitine is present in the form of free ester and acylcarnitine ester. total carnitine is present as free and acylcarnitine ester forms (Vieira -Neto et al., 2012). Acylcarnitine esters with short and medium chain length are formed in peroxisomes, then they are oxidized in mitochondria.

Acylcarnitine profiling is a powerful tool for diagnosis and neonatal screening of disorders of fatty acid oxidation and organic acid metabolism (Vieira- Neto et al., 2012). Age reference cutoff ranges for each analyte should be established for each population prior to screening/diagnosis of patients (Sarker et al., 2019). Threshold values for free carnitine and acylcarnitine esters in DBS have been published in the evaluation of newborn screening programs (Vieira Neto et al., 2012; He et al., 2021; Al-Riyami et al., 2022). However, these cutoffs are often very high percentiles (eg, 99.5th or 99.98th), which do not necessarily indicate reference intervals that can be used for diagnosis in neonates with suspected IEM. Some studies have established reference intervals of 5.0 to 95.0 for acylcarnitines in dried blood spots (Cavedon et al., 2005).

Identification of normal levels of acylcarnitine concentrations is necessary to use them as a reference for establishing a metabolic screening program for newborns (Céspedes et al., 2017). Some researchers use umbilical cord blood to determine reference intervals for acylcarnitines and create metabolic profiles (Walter et al., 2009; Vieira Neto et al., 2012), however, most define reference intervals in blood collected during the first three days of life from the heel of a newborn. Naturally, there will be significant differences between the acylcarnitine profiles of umbilical blood and those of blood collected from neonates after birth from the heel due to adaptation from a continuous supply of glucose in utero to a neonatal diet based on breast milk and therefore fat as a source of nutrition (Walter et al., 2009; Vieira -Neto et al., 2012).

Currently in Kazakhstan there are no developed reference intervals for the concentrations of acylcarnitines in dried blood spots for different age groups of the child population, including newborns. We initiated selective screening to obtain data on the frequency of IEM in children at risk in Western Kazakhstan. From October 2022 to December 2024, the incidence of 37 inborn errors of metabolism including amino acid disorders (AAD), organic acidemias (OA) and fatty acid oxidation disorders (FAOD) was assessed using LC-MS/MS technology in a group of high-risk children. The results of selective screening tests in different age groups of children examined should be interpreted by comparison with reference values and/or threshold levels established for these groups.

Therefore, one of the objectives of this study is to establish reference intervals for the concentration of acylcarnitines in dried blood spots in newborn children of Western Kazakhstan. Due to the urgent need for a highly sensitive diagnostic method and effective screening for IEM in Western Kazakhstan, this study focused on establishing the concentration values of free carnitine and short chain acylcarnitines in dried blood spot samples of Western Kazakhstan newborns using MS/MS technology.

Study Objectives

To establish reference values for free carnitine(C0) and short chain acylcarnitines (ACs) concentrations in samples of DBS from newborns in Western Kazakhstan using LC-MS/MS (liquid chromatography-tandem mass spectrometry) technology.

Tasks

1. To set reference ranges of C0 and short chain ACs concentrations in samples of DBS of 250 newborns of Western Kazakhstan aged 1-3 days using LC-MS/MS technology.
2. To evaluate factors that may affect C0 and short chain ACs levels.
3. To compare findings of the determined analytes in newborns of Western Kazakhstan DBS with the results of previously published studies in other populations.

Methods

Data Sources

The data of this study were obtained during the examination of 250 healthy newborns aged 1-3 days to establish reference values of C0 and short chain ACs (Figure 1).

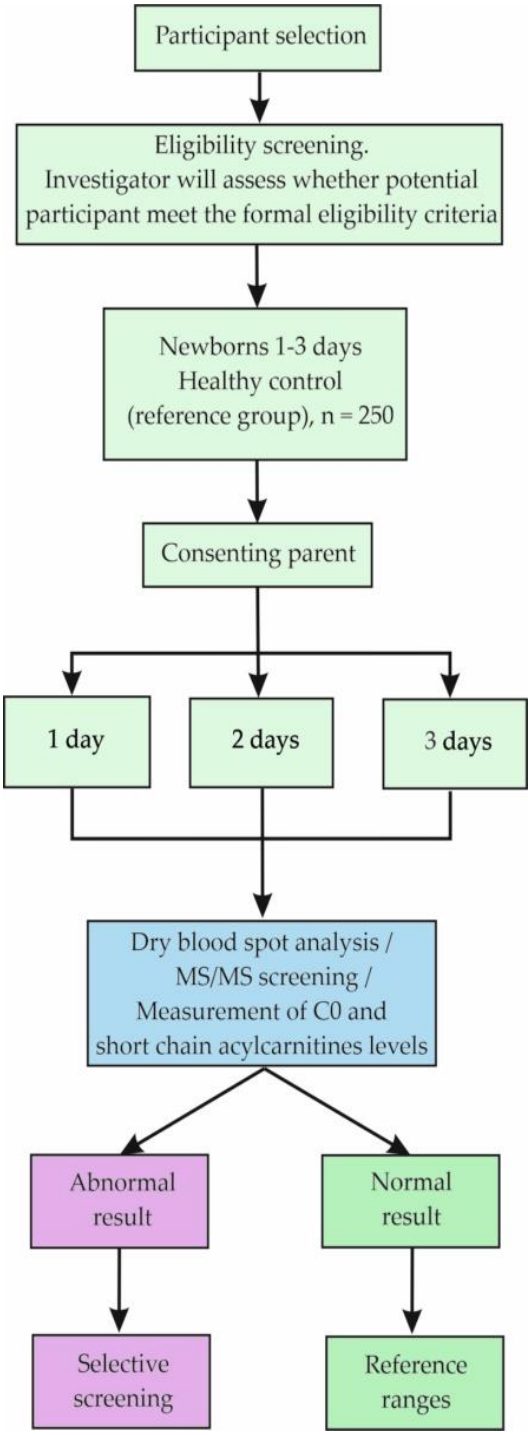


Figure 1. Study flowchart

The study was approved by the Bioethics Committee of the West Kazakhstan Marat Ospanov Medical University (Ref. No. 7, 09/09/2020.) Written informed consent (IS) was obtained from the parents and/or legal guardians of children after birth to collect a DBS sample. Demographic and anthropometric data of newborns are presented in Table 1.

Table 1. Demographic and anthropometric data of study participants

	Healthy newborns (n=250)			
	Group A 1 day (n=36)	Group B 2 days (n=116)	Group C 3 days (n=98)	The whole sample
Weight in grams, Median (IQR)	3434 (3180;3560)	3600 (3300;3833)	3615 (3470;3860)	3560 (3298;3830)
Gender				
Male, n, %	16 (44,4 %)	63 (54,3 %)	44 (44,9 %)	123 (49,2 %)
Female, n, %	20 (55,6 %)	53 (45,7 %)	54 (55,1 %)	127 (50,2 %)
Geographic distribution				
Urban population, n, %	22 (61,1 %)	68 (58,6 %)	54 (55,1 %)	144 (57,6 %)
Rural population, n, %	14 (38,9 %)	48 (41,4 %)	44 (44,9 %)	106 (42,4 %)

Criteria for Inclusion in the Study

Pediatricians examine all children in this study to ensure they do not suffer from any disorder or chronic disease. Healthy male and female newborns born after an uncomplicated pregnancy and vaginal delivery should have a body weight of 2500–4000 g, gestational age of 37–42 weeks, and an APGAR score greater than 7 in 10 minutes after birth. None should be diagnosed with birth asphyxia, defined as an Apgar score ≤6 at 5 min. All newborns must be breastfed, and their mothers must be healthy between 24 and 36. They must not have any food restrictions (vegetarian, vegan, etc.). Echograms of the placenta and fetus, as well as laboratory tests, should be normal throughout pregnancy.

Mass Spectrometry Analysis

Specimen Collection and Storage

Neonatal whole blood samples were collected from infants no earlier than 3 hours after feeding by heel prick using a heel stick. Five drops of whole blood (each ~75 µl) were applied to Guthrie cards, Ahlstrom 226 filter paper, and PerkinElmer 226 Five-Spot Card (PerkinElmer Health Sciences, Greenville, USA) to form dried blood spots (DBSs) for LC-MS/MS analysis. Samples were dried for 4 hours at room temperature and then stored at 4°C in labeled individual zip-lock plastic envelopes with desiccants until analyzed by LC-MS/MS. Samples were sent to the laboratory within five days. In the case of long-term storage of samples, it was carried out at a temperature of –20°C.

Specimen Preparation and LC-MS/MS Analysis

The Neobase2 TM Non-derivatized MSMS kit (PerkinElmer, Wallac Oy, Turku, Finland) will be used to quantify free carnitine, 10 short chain acylcarnitines in dried blood spots according to the manufacturer's instructions. Vial with lyophilized isotope-labeled internal standards (IS) containing 2H9-free carnitine (C0 IS), 2H3-Acetylcarnitine (C2 IS), 2H3-Propionylcarnitine (C3 IS), 2H3-C4-Malonylcarnitine+3-Hydroxybutyrylcarnitine (C3DC/C4OH IS), 2H3-Butyrylcarnitine (C4 IS), 2H9-C5-Methylmalonylcarnitine+3-Hydroxyisovalerylcarnitine (C4DC/C5OH IS), 2H9-Isovalerylcarnitine (C5 IS), 2H9-C5-Tiglylcarnitine (C5:1 IS), 2H3-Glutarylcarnitine (C5DC IS), was being recovered by adding 1.4 ml of the extraction solution that is included in the Neobase 2 kit. The Extraction Working Solution (EWS) IS was prepared by diluting the recovered internal standards with the extraction solution of 1:100 (v/v).

DBS were analyzed using a Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer (Shimadzu Corporation, Kyoto, Japan). Level I and Level II (low standard and high standard) dried blood drops were included with each assay lot of the Neobase2 TM Non-derivatized MSMS kit to monitor system accuracy and precision.

To analyze free carnitine and short chain acylcarnitines, stored DBS card samples are brought to room temperature (+18 to +25°C) before extraction. A 3.2 mm disc (equivalent to ~3.1 µl of whole blood) is punched out of one dried blood spot with a diameter of 3.2 mm using a Wallac DBS Puncher (PerkinElmer, Wallac Oy, Mustionkatu 6, FI-20750 Turku, Finland) into the well of the 96-well polystyrene U-bottom microplate supplied

with the Neobase2 TM Non-derivatized MSMS kit. After adding 125 μL of working extraction solution to each well of the microplate, the plate is covered with an adhesive aluminum film and incubated for 30 minutes at room temperature on a microplate shaker with a shaking speed of 650 rpm. After incubation, 100 μL of the supernatant is transferred to a new 96-well U-bottom microplate, covered with aluminum foil to reduce evaporation, and incubated for 1 hour. The plate is then placed into the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer autosampler, and 5 μL of supernatant is injected into the LCMS for analysis.

Metabolites to Measure

Free carnitine (C0), Acetylcarnitine (C2), Propionylcarnitine (C3), Malonylcarnitine+3-Hydroxybutyrylcarnitine (C3DC/C4OH), Butyrylcarnitine (C4), 2H9-C5-Methylmalonylcarnitine+3-Hydroxyisovalerylcarnitine (C4DC/C5OH), Isovalerylcarnitine (C5), Tiglylcarnitine (C5:1), Glutarylcarnitine (C5DC).

Statistical Analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to check the normality of the distribution. The data obtained in the study demonstrated that the distribution of free carnitine and short chain acylcarnitines in DBS differs from normal. Me (median) and quartiles (IQR interquartile range) were used for descriptive statistics of the samples. Nonparametric tests (Mann-Whitney U test, Kruskal-Wallis H test) were used to test differences in C0 and short chain ACs concentrations depending on various factors (gender, age, place of residence). Reference ranges in the group of healthy newborns aged 1-3 days were determined non-parametrically and corresponded to the 2.5-97.5th percentile of the experimental distribution. Considering the skewed distribution, correlations between body weight, age, and the concentration of C0 and short chain ACs in dry blood spots were performed using Spearman's test. Two-sided levels <0.05 are assumed to be statistically significant. Statistical analysis was done using the software IBM SPSS v. 23.0 (IBM, Armonk, NY, USA) and Statistica (StatSoft, Inc., Tulsa, OK, USA, v. 10).

Results and Discussion

Descriptive statistics and reference intervals for the concentrations of C0 and short chain ACs in whole blood of healthy newborns divided into subgroups according to age are presented in Table 2. For each analyte, the upper cut-off limit is set above the 97.5th percentile, while the lower limit is set below 2.5th percentile. Differences in the distribution of free carnitine and short chain acylcarnitines levels in DBS between groups of newborns aged 1, 2 and 3 days, determined using the Kruskal-Wallis test, are noted in Table 2. Statistically significant differences between age groups are noted in concentration malonylcarnitine (C3DC), butyrylcarnitine (C4), isovalerylcarnitine (C5) and glutarylcarnitine (C5DC) (Table 2). In addition, significant weak negative correlations with age were established for the concentrations in DBS of malonylcarnitine (C3DC), butyrylcarnitine (C4), isovalerylcarnitine (C5) and glutarylcarnitine (C5DC) (Table 3).

Significant differences between the groups of female and male newborns were established by the concentration of acetylcarnitine (C2), butyrylcarnitine (C4), and tiglylcarnitine (C5:1) in DBS (Table 3). In a study by Ruoppolo et al. (2015), who examined the metabolome of newborns, including blood acylcarnitine profiles, statistically significant differences were also found between male and female newborns in the level of free carnitine and short chain acylcarnitines in DBS.

Assessing the effect of newborn body weight on the level of free carnitine and short chain acylcarnitines, no significant correlations were found between the concentration of free carnitine and short chain acylcarnitines in dry blood spots and newborn body weight. Reference values for free carnitine and short chain acylcarnitines, like other blood metabolites, are highly dependent on various factors. such as genetic background, geographical location of the population, diet and age (Cavedon et al., 2005; Ruoppolo et al., 2015; Dogan et al., 2017; Sarker et al., 2019). We compared the results of measuring free carnitine and short chain acylcarnitines levels in DBS in newborns of western Kazakhstan with the results of previously published studies in other populations. A significant number of researchers confirm the relationship between gender and the level of certain acylcarnitines in DBS (Ruoppolo et al., 2015; He et al., 2021; Al-Riyami et al., 2022), but denies Céspedes et al. (2017).

The relationship between free carnitine and short chain acylcarnitines levels and birth weight was confirmed by Ruoppolo et al. (2015), Manta-Vogli et al. (2020), He et al. (2021), but is not confirmed by Céspedes et al. (2017).

Table 2. C0 and short chain ACs levels in dried blood spots of 250 healthy newborns aged 1-3 days in Western Kazakhstan

Amino acid, μmol/l		All children 1-3 days (n = 250)	Group A 1 day (n = 36)	Group B 2 days (n = 116)	Group C 3 days (n = 98)	Kruskal – Wallis *H* test	p-values
C0	Median	29.35	28.90	31.62	27.82	4,66	0.097
	Range	24.92;37.13	23.53;35.18	25.35;38.39	25.39;34.30		
	2.5th-97.5th	15.79-55.32	21.81-51.32	17.89-64.62	15.25-45.52		
C2	Median	33.54	31.99	33.89	33.54	0.917	0.623
	Range	26.44;40.38	23.90;38.78	26.38;42.34	27.41;39.49		
	2.5th-97.5th	13.20-61.58	19.68-49.75	10.53-68.94	15.87-56.46		
C3	Median	1.78	1.67	1.89	1.62	4.18	0.124
	Range	1.37;2.34	1.28;2.42	1.45;2.50	1.34;2.11		
	2.5th-97.5th	0.926-3.72	0.928-4.14	0.994-4.13	0.796-2.90		
C3DC	Median	0.157	0.184	0.158	0.148	8.31	0.016
	Range	0.130;0.185	0.138;0.206	0.131;0.187	0.124;0.167		
	2.5th-97.5th	0.080-0.255	0.082-0.271	0.071-0.246	0.084-0.255		
C4	Median	0.290	0.378	0.290	0.278	18.86	0.0001
	Range	0.244;0.364	0.285;0.454	0.240;0.369	0.231;0.330		
	2.5th-97.5th	0.160-0.607	0.191-0.690	0.146-0.771	0.160-0.467		
C4DC	Median	0.504	0.497	0.498	0.513	2.11	0.348
	Range	0.419;0.592	0.404;0.573	0.410;0.589	0.445;0.645		
	2.5th-97.5th	0.285-0.872	0.311-0.872	0.271-0.922	0.285-0.819		
C4OH	Median	0.147	0.154	0.142	0.149	1.89	0.388
	Range	0.115;0.175	0.122;0.178	0.106;0.171	0.115;0.177		
	2.5th-97.5th	0.066-0.283	0.075-0.327	0.047-0.286	0.066-0.253		
C5	Median	0.144	0.166	0.142	0.139	8.47	0.015
	Range	0.121;0.174	0.141;0.212	0.118;0.181	0.115;0.167		
	2.5th-97.5th	0.076-0.289	0.096-0.370	0.072-0.281	0.076-0.288		
C5:1	Median	0.060	0.059	0.063	0.059	5.80	0.055
	Range	0.046;0.069	0.044;0.073	0.048;0.076	0.043;0.067		
	2.5th-97.5th	0.021-0.093	0.005-0.080	0.024-0.131	0.026-0.082		
C5DC	Median	0.135	0.153	0.139	0.126	7.05	0.029
	Range	0.112;0.157	0.115;0.183	0.116;0.159	0.109;0.148		
	2.5th-97.5th	0.075-0.233	0.071-0.273	0.065-0.233	0.078-0.225		
C5OH	Median	0.240	0.249	0.273	0.249	4.50	0.105
	Range	0.220;0.278	0.228;0.278	0.214;0.274	0.223;0.282		
	2.5th-97.5th	0.170-0.349	0.164-0.336	0.163-0.333	0.197-0.352		

Table 3. Statistical analysis according to age (Spearman's correlation) and gender (Mann Whitney U test)

Analyte	Spearman correlation		Male N=123		Female N=127		p-values
	ρ	p-values	Median (μmol/L)	Range	Median (μmol/L)	Range	
C0	-0.096	0.065	30.35	17.36;59.01	28.09	15.58;50.68	0.061
C2	0.013	0.419	31.32	15.87;55.16	35.14	13.20;68.93	0.017
C3	-0.070	0.135	1.76	0.956;3.58	1.78	0.755;3.72	0.370
C3DC	-0.174	0.006	0.156	0.081;0.231	0.159	0.080;0.271	0.125
C4	-0.240	0.000	0.273	0.164;0.558	0.308	0.151;0.690	0.020
C4DC	0.089	0.163	0.502	0.285;0.883	0.512	0.290;0.872	0.751
C4OH	-0.006	0.927	0.142	0.065;0.249	0.151	0.071;0.283	0.460
C5	-0.157	0.013	0.135	0.083;0.274	0.148	0.073;0.289	0.395
C5:1	0.071	0.262	0.057	0.015;0.088	0.064	0.031;0.105	0.003
C5DC	-0.169	0.007	0.137	0.075;0.216	0.133	0.071;0.237	0.786
C5OH	0.072	0.260	0.243	0.170;0.352	0.293	0.180;0.336	0.829

Conclusion

The present study established age- and sex-specific concentrations of free carnitine and short-chain acylcarnitines that can be used as reference standards in a newborn screening program for inherited metabolic diseases in Kazakhstan.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, grant No. AP14869996.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as a poster presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

References

Al-Riyami, S., Al-Manei, M., Al-Fahdi, A., & Al-Thihli, K. (2022). Establishment and validation of reference values for amino acids and acylcarnitines in dried blood spots for Omani newborns using tandem mass spectrometry. *Oman Medical Journal*, *37*(5), e426.

Cavedon, C. T., Bourdoux, P., Mertens, K., Van Thi, H. V., Herremans, N., de Laet, C., & Goyens, P. (2005). Age-related variations in acylcarnitine and free carnitine concentrations measured by tandem mass spectrometry. *Clinical Chemistry*, *51*(4), 745–752

Céspedes, N., Valencia, A., Echeverry, C. A., Arce-Plata, M. I., Colón, C., Castiñeiras, D. E., Hurtado, P. M., Cocho, J. A., Herrera, S., & Arévalo-Herrera, M. (2017). Reference values of amino acids, acylcarnitines and succinylacetone by tandem mass spectrometry for use in newborn screening in southwest Colombia. *Colombia Medica (Cali, Colombia)*, *48*(3), 113–119.

Dogan, E., Uysal, S., Ozturk, Y., Arslan, N., & Coker, C. (2017) Selective screening for inborn errors of metabolism: A Report of Six Years Experience. *Iranian Journal of Pediatrics*, *27*(5), e11323

He, F., Yang, R., Huang, X., Tian, Y., Pei, X., Bohn, M. K., Zou, L., Wang, Y., Li, H., Wang, T., Gu, M., Jiang, T., Chen, X., Zou, H., Wei, H., Tian, W., Tang, T., Adeli, K., & Wang, Z. (2021). Reference standards for newborn screening of metabolic disorders by tandem mass spectrometry: A nationwide study on millions of Chinese neonatal populations. *Frontiers in Molecular Biosciences*, *8*, 719866.

Mak, C. M., Lee, H. C., Chan, A. Y., & Lam, C. W. (2013). Inborn errors of metabolism and expanded newborn screening: review and update. *Critical Reviews in Clinical Laboratory Sciences*, *50*(6), 142–162.

Manta-Vogli, P. D., Schulpis, K. H., Loukas, Y. L., & Dotsikas, Y. (2020). Perinatal free carnitine and short chain acylcarnitine blood concentrations in 12,000 full-term breastfed newborns in relation to their birth weight. *Pediatrics and Neonatology*, *61*(6), 620–628.

Ruoppolo M, Scolamiero E, Caterino M, Mirisola V, Franconi F, Campesi I. Female and male human babies have distinct blood metabolomic patterns. *Molecular Biosystems*,*11*(9), 2483-2492.

Sarker, S. K., Islam, M. T., Biswas, A., Bhuyan, G. S., Sultana, R., Sultana, N., Rakhshanda, S., Begum, M. N., Rahat, A., Yeasmin, S., Khanam, M., Saha, A. K., Noor, F. A., Sajib, A. A., Islam, A. B. M. M. K., Qadri, S. K., Shahidullah, M., Mannan, M. A., Muraduzzaman, A. K. M., Shirin, T., …& Mannoor, K. (2019). Age-specific cut-off values of amino acids and acylcarnitines for diagnosis of inborn errors of

metabolism using liquid chromatography tandem mass spectrometry. *BioMed Research International*, *2019*, 3460902.

Scolamiero, E., Cozzolino, C., Albano, L., Ansalone, A., Caterino, M., Corbo, G., di Girolamo, M. G., Di Stefano, C., Durante, A., Franzese, G., Franzese, I., Gallo, G., Giliberti, P., Ingenito, L., Ippolito, G., Malamisura, B., Mazzeo, P., Norma, A., Ombrone, D., Parenti, G., …& Ruoppolo, M. (2015). Targeted metabolomics in the expanded newborn screening for inborn errors of metabolism. *Molecular Bio Systems*, *11*(6), 1525–1535.

Vieira Neto, E., Fonseca, A. A., Almeida, R. F., Figueiredo, M. P., Porto, M. A., & Ribeiro, M. G. (2012). Analysis of acylcarnitine profiles in umbilical cord blood and during the early neonatal period by electrospray ionization tandem mass spectrometry. *Brazilian Journal of Medical and Biological Research, 45*(6), 546–556.

Walter, J. H., Patterson, A., Till, J., Besley, G. T., Fleming, G., & Henderson, M. J. (2009). Bloodspot acylcarnitine and amino acid analysis in cord blood samples: Efficacy and reference data from a large cohort study. *Journal of Inherited Metabolic Disease*, *32*(1), 95–101.

Author Information

Lyazzat Syrlybayeva
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan
Contact e-mail: *micropaleontolog@yandex.kz*

Gulmira Zharmakhanova
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

Victoria Kononets
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

Zhanylsyn Gaisiyeva
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

To cite this article:

Syrlybayeva, L., Zharmakhanova, G., Kononets, V., & Gaisiyeva, Z. (2024). Age-specific reference values for free carnitine and short chain acylcarnitines content in dried blood spots in newborns in Western Kazakhstan: A tandem mass spectrometry measurement. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 103-110.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 111-118

ICGeHeS 2024: International Conference on General Health Sciences

Immunoglobulin Heavy Chain Gene Mutations in Chronic Lymphoid Leukemia

Ayse Dalyan
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract Chronic lymphoid leukemia (CLL) is the most common type of leukemia in adults in western countries and is a clinically and genetically heterogeneous disease. Although the disease usually follows a slow course, significant survival differences are observed depending on clinical and biological factors. Some patients with CLL do not need treatment for many years, while others need urgent treatment. It is noteworthy that staging systems are inadequate in patient follow-up and predicting the course of the disease. Therefore, parameters that determine prognosis in CLL independent of disease stage have been developed. In recent years, mutation status of Immunoglobulin heavy chain variable region (IgVH) genes has emerged as a strong marker for prognosis in CLL. Patients with CLL are divided into 2 subgroups with different clinical courses according to the mutation status in the IgVH genes: one refers to mutated IgVH segments with a more favorable clinical course and the other refers to non-mutated IgVH segments associated with a poor outcome. In this study, we will try to clarify the relationship between IgVH mutation status and CLL prognosis and survival.

Keywords: CLL, Mutation IgVH status, Somatic hypermutation

Introduction

CLL is a lymphatic system neoplasm characterized by the proliferation and accumulation of monoclonal B lymphocytes (Herishanu et al., 2011; Urbaniak et al., 2022). CLL is a heterogeneous disease with highly variable prognostic features. In some patients, the disease progresses very slowly and they can live their entire lives without any symptoms; in others, it progresses in an rapid form and they have a short survival (Motta et al., 2009). Rai and Binet staging systems, which are used as clinical staging systems in CLL patients, form the basis of prognosis evaluation (Moreno & Montserrat, 2008). According to these staging systems, the risk stratification of CLL patients is based on the measurement of the disease mass at the time of diagnosis, and the average survival time of patients in the advanced stage of the disease is approximately 1-2 years, while the average survival time of those in the lower stage is expressed as approximately over 10 years (Abbott, 2006). Although staging systems form the basis of evaluating prognosis, it has been reported that they are not effective in predicting patients with good prognosis (Mirzaei et al., 2018). Many biological and classical prognostic markers, including these systems, have been identified to determine prognosis (Moreno & Montserrat, 2008; Ivanescu et al., 2012). The main prognostic markers in CLL are; chromosomal abnormalities (17q13, 6q21, 11q23, 13q14, and 17p13), ZAP70 and CD38 expression levels, beta-2-microglobulin levels, IgVH mutation status, V3-21 gene usage, lymphocyte doubling time (LDT), peripheral blood lymphocyte count, morphology of lymphocytes in peripheral blood, BM histology, age, gender, serum thymidine kinase or soluble CD23 and TP53 mutations (Moreno & Montserrat, 2008; Mirzaei et al., 2018; Ivanescu et al., 2012). Among these prognostic markers in CLL, the mutation status of the immunoglobulin heavy chain gene variable region plays an important role in detecting differences in disease outcomes and guiding treatment decisions (Balla et al., 2024).

Immunoglobulin Genes and IgVH Mutation Status

The immunoglobulin (Ig) molecule is identified on the surface of all mature B cells, consisting of two homologous heavy (H) chains and two homologous light (L) chains linked to them by covalent bonds. Both heavy and light chains contain the N-terminal variable (V) region responsible for antigen binding and the C-terminal constant (C) region that decides the effector site (Tobin, 2005; Karan-Djurasevic & Pavlovic, 2017). The heavy chain consists of approximately 450-600 amino acid residues and the light chain consists of approximately 230 amino acid residues (Male, 2021). The heavy chain locus (IgH) is located on chromosome 14 (14q32.33), while the two immunoglobulin light chain (IgL) loci are located on chromosome 2 (2p11.3) kappa chain and chromosome 22 (22q11.2) lambda chain (Mikocziova et al., 2021). The IgH locus is estimated to be approximately 1250 kb in length (van Dongen et al., 2003). It occur of gene segment clusters containing almost 51 functional VHs, 30 diversity (D), 6 joining (JH) and 10 constant (C) genes, which are divided into 6 or 7 subgroups depending on sequence homology. The IgL locus is composed of VL and JL gene segments, but lacks D gene segments and contains 29-33 functional V(λ) and 36-40 functional V(κ) genes (Tobin, 2005; Tobin & Rosenquist 2005; Karan-Djurasevic & Pavlovic, 2017).

According to their various biochemical properties, immunoglobulins are divided into different isotypes or classes. Light chain isotypes come in two forms, kappa (κ) and lambda (λ). Heavy chain isotypes are γ, α, μ, δ, ε, and they are in related with kappa or lambda light chains. The name of a structurally complete imminoglobulin (Ig) isotype is determined by the heavy chain; Ig G, Ig A, Ig M, Ig D and Ig E molecules contain γ, α, μ, δ, ε heavy chains, respectively (Lodish et al., 2018). The heavy chain constant region, which defines Ig isotypes, is accountable for from fixing membrane-bound Ig to the plasma membrane of B cells. The variable region of each Ig chain occur of the three hypervariable complementarity determinant regions (CDRs) CDR1, CDR2, and CDR3, and the four relatively invariant framework regions (FRs) FR1, FR2, FR3, and FR4 (Karan-Djurasevic & Pavlovic, 2017). (Figure 1) While FRs are similar across various VH segments, CDRs vary considerably even within the same VH family. Moreover, CDRs express preferential target sequences of somatic hypermutations along the course of the germinal center reaction. Despite the fact that FRs are generally much less affected by somatic mutations, nucleotide changes can emerge in these regions, particularly in B cells under heavy mutational process (van Dongen et al., 2003). The heavy chain CDR3 region (VH CDR3) has great diversity potential and is the main determinant of the antigen specificity of the antibody (Arnaout et al., 2011).

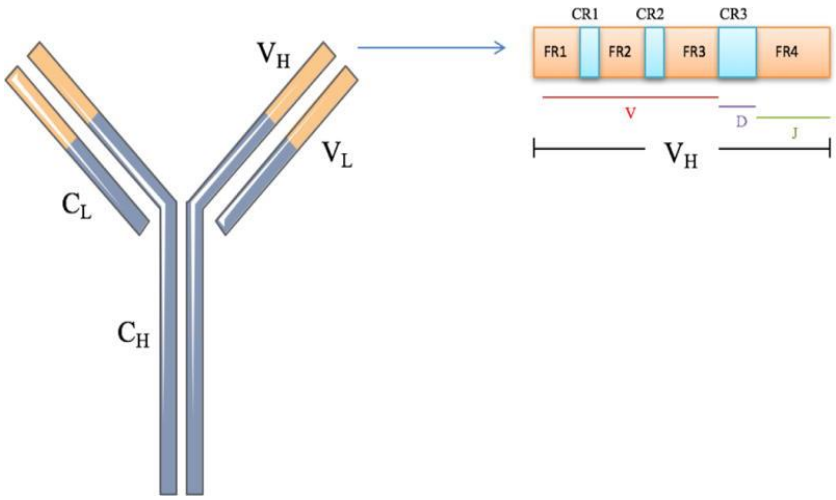


Figure 1. Schematic of an immunoglobulin molecule (Crombie & Davids, 2017).

B cells begin their development in the fetal liver and bone marrow and have the capacity to recognize a wide variety of antigens and make antibodies during their development (LeBien & Tedder, 2008). In the early developmental stage of B-cells, V, D, J genes recombine uniquely in the IgVH region and lymphocytes form a new VDJ genetic structure (Alkan, 2006). B lymphocytes that encounter the antigen undergo somatic mutation in the germinal center to provide the codes required for the immunoglobulin molecule in the IgVH gene segments. With point mutations in the nucleotide sequence, antibodies fully compatible with the antigen are synthesized and the Ig class changes (Meffre et al., 2000; Lu et al., 2020; Gupta et al., 2020).

In 1999, independent studies reported that IgVH mutation status differentiated CLL into two distinct clinical forms with different prognoses (Hamblin et al., 1999; Damle et al., 1999). The mutation status of IgVH genes defines depending on the cut-off value of 98% identity with the nearest germline IgVH genes; CLL cases with

≥98% identity are considered unmutated CLL (U-CLL), while those with <98% identity are considered mutated CLL (M-CLL). Somatic mutations of IgVH occur in about half of cases and have a favorable clinical course, require less treatment and have a longer treatment-free period than those without mutations, and have a significantly longer survival than those without mutations (Hamblin et al., 1999; Damle et al., 1999; González-Gascón Y Marín et al, 2014; Langerak et al., 2020). While CLL patients have positive results with a median survival of 10-20 years from the time of diagnosis; It is known that patients with unmutated IgVH have significantly worse outcomes, with an average survival time of 5-10 years (Kay et al., 2007). According to studies, it has been determined that the mutation status of IgVH genes is associated with a specific clinical response to chemoimmunotherapy with fludarabine, cyclophosphamide and rituximab (FCR); fit M-CLL patients treated with FCR had longer sustained responses, progression-free survival, and substantial improvement in overall survival (OS) compared to U-CLL patients treated with the same therapy (Rosenquist et al., 2017; Thompson et al., 2016; Rossi et al., 2015; Fischer et al., 2016). It has also been reported that the risk of relapse after stem cell transplantation is quite high in patients with U-CLL (Ritgen et al., 2003).

Immunoglobulin heavy chain variable region gene repertoire: The repertoire of IgVH used in CLL is different from normal B cells. VH1, VH3 and VH4 gene families are common in B cells of CLL. Among B cells, the VH1 gene family is expressed more frequently than the VH3 gene family, and furthermore, these gene families exhibit a hierarchy of mutations among themselves; VH3 > VH4 > VH1 (Fais et al., 1998; Ghia et al., 2005). In most CLL studies, just 6-7 individual IgVH genes were used in more than half of the VDJ rearrangements. The most commonly used are IgVH1-69, IgVH3-7, IgVH3-23 and IgVH4-34, followed by a few other genes (IgVH3-30, IgVH3-30.3, IgVH4-59, IgVH3-48, IgVH1-2, IgVH4-39, IgVH1-3 and IgVH1-18), depending on the groups (Karan-Djurasevic, & Pavlovic, 2017). The frequency of somatic mutations is not the same among individual IgVH genes in CLL cells; IgVH3-7, IgVH3-23 and I IgVH4-34 show a high rate of somatic mutations, while IgVH1-69 may have few or no mutations (Fais et al., 1998; Ghia et al., 2005; Zhang & Kipps 2014).

Some IgVH genes may show different characteristics according to specific clinical features and different geographical regions, regardless of mutation status. The IgVH genes frequently used in Western patients are IgVH1-69, IgVH3-23, IgVH4-34 and IgVH3-07. The IgVH1-69 gene has been observed to be one of the most common rearranged genes in Western CLL patients and is mostly related with the U-CLL subset, but a very low frequency of IgVH1-69 usage has been reported in Asian cohorts. The IgVH3-21 gene has been reported to be more represented in CLL patients in northern European countries compared to the Mediterranean region and has been related with poor prognosis regardless of mutation status (Ghia et al., 2005; González-Gascón Y Marín et al., 2014; Stanganelli et al., 2013).

BCR stereotypy: Studies of VDJ recombinations of the Ig gene in CLL suggest that certain gene segments are very frequent in different patients, show stereotyped use of gene segments. A much higher ratio of unmutated CLL patients carry stereotypical VDJ recombinations that lead to alike CDRs (Jeyakumar & O' Brien, 2018). The antigen receptor on the surface membrane of the B cell (BCR) plays a significantly role in the progression and evolution of CLL (Vergani et al., 2023). In about 30% of CLLs, BCRs clustered into stereotyped subsets, each based on a distinct amino acid pattern in highly variable complementarity-determining regions (VH CDR3), some of which were named major because they were more common (Agathangelidis et al., 2012; Agathangelidis et al., 2021; Koehrer & Burger 2024). Just 6 of the IgVH genes; IgVH1-3, IgVH1-2, IgVH3-21, IgVH1-69, IgVH4-39, and IgVH4-34 constitute almost 80% of the major stereotyped subsets. It was stated that some IgVH genes, IgVH1-69, IgVH3-21, IgVH1-2, were highly represented, while IgVH3-7, IgVH3-23, IgVH3-30 were less represented (Koehrer & Burger 2024). Regardless of the mutation status of the IgVH3-21 gene, it is associated with an aggressive clinical course and poor overall survival; This indicates that the use of stereotypic IgVH genes affects prognosis (Tobin et al., 2003; Jeyakumar & O' Brien, 2018).

A consensus was reached in the ERIC (Europian Research Initiative on CLL www.ericll.org) structure for reliable and reproducible analysis of the IgVH gene mutation status in CLL, and the first recommendations on this subject were published in 2007 (Ghia et. al., 2007). As a result of increased knowledge and work, ERIC recommendations have been updated (Rosenquist et al., 2017; Langerak et al., 2011; Agathangelidis et al., 2022). IgVH mutation status is considered a strong prognostic factor in CLL and has been involved in the disease-specific International Prognostic Index (CLL-IPI) (International CLL-IPI working group, 2016; Rotbain et al., 2020). IgVH mutation status should be determined at the beginning of first-line treatment for its prognostic significance. It is routinely determined by Sanger sequencing and, unlike mutation burden, mutation status does not change over time. Unlike other types of mutations, repeated testing is not required for its evaluation (Pula et al., 2022). Recently, studies have been conducted with next generation sequencing (NGS) methods, which are reported to provide more accurate results than traditional methods (Crombie & Davids,

2017). Although Sanger sequencing is used as the standard method for IgVH mutation status, NGS is seen as the harbinger of a new era in medical diagnostic (Davi et al., 2020). The use of NGS to determine IgVH mutation status has not been validated for clinical routine testing due to absence of standardization and uncertain results; However, studies on the application of this methodology in CLL patients with additional applications are ongoing. (Pula et al., 2022).

Relationship between IgVH Mutation Status and Other Prognostic Markers

It has been stated that prognostic markers ZAP70, CD38, activation-induce cytidine deaminase (AID) mRNA, serum thymidine kinase, soluble CD23, lipoprotein lipase A and ADAM 29 are associated with IgVH mutation status (Gribben, 2008; Moreno &Montserrat, 2008; Pashaei et al., 2017). Among these, CD38 and ZAP70 are prominent markers.

CD38: CD38 is a glycoprotein with ectoenzymatic properties that is expressed in B, T, NK and other lymphoid and myeloid cells (Morandi et al., 2018; Pashaei et al., 2017). CD38 expression is related with neoplastic cells stating diffuse bone marrow infiltration, atypical morphology, elevated peripheral blood lymhocytosis and a less positive prognosis (Kostareli et al., 2008). High expression of CD38 has been reported to be related with unmutated IgVH (Damle et al., 1999; Hamblin et al., 2002; Thunberg et al., 2001). Although CD38 and IgVH mutation status are generally associated, CD38 expression changes have been reported to be observed in some patients throughout the course of the disease (Hamblin et al., 2002). This may limit the use of CD38 as a marker instead of IgVH mutation status. It is stated that CD38 can be a prognostic factor with its own biological and clinical value, independent of IgVH mutation (Kostareli et al., 2008).

ZAP70: ZAP70 (zeta transmitted protein 70) is a protein tyrosine kinase expressed primarily in T and NK cells (Guillaume et al., 2005). In CLL cells, a strong related between ZAP70 expression and unmutated IgVH genes, and patients positive for ZAP70 showed rapid progression, need for treatment in a short time, longer duration of treatment, and shorter survival rates (Wiestner et al., 2003; Crespo et al., 2003; Rassenti et al., 2004). It has also been suggested that determining ZAP70 expression at diagnosis is an important prognostic marker in the evaluation of disease progression in the early stages (Rassenti et al., 2004).

Conclusion

In conclusion, IgVH mutation status in CLL patients can be considered as an effective marker for the accurate determination of prognosis among prognostic markers. The IgVH mutation status determined at the start of treatment will provide accurate information about disease progression and survival. Accordingly, further studies on IgVH mutation status may lead to a new treatment strategy.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

* This work was supported by Gaziantep University Scientific Research Projects Coordination Unit. Project Number: FEF.DT.22.14

References

Abbott B. L. (2006). Chronic lymphocytic leukemia: recent advances in diagnosis and treatment. *The Oncologist*, *11*(1), 21–30.

Agathangelidis, A., Darzentas, N., Hadzidimitriou, A., Brochet, X., Murray, F., Yan, X. J., Davis, Z., van Gastel-Mol, E. J., Tresoldi, C., Chu, C. C., Cahill, N., Giudicelli, V., Tichy, B., Pedersen, L. B., Foroni, L., Bonello, L., Janus, A., Smedby, K., Anagnostopoulos, A., Merle-Beral, H., … Stamatopoulos, K. (2012). Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. *Blood*, *119*(19), 4467–4475

Agathangelidis, A., Chatzidimitriou, A., Gemenetzi, K., Giudicelli, V., Karypidou, M., Plevova, K., Davis, Z., Yan, X. J., Jeromin, S., Schneider, C., Pedersen, L. B., Tschumper, R. C., Sutton, L. A., Baliakas, P., Scarfò, L., van Gastel, E. J., Armand, M., Tausch, E., Biderman, B., Baer, C., … Stamatopoulos, K. (2021). Higher-order connections between stereotyped subsets: implications for improved patient classification in CLL. *Blood*, *137*(10), 1365–1376.

Agathangelidis, A., Chatzidimitriou, A., Chatzikonstantinou, T., Tresoldi, C., Davis, Z., Giudicelli, V., Kossida, S., Belessi, C., Rosenquist, R., Ghia, P., Langerak, A. W., Davi, F., Stamatopoulos, K., & ERIC, the European Research Initiative on CLL (2022). Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: the 2022 update of the recommendations by ERIC, the European Research Initiative on CLL. *Leukemia*, *36*(8), 1961–1968.

Alkan, S. (2006). *Kronik lenfositik losemi (KLL): Patogenezi ve biyolojisi.* Turk Hematoloji Dernegi. Retrieved from http://www.thd.org.tr/thdData/userfiles/file/kroniklenfositik.pdf

Arnaout, R., Lee, W., Cahill, P., Honan, T., Sparrow, T., Weiand, M., Nusbaum, C., Rajewsky, K., & Koralov, S. B. (2011). High-resolution description of antibody heavy-chain repertoires in humans. *PloS one*, *6*(8), e22365.

Balla, B., Tripon, F., Lazar, E., & Bănescu, C. (2024). Analysis of mutational status of *IGHV*, and cytokine polymorphisms as prognostic factors in chronic lymphocytic leukemia: The Romanian experience. *International Journal of Molecular Sciences*, *25*(3), 1799.

Crespo, M., Bosch, F., Villamor, N., Bellosillo, B., Colomer, D., Rozman, M., Marcé, S., López-Guillermo, A., Campo, E., & Montserrat, E. (2003). ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *The New England Journal of Medicine*, *348*(18), 1764–1775.

Crombie, J., & Davids, M. S. (2017). IGHV mutational status testing in chronic lymphocytic leukemia. *American Journal of Hematology*, *92*(12), 1393-1397.

Damle, R. N., Wasil, T., Fais, F., Ghiotto, F., Valetto, A., Allen, S. L., Buchbinder, A., Budman, D., Dittmar, K., Kolitz, J., Lichtman, S. M., Schulman, P., Vinciguerra, V. P., Rai, K. R., Ferrarini, M., & Chiorazzi, N. (1999). Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*, *94*(6), 1840–1847.

Davi, F., Langerak, A. W., de Septenville, A. L., Kolijn, P. M., Hengeveld, P. J., Chatzidimitriou, A., Bonfiglio, S., Sutton, L. A., Rosenquist, R., Ghia, P., Stamatopoulos, K., & ERIC, the European Research Initiative on CLL, and the EuroClonality-NGS Working Group (2020). Immunoglobulin gene analysis in chronic lymphocytic leukemia in the era of next generation sequencing. *Leukemia*, *34*(10), 2545–2551.

Fais, F., Ghiotto, F., Hashimoto, S., Sellars, B., Valetto, A., Allen, S. L., Schulman, P., Vinciguerra, V. P., Rai, K., Rassenti, L. Z., Kipps, T. J., Dighiero, G., Schroeder, H. W., Jr, Ferrarini, M., & Chiorazzi, N. (1998). Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *The Journal of Clinical İnvestigation*, *102*(8), 1515–1525.

Fischer, K., Bahlo, J., Fink, A. M., Goede, V., Herling, C. D., Cramer, P., Langerbeins, P., von Tresckow, J., Engelke, A., Maurer, C., Kovacs, G., Herling, M., Tausch, E., Kreuzer, K. A., Eichhorst, B., Böttcher, S., Seymour, J. F., Ghia, P., Marlton, P., Kneba, M., … & Hallek, M. (2016). Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*, *127*(2), 208–215.

Ghia, P., Stamatopoulos, K., Belessi, C., Moreno, C., Stella, S., Guida, G., Michel, A., Crespo, M., Laoutaris, N., Montserrat, E., Anagnostopoulos, A., Dighiero, G., Fassas, A., Caligaris-Cappio, F., & Davi, F. (2005). Geographic patterns and pathogenetic implications of IGHV gene usage in chronic lymphocytic leukemia: the lesson of the IGHV3-21 gene. *Blood*, *105*(4), 1678–1685.

Ghia, P., Stamatopoulos, K., Belessi, C., Moreno, C., Stilgenbauer, S., Stevenson, F., Davi, F., Rosenquist, R., & European Research Initiative on CLL (2007). ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. *Leukemia*, *21*(1), 1–3.

González-Gascón Y Marín, I., Hernández, J. A., Martín, A., Alcoceba, M., Sarasquete, M. E., Rodríguez-Vicente, A., Heras, C., de Las Heras, N., Fisac, R., García de Coca, A., de la Fuente, I., Hernández-Sánchez, M., Recio, I., Galende, J., Martín-Núñez, G., Alonso, J. M., Hernández-Rivas, J. M., & González, M. (2014). Mutation status and immunoglobulin gene rearrangements in patients from northwest and central region of Spain with chronic lymphocytic leukemia. *Biomed Research International*, *2014*, 257517.

Gribben J. G. (2008). Molecular profiling in CLL. *Hematology. American Society of Hematology. Education Program*, 444–449.

Guillaume, N., Alleaume, C., Munfus, D., Capiod, J. C., Touati, G., Pautard, B., Desablens, B., Lefrère, J. J., Gouilleux, F., Lassoued, K., & Gouilleux-Gruart, V. (2005). ZAP-70 tyrosine kinase is constitutively expressed and phosphorylated in B-lineage acute lymphoblastic leukemia cells. *Haematologica*, *90*(7), 899–905.

Gupta, S. K., Viswanatha, D. S., & Patel, K. P. (2020). Evaluation of somatic hypermutation status in chronic lymphocytic leukemia (CLL) in the era of next generation sequencing. *Frontiers in Cell and Developmental Biology*, *8*, 357.

Herishanu, Y., Pérez-Galán, P., Liu, D., Biancotto, A., Pittaluga, S., Vire, B., ... & Wiestner, A. (2011). The lymph node microenvironment promotes B-cell receptor signaling, NF-κB activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood, The Journal of the American Society of Hematology*, *117*(2), 563-574.

Hamblin, T. J., Davis, Z., Gardiner, A., Oscier, D. G., & Stevenson, F. K. (1999). Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*, *94*(6), 1848–1854.

Hamblin, T. J., Orchard, J. A., Ibbotson, R. E., Davis, Z., Thomas, P. W., Stevenson, F. K., & Oscier, D. G. (2002). CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. *Blood*, *99*(3), 1023–1029.

International CLL-IPI working group (2016). An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): A meta-analysis of individual patient data. *The Lancet. Oncology*, *17*(6), 779–790.

Ivanescu, A. M., Oprea, M., Momanu, R., Coles, E., Colita, A., & Lupu, A. R. (2012). Prognosis factors in chronic lymphocytic leukemia. *Journal of Medicine and Life*, *5*(Spec Issue), 48–53.

Jeyakumar, D., & O'Brien, S. (2018). Treatment of chronic lymphocytic leukemia and related disorders. In: P. Wiernik, J. Dutcher, M. Gertz (Eds.). *Neoplastic diseases of the blood* (pp.117-134). New York, NY: Springer

Karan-Djurasevic, T., & Pavlovic, S. (2017). Somatic hypermutational status and gene repertoire of immunoglobulin rearrangements in chronic lymphocytic leukemia. In G. Isvoranu (Ed.), *Lymphocyte Updates-Cancer, Autoimmunity and Infection* (pp. 49-78.). Rijeka, Croatia, NY: InTech.

Kay, N. E., O'Brien, S. M., Pettitt, A. R., & Stilgenbauer, S. (2007). The role of prognostic factors in assessing 'high-risk' subgroups of patients with chronic lymphocytic leukemia. *Leukemia*, *21*(9), 1885–1891.

Koehrer, S., & Burger, J. A. (2024). Chronic lymphocytic leukemia: disease biology. *Acta Haematol*, *147*, 8-21.

Kostareli, E., Smilevska, T., Stamatopoulos, K., Kouvatsi, A., & Anagnostopoulos, A. (2008). Chronic lymphocytic leukaemia: An immunobiology approach. *Srpski Arhiv za Celokupno Lekarstvo*, *136*(5-6), 319-323.

Langerak, A. W., Davi, F., & Stamatopoulos, K. (2020). Immunoglobulin heavy variable somatic hyper mutation status in chronic lymphocytic leukaemia: on the threshold of a new era?. *British Journal of Haematology*, *189*(5), 809–81.

Langerak, A. W., Davi, F., Ghia, P., Hadzidimitriou, A., Murray, F., Potter, K. N., Rosenquist, R., Stamatopoulos, K., Belessi, C., & European Research Initiative on CLL (ERIC) (2011). Immunoglobulin sequence analysis and prognostication in CLL: guidelines from the ERIC review board for reliable interpretation of problematic cases. *Leukemia*, *25*(6), 979–984.

LeBien, T. W., & Tedder, T. F. (2008). B lymphocytes: How they develop and function. *Blood*, *112*(5), 1570–1580.

Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Scott, M. P., Bretscher, A., Ploegh, H., & Matsudaira, P. (2018). H. Geckil, M. Ozmen, O. Yesilada (Eds.). In *Molekuler hucre biyolojisi* (pp. 1055-1107). Ankara, *Palme Yayıncılık*.

Lu, R. M., Hwang, Y. C., Liu, I. J., Lee, C. C., Tsai, H. Z., Li, H. J., & Wu, H. C. (2020). Development of therapeutic antibodies for the treatment of diseases. *Journal of Biomedical Science*, *27*(1), 1.

Male, D. (2021). *Immunology: An Illustrated Outline* (6th ed.). Boca Raton, NY: CRC Press.

Meffre, E., Casellas, R., & Nussenzweig, M. C. (2000). Antibody regulation of B cell development. *Nature Immunology*, *1*(5), 379–385.

Mikocziova, I., Greiff, V., & Sollid, L. M. (2021). Immunoglobulin germline gene variation and its impact on human disease. *Genes and Immunity*, *22*(4), 205–217.

Mirzaei, H., Fathullahzadeh, S., Khanmohammadi, R., Darijani, M., Momeni, F., Masoudifar, A., Goodarzi, M., Mardanshah, O., Stenvang, J., Jaafari, M. R., & Mirzaei, H. R. (2018). State of the art in microRNA as diagnostic and therapeutic biomarkers in chronic lymphocytic leukemia. *Journal of Cellular Physiology*, *233*(2), 888–90.

Morandi, F., Horenstein, A. L., Costa, F., Giuliani, N., Pistoia, V., & Malavasi, F. (2018). CD38: A target for ımmunotherapeutic approaches in multiple myeloma. *Frontiers in Immunology*, *9*, 2722.

Moreno, C., & Montserrat, E. (2008). New prognostic markers in chronic lymphocytic leukemia. *Blood Reviews*, *22*(4), 211–219.

Motta, M., Wierda, W. G., & Ferrajoli, A. (2009). Chronic lymphocytic leukemia: treatment options for patients with refractory disease. *Cancer*, *115*(17), 3830–3841

Pashaei, M., Shokrollahi, M., & Kokhaei, P. (2016). Somatic mutation in immunoglobulin gene variable region in patients with chronic lymphocytic leukemia and prognostic impact. *Basic & Clinical Cancer Research*, *8*(4), 29-36.

Puła, B., Jamroziak, K., Wróbel, T., Giannopoulos, K., & Hus, I. (2022). IGHV mutational status and the choice of first-line therapy for patients with chronic lymphocytic leukaemia. *Hematology in Clinical Practice*, *13*(1), 7-14.

Rassenti, L. Z., Huynh, L., Toy, T. L., Chen, L., Keating, M. J., Gribben, J. G., Neuberg, D. S., Flinn, I. W., Rai, K. R., Byrd, J. C., Kay, N. E., Greaves, A., Weiss, A., & Kipps, T. J. (2004). ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *The New England Journal of Medicine*, *351*(9), 893–901.

Ritgen, M., Lange, A., Stilgenbauer, S., Dohner, H., Bretscher, C., Bosse, H., Stuhr, A., Kneba, M., & Dreger, P. (2003). Unmutated immunoglobulin variable heavy-chain gene status remains an adverse prognostic factor after autologous stem cell transplantation for chronic lymphocytic leukemia. *Blood*, *101*(5), 2049–2053.

Rosenquist, R., Ghia, P., Hadzidimitriou, A., Sutton, L. A., Agathangelidis, A., Baliakas, P., Darzentas, N., Giudicelli, V., Lefranc, M. P., Langerak, A. W., Belessi, C., Davi, F., & Stamatopoulos, K. (2017). Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: Updated ERIC recommendations. *Leukemia*, *31*(7), 1477–1481.

Rossi, D., Terzi-di-Bergamo, L., De Paoli, L., Cerri, M., Ghilardi, G., Chiarenza, A., Bulian, P., Visco, C., Mauro, F. R., Morabito, F., Cortelezzi, A., Zaja, F., Forconi, F., Laurenti, L., Del Giudice, I., Gentile, M., Vincelli, I., Motta, M., Coscia, M., Rigolin, G. M., …& Gaidano, G. (2015). Molecular prediction of durable remission after first-line fludarabine-cyclophosphamide-rituximab in chronic lymphocytic leukemia. *Blood*, *126*(16), 1921–1924.

Rotbain, E. C., Frederiksen, H., Hjalgrim, H., Rostgaard, K., Egholm, G. J., Zahedi, B., Poulsen, C. B., Enggard, L., da Cunha-Bang, C., & Niemann, C. U. (2020). IGHV mutational status and outcome for patients with chronic lymphocytic leukemia upon treatment: a Danish nationwide population-based study. *Haematologica*, *105*(6), 1621–1629.

Stanganelli, C., Travella, A., Bezares, R., & Slavutsky, I. (2013). Immunoglobulin gene rearrangements and mutational status in argentinian patients with chronic lymphocytic leukemia. *Clinical Lymphoma, Myeloma & Leukemia*, *13*(4), 447–457.

Thunberg, U., Johnson, A., Roos, G., Thorn, I., Tobin, G., Sällstrom, J., Sundström, C., & Rosenquist, R. (2001). CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. *Blood*, *97*(6), 1892–1894.

Thompson, P. A., Tam, C. S., O'Brien, S. M., Wierda, W. G., Stingo, F., Plunkett, W., Smith, S. C., Kantarjian, H. M., Freireich, E. J., & Keating, M. J. (2016). Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. *Blood*, *127*(3), 303–309.

Tobin, G., Thunberg, U., Johnson, A., Eriksson, I., Soderberg, O., Karlsson, K., Merup, M., Juliusson, G., Vilpo, J., Enblad, G., Sundström, C., Roos, G., & Rosenquist, R. (2003). Chronic lymphocytic leukemias utilizing the VH3-21 gene display highly restricted Vlambda2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. *Blood*, *101*(12), 4952–4957.

Tobin, G. (2005). The immunoglobulin genes and chronic lymphocytic leukemia (CLL). *Upsala Journal of Medical Sciences*, *110*(2), 97-114

Tobin, G., & Rosenquist, R. (2005). Prognostic usage of VH gene mutation status and its surrogate markers and the role of antigen selection in chronic lymphocytic leukemia. *Medical Oncology*, *22*, 217-228.

Urbaniak, M., Iskierka-Jażdżewska, E., Majchrzak, A., & Robak, T. (2022). Atypical immunophenotype of chronic lymphocytic leukemia. *Acta Haematologica Polonica*, *53*, 48–52.

van Dongen, J. J., Langerak, A. W., Brüggemann, M., Evans, P. A., Hummel, M., Lavender, F. L., Delabesse, E., Davi, F., Schuuring, E., García-Sanz, R., van Krieken, J. H., Droese, J., González, D., Bastard, C., White, H. E., Spaargaren, M., González, M., Parreira, A., Smith, J. L., Morgan, G. J., …& Macintyre, E. A. (2003). Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*, *17*(12), 2257–2317.

Vergani, S., Bagnara, D., Agathangelidis, A., Ng, A. K. Y., Ferrer, G., Mazzarello, A. N., Palacios, F., Yancopoulos, S., Yan, X. J., Barrientos, J. C., Rai, K. R., Stamatopoulos, K., & Chiorazzi, N. (2023). CLL stereotyped B-cell receptor immunoglobulin sequences are recurrent in the B-cell repertoire of healthy individuals: Apparent lack of central and early peripheral tolerance censoring. *Frontiers in Oncology*, *13*, 1112879.

Wiestner, A., Rosenwald, A., Barry, T. S., Wright, G., Davis, R. E., Henrickson, S. E., Zhao, H., Ibbotson, R. E., Orchard, J. A., Davis, Z., Stetler-Stevenson, M., Raffeld, M., Arthur, D. C., Marti, G. E., Wilson, W. H., Hamblin, T. J., Oscier, D. G., & Staudt, L. M. (2003). ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. *Blood*, *101*(12), 4944–4951.

Zhang, S., & Kipps, T. J. (2014). The pathogenesis of chronic lymphocytic leukemia. *Annual Review of Pathology*, *9*, 103–118.

Author Information

Ayse Dalyan
University of Gaziantep, Department of Biology, 27310 Gaziantep, Turkiye
Contact e-mail: *agurlekdalyan@gmail.com*

Mehmet Ozaslan
University of Gaziantep, Department of Biology, 27310 Gaziantep, Turkiye

To cite this article:

Dalyan, A. & Ozaslan, M. (2024). Immunoglobulin heavy chain gene mutations in chronic lymphoid leukemia. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)*, *13*, 111-118.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 119-127

ICGeHeS 2024: International Conference on General Health Sciences

Essential and Conditionally Essential Amino Acid Profile in West Kazakhstan Children with Suspected Inborn Errors of Metabolism

Gulmira Zharmakhanova
West Kazakhstan Marat Ospanov Medical University;

Victoria Kononets
West Kazakhstan Marat Ospanov Medical University

Lyazzat Syrlybayeva
West Kazakhstan Marat Ospanov Medical University

Zhanylsyn Gaisiyeva
West Kazakhstan Marat Ospanov Medical University

Abstract: Measuring the concentration of amino acids in the blood and compiling a metabolic profile of amino acids, taking into account the influence of factors such as age, gender, body weight, region of residence, is extremely important in the diagnosis of amino acid metabolic disorders, especially when conducting selective screening for inborn errors of metabolism (IEM). Aims: To describe the metabolic profile of essential and conditionally essential amino acids in samples of dried blood spots from children in Western Kazakhstan with suspected IEM using LC-MS/MS technology (liquid chromatography-tandem mass spectrometry). Methods: The cross-sectional study included 200 clinical-risk children of West Kazakhstan aged one day to 18 years, 52.5 % male and 47.5 % female. Depending on their age, the children were divided into the following groups: group A (newborns, age 1-30 days), group B (age 1 month -7 years) and group C (age 8-18 years). Blood samples on Guthrie cards were collected and quantified by LC-MS/MS. Nonparametric statistical approaches were used. The concentrations of arginine, glycine, leucine, isoleucine, hydroxyproline, methionine, phenylalanine, proline, tyrosine, valine in dried blood spots of children with suspected IEM were determined. Results: Significant differences were established between children with suspected IEM of different age groups in the concentrations of hydroxyproline, arginine, glycine, isoleucine, leucine, phenylalanine, proline, tyrosine and valine. A negative correlation with age for the majority of essential and conditionally essential Amino Acids indicates their decline with age in children with suspected hereditary metabolic diseases. Significant differences between groups of female and male children with suspected IEM were established only in the concentration of methionine in dried blood spots. The highest values were determined in female group. Conclusion: The results of this study may be important in conducting selective screening for IEM in various age groups of the pediatric population.

Keywords: Essential amino acids, Selective screening, Inborn errors of metabolism, Tandem mass spectrometry

Introduction

Inborn errors of metabolism (IEMs) are a large class of genetic disorders that result from defects in enzymes involved in energy production and nutrient metabolism (Phipps, Jones & Patel, 2019). Deficiency or alteration in the activity of essential enzymes or proteins in metabolic intermediate pathways results in a wide range of diseases with clinical heterogeneity (Golbahar et al., 2013; Han et al., 2015; Sarker et al., 2019). Disorders of amino acid metabolism, organic acid metabolism and the urea cycle make up a significant part of IEM (Phipps et al., 2019).

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

Amino acids serve as key building blocks and energy sources for cell repair, survival, regeneration and growth. In addition to their role as building blocks of protein, amino acids are also a source of energy (ketogenic, glucogenic, or both), are building blocks of Krebs cycle intermediates (also known as the tricarboxylic acid cycle) and other metabolites, and are metabolized as needed. Nine amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine) are considered "essential", i.e. essential in the diet, since humans are not able to synthesize them endogenously (Aliu et al., 2018).

Diagnosis of hereditary disorders of amino acid (AA) metabolism is based on qualitative and/or quantitative analysis of AA, mainly in blood and urine (Piraud et al., 2011). For many years, the most common method was ion exchange chromatography followed by post-column derivatization with ninhydrin. The advent of tandem mass spectrometry (MS/MS) coupled with liquid chromatography (LC) has made it possible to measure many metabolites for the diagnosis of inborn errors of metabolism (Piraud et al., 2011). High-performance technologies based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) are now widely used, which allow the simultaneous quantification of several metabolites, such as amino acids and acylcarnitines, from a very small amount of a biological sample (Sarker et al., 2019).

Amino acid profiles are important tools needed to diagnose inherited disorders of amino acid metabolism. Age reference cutoff ranges for each analyte should be established first for each population by individual laboratories prior to screening/diagnosis of patients (Yang et al., 2018; Sarker et al., 2019), as cutoff values are highly dependent on various factors. such as genetic background, geographical location of the population, diet and age (Dietzen et al., 2016; Sarker et al., 2019). According to Uaariyapanichkul et al. (2018), compared with Caucasians, the levels of most amino acids in the blood of Thai children were higher.

Newborns are currently screened or diagnosed with more than 30 IEMs using LC-MS/MS in newborn screening programs in most developed countries and some developing countries around the world (Mak et al., 2013). Currently, screening of newborns using MS/MS is not mandatory in Kazakhstan, but we have developed and are conducting a pilot project for selective screening for hereditary metabolic disorders using MS/MS. During the study, reference intervals were developed for the concentration of 57 metabolites, including amino acids and acylcarnitines in dried blood spots for different age groups of the pediatric population, including newborns (Syrlybayeva, Zharmakhanova & Kononets, 2023; Zharmakhanova et al., 2023). From October 2022 to December 2024, we assessed the frequency of 37 inherited metabolic disorders using LC-MS/MS technology in a group of high-risk children in Western Kazakhstan. The data obtained from examining a group of high-risk children will be compared with the reference intervals we previously established.

Study Objectives

To describe the metabolic profile of essential and conditionally essential amino acids in samples of dried blood spots from children in Western Kazakhstan with suspected IEM using LC-MS/MS technology.

Tasks

1. To determine the level of essential and conditionally essential amino acids in samples of dried blood spots in children with suspected IEM, taking into account age and gender.
2. To evaluate factors that may affect essential and conditionally essential amino acids levels.
3. To compare findings of the determined analytes in newborns of Western Kazakhstan DBS with the results of previously published studies in other populations.

Methods

Data Sources

The data of this study were obtained during the examination of 200 clinical-risk children aged one day to 18 years to describe the metabolic profile of essential and conditionally essential amino acids (Figure 1). In this research, we recruited a sample of eligible 200 children across the region (200 children aged one day to 18 years suspected of IEM). Western Kazakhstan is divided into four regions (provinces, oblasts) according to the administrative division: Aktobe, West Kazakhstan, Atyrau and Mangystau (Figure 2).

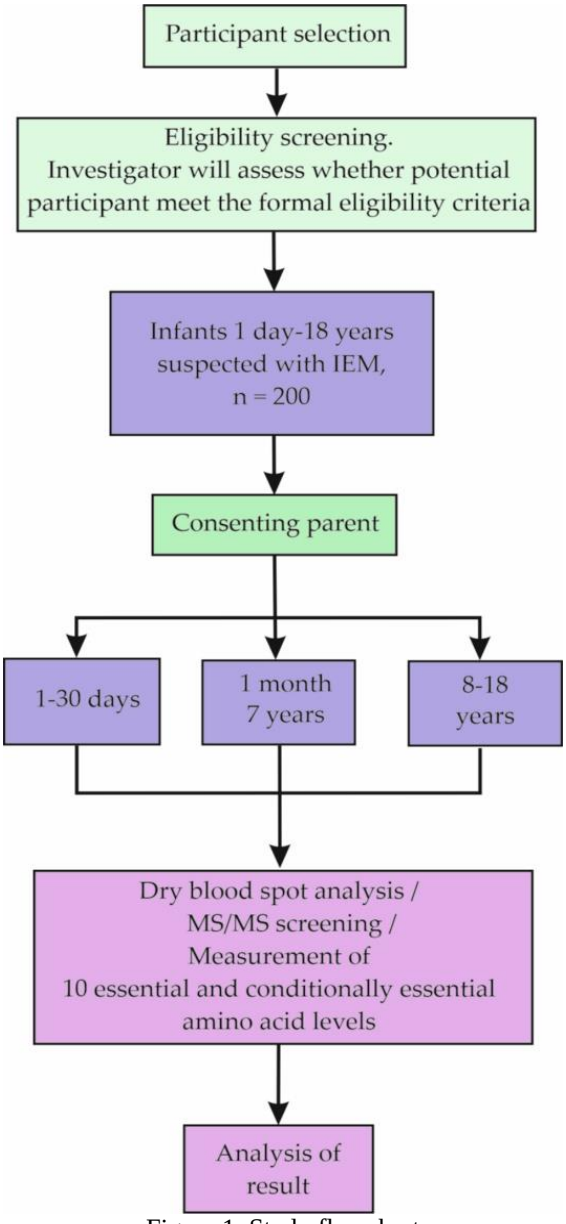


Figure 1. Study flowchart

Figure 2. Sites for data collection throughout western Kazakhstan.

The study was approved by the Bioethics Committee of the West Kazakhstan Marat Ospanov Medical University (Ref. No. 7, 09/09/2020.) Written informed consent (IS) was obtained from the parents and/or legal guardians of children after birth to collect a DBS sample. Demographic and anthropometric data of study participants are presented in Table 1.

Table 1. Demographic and anthropometric data of study participants children suspected IEM (n=200)

	Group A newborns 1-30 days (n=130)	Group B 1 months-7 years (n=52)	Group C 8-18 years (n=18)	The whole sample (n=200)
Weight in grams, by birth Median (IQR)	3180 (2500;3725)	3095 (2300;3465)	3200 (2950;3400)	3155 (2480;3628)
Gender				
Male, n, %	71 (54.6 %)	24 (46.2 %)	10 (56.6 %)	105 (52.5 %)
Female, n, %	59 (45.4 %)	28 (53.8 %)	8 (44.4 %)	95 (47.5 %)
Geographic distribution				
Urban population, n, %	68 (52.3 %)	27 (51.9 %)	11 (61.1 %)	124 (62,0 %)
Rural population, n, %	62 (47.7 %)	25 (48.1 %)	7 (38.9 %)	76 (38,0 %)

Depending on their age, the children were divided into the following groups: group A (newborns, age 1-30 days), group B (age 1 month -7 years) and group C (age 8-18 years). Children from a West Kazakhstan population with suspected metabolic disorders were assessed for inborn metabolic disorders and referred by primary care neonatologists and pediatric consultants between October 2022 and August 2024 based on clinical symptoms associated with metabolic disorders. The study included newborns and children undergoing treatment in maternity hospitals and pediatric clinics of 7 children's hospitals in the region. Patient information was obtained from Individual Record Cards (IRCs) completed by parents or guardians of study participants. The completed questionnaires were reviewed by the researchers. In cases of improper collection, data were removed from the study if they could not be adequately verified. A sample IRC, as well as other supplementary materials, is available at the registry at clini-cal.trials.gov (https://www.clinicaltrials.gov/study/NCT05910151).

Criteria for Inclusion in the Study

Children from 1 day to 18 years of age enrolled for selective screening for IEM if one of the main criteria or two or more additional criteria (symptoms) are identified. The inclusion criteria for IEM selective screening are:

Main Criteria (Symptoms)

Sudden deterioration of the child's clinical condition after a period of normal development (days, weeks, months):
• acute metabolic encephalopathy,
• lethargy (coma),
• seizures resistant to antiepileptic therapy;
Hepatomegaly (hepatosplenomegaly);
Metabolic acidosis with increased anion gap;
Multiple fractures;
Child mortality in the family from diseases with similar symptoms.

Additional Criteria (Symptoms)

Treatment-resistant seizures;
Abnormal muscle tone: dystonia, hyperkinesis, hypotension;
Speech retardation;
Mental retardation of unknown cause;
Cardiomyopathy;
Tachypnea;
Frequent spitting up (vomiting);
Osteoarticular anomalies (joint stiffness, chest deformity, rickets-like changes);
Hernias (umbilical, inguinal-scrotal);

Persistent or recurrent hypoglycemia;
Metabolic alkalosis;
Hyperammonemia;
Thrombocytopenia;
Abnormal odor of urine, body, earwax, any unusual odor;
Hair growth disorders, alopecia;
Ophthalmic anomalies;
Unusual appearance, dysmorphic features;
Parents' consanguinity;
Positive family history with metabolic disorders.

Mass Spectrometry Analysis

Specimen Collection and Storage

Whole blood samples were collected from infants no earlier than 3 hours after feeding by heel prick using a heel stick. Five drops of whole blood (each ~75 µl) were applied to Guthrie cards, Ahlstrom 226 filter paper, and PerkinElmer 226 Five-Spot Card (PerkinElmer Health Sciences, Greenville, USA) to form dried blood spots (DBSs) for LC-MS/MS analysis. Whole blood samples from older children at high-risk were collected after a 4-hour fasting using a standard venipuncture method.

Samples were dried for 4 hours at room temperature and then stored at 4°C in labeled individual zip-lock plastic envelopes with desiccants until analyzed by LC-MS/MS. Samples were sent to the laboratory within five days. In the case of long-term storage of samples, it was carried out at a temperature of –20°C.

Specimen Preparation and LC-MS/MS Analysis

The Neobase2 TM Non-derivatized MSMS kit (PerkinElmer, Wallac Oy, Turku, Finland) was used to quantify 5 essential amino acids and 5 conditionally essential amino acids in dried blood spots according to the manufacturer's instructions. Vial with lyophilized isotope-labeled internal standards (IS) containing $^{2}H_3$-Leucine (Leu IS), $^{2}H_3$-Isoleucine (Leu IS), $^{2}H_3$-Methionine (Met IS), $^{13}C_6$-Phenylalanine (Phe IS), ^{15}N-$^{13}C_5$-Valine (Val IS), $^{2}H_4$- ^{13}C-Arginine (Arg IS), ^{15}N,2-^{13}C-Glycine (Gly IS), $^{2}H_3$-Hydroxyproline (Leu IS), $^{13}C_5$-Proline (Pro IS), $^{13}C_6$-Tyrosine (Tyr IS), was being recovered by adding 1.4 ml of the extraction solution that is included in the Neobase 2 kit. The Extraction Working Solution (EWS) IS was prepared by diluting the recovered internal standards with the extraction solution of 1:100 (v/v).

DBS were analyzed using a Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer (Shimadzu Corporation, Kyoto, Japan). Level I and Level II (low standard and high standard) dried blood drops were included with each assay lot of the Neobase2 TM Non-derivatized MSMS kit to monitor system accuracy and precision.

To analyze essential and conditionally essential amino acids, stored DBS card samples are brought to room temperature (+18 to +25°C) before extraction. A 3.2 mm disc (equivalent to ~3.1 µl of whole blood) is punched out of one dried blood spot with a diameter of 3.2 mm using a Wallac DBS Puncher (PerkinElmer, Wallac Oy, Mustionkatu 6, FI-20750 Turku, Finland) into the well of the 96-well polystyrene U-bottom microplate supplied with the Neobase2 TM Non-derivatized MSMS kit. After adding 125 µL of working extraction solution to each well of the microplate, the plate is covered with an adhesive aluminum film and incubated for 30 minutes at room temperature on a microplate shaker with a shaking speed of 650 rpm. After incubation, 100 µL of the supernatant is transferred to a new 96-well U-bottom microplate, covered with aluminum foil to reduce evaporation, and incubated for 1 hour. The plate is then placed into the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer autosampler, and 5 µL of supernatant is injected into the LCMS for analysis.

Metabolites to Measurement

Arginine (Arg), Glycine (Gly), Leucine (Leu), Isoleucine (Ile), Hydroxyproline (Pro-OH), Methionine (Met), Phenylalanine (Phe), Proline (Pro), Tyrosine (Tyr), Valine (Val).

Statistical Analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to check the normality of the distribution. The data obtained in the study demonstrated that the distribution of essential and conditionally essential amino acids in DBS differs from normal. Me (median) and quartiles (IQR interquartile range) were used for descriptive statistics of the samples. Nonparametric tests (Mann-Whitney U test, Kruskal-Wallis H test) were used to test differences in essential and conditionally essential amino acids concentrations depending on various factors (gender, age, place of residence). Considering the skewed distribution, correlations between body weight, age, and the concentration of essential and conditionally essential amino acids in dry blood spots were performed using Spearman's test. Two-sided levels <0.05 are assumed to be statistically significant. Statistical analysis was done using the software IBM SPSS v. 23.0 (IBM, Armonk, NY, USA) and Statistica (StatSoft, Inc., Tulsa, OK, USA, v. 10).

Results and Discussion

Descriptive statistics on the concentrations of 10 essential and conditionally essential amino acids in samples of dried blood spots from children in Western Kazakhstan with suspected IEM, divided into subgroups according to age, are presented in Table 2.

Table 2. Essential and conditionally essential amino acid profile in dried blood spots of 200 children with suspected IEM in Western Kazakhstan.

Amino acid, µmol/l		All children (n = 200)	Group A newborns 1-30 days (n=130)	Group B 1 months-7 years (n=52)	Group C 8-18 years (n=18)	Kruskal – Wallis *H* test	p-values
5-Oxo Pro	Median	30.76	66.68	19.90	23.34	28.43	0.000
	Range	19.62;93.66	22.01;106.76	16.44;31.18	18.23;62.59		
Arg	Median	23.18	17.89	45.23	44.38	75.38	0.000
	Range	15.73;39.03	13.87;24.38	32.78;55.11	35.02;56.95		
Gly	Median	493.51	547.56	311.37	315.84	73.99	0.000
	Range	362.36;632.40	472.18;660.36	251.52;423.37	273.64;407.04		
Ile	Median	56.12	51.41	34.15	54.49	18.12	0.000
	Range	49.22;65.13	47.82;59.52	25.12;44.53	47.19;62.24		
Leu	Median	144.49	156.21	115.46	129.50	34.25	0.000
	Range	117.08;176.12	130.20;186.43	98.94;145.51	107.94;141.56		
Met	Median	29.02	30.58	23.15	21.92	5.39	0.068
	Range	21.38;57.25	24.31;54.42	18.53;83.94	16.68;31.68		
Phe	Median	54.50	58.51	43.95	46.83	54.14	0.000
	Range	44.38;64.77	51.01;68.82	37.68;49.99	35.66;55.12		
Pro	Median	148.89	157.84	117.86	128.10	32.78	0.000
	Range	120.27;174.98	134.29;185.77	93.29;156.40	104.76;138.22		
Tyr	Median	85.33	104.83	57.57	59.26	70.87	0.000
	Range	60.42;126.79	83.39;149.64	46.74;69.35	19.80;70.65		
Val	Median	124.98	127.04	119.68	157.59	8.77	0.013
	Range	105.62;151.65	105.95;148.56	103.25;147.28	124.57;182.02		

Differences in the distribution of the level of essential and conditionally essential amino acids in dried blood spots between groups of children with suspected IEM of different ages, determined using the Kruskal-Wallis test, are noted in Table 2. Statistically significant differences between age groups were noted in the concentration of hydroxyproline, arginine , glycine, isoleucine, leucine, phenylalanine, proline, tyrosine and valine (Table 2).

In addition, significant negative correlations of moderate strength with age were established for the concentrations in dried blood spots of glycine, phenylalanine and tyrosine, weak negative correlations for hydroxyproline, isoleucine, leucine, methionine, proline and an moderate positive correlation with age for arginine (Table 3).

Age had a significant effect on the concentrations of most essential and conditionally essential amino acids, with Spearman's r correlation coefficients ranging from 0.053 to 0.619 for positive relationships and from -0.153 to -0.613 for negative relationships (Table 3). Only arginine and valine had a positive correlation with age. Most essential and conditionally essential amino acids showed a negative correlation with age, indicating a decrease in these amino acids with age in children with suspected hereditary metabolic diseases (Table 3).

Table 3. Statistical analysis according to age (Spearman's correlation) and gender (Mann-Whitney U test)

Analyte	Spearman correlation		Male N=105		Female N=95		p-values
	ρ	p-values	Median (μmol/L)	Range	Median (μmol/L)	Range	
5-Oxo Pro	-0,364	0.000	39.88	20.30;103.51	26.76	19.09;90.46	0.137
Arg	0.619	0.000	22.99	14.99;37.55	23.36	15.94;46.42	0.676
Gly	-0.613	0.000	473.46	325.25;629.45	505.17	384.91;632.76	0.324
Ile	-0.196	0.005	57.28	53.07;62.04	58.61	50.11;67.07	0.233
Leu	-0.411	0.000	144.02	114.61;172.50	146.67	125.56;183.24	0.407
Met	-0.153	0.032	26.32	20.32;53.62	32.26	22.90;62.16	0.035
Phe	-0.514	0.000	54.16	43.70;62.82	55.80	45.10;67.10	0.262
Pro	-0.402	0.000	148.43	115.95;175.46	150.76	122.32;171.50	0.544
Tyr	-0.598	0.000	83.81	56.57;120.23	89.03	61.95;140.54	0.058
Val	0.053	0.459	124.03	104.00;145.08	125.82	106.94;155.84	0.326

Significant differences between groups of children with suspected female and male IEM were found only in the concentration of methionine in dried blood spots (Table 4). The highest values were determined in female group.

Table 4. Statistical analysis according to body weigh (Spearman's correlation) in group A.

Analyte	5-Oxo Pro	Arg	Gly	Ile	Leu	Met	Phe	Pro	Tyr	Val
ρ	0.463	-0.011	0.129	0.075	0.068	-0.181	0.066	0.118	0.008	0.236
p-values	0.000	0.813	0.075	0.301	0.389	0.009	0.405	0.078	0.857	0.000

The effect of body weight on the concentration of essential and conditionally essential amino acids in dried blood spots of children with suspected IEM was assessed (Table 4). There were no significant correlations between amino acid concentrations and body weight in groups B and C. In group A (newborns), significant weak positive correlations were noted between body weight and the concentration of hydroxyproline and valine and a weak negative correlation for the concentration of methionine.

The influence of factors such as age, gender, and body weight on the concentration of metabolites, including amino acids, in the blood of children was reported by Manta-Vogli et al. (2020), Sarker et al. (2019), Dietzen et al. (2016), Yu et al. (2018), Uaariyapanichkul et al. (2018). In a study by Manta-Vogli et al. (2020) found an association between a number of amino acids, including essential and conditionally essential phenylalanine, leucine, tyrosine and glycine, in breastfed full-term infants and their birth weight. On the contrary, they found no relationship between birth weight and blood concentrations of the amino acids valine, methionine, citrulline and arginine (Manta-Vogli et al., 2020). However, in our study, no relationship was found between the levels of glycine, leucine, tyrosine and phenylalanine and body weight, but the concentration of hydroxyproline, methionine and valine in dried blood spots was found to be related to birth weight.

Conclusion

The results of this study may be important in determining thresholds for newborn screening and in selective screening for IEM in different age groups of the pediatric population.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, grant No. AP14869996.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as a poster presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

References

Aliu, E., Kanungo, S., & Arnold, G. L. (2018). Amino acid disorders. *Annals of Translational Medicine*, *6*(24), 471.

Dietzen, D. J., Bennett, M. J., Lo, S. F., Grey, V. L., & Jones, P. M. (2016). Dried blood spot reference intervals for steroids and amino acids in a neonatal cohort of the national children's study. *Clinical Chemistry*, *62*(12), 1658–1667.

Golbahar, J., Al-Jishi, E. A., Altayab, D. D., Carreon, E., Bakhiet, M., & Alkhayyat, H. (2013). Selective newborn screening of inborn errors of amino acids, organic acids and fatty acids metabolism in the Kingdom of Bahrain. *Molecular Genetics and Metabolism*, *110*(1-2), 98–101.

Han, L., Han, F., Ye, J., Qiu, W., Zhang, H., Gao, X., Wang, Y., Ji, W., & Gu, X. (2015). Spectrum analysis of common inherited metabolic diseases in Chinese patients screened and diagnosed by tandem mass spectrometry. *Journal of Clinical Laboratory Analysis*, *29*(2), 162–168.

Mak, C. M., Lee, H. C., Chan, A. Y., & Lam, C. W. (2013). Inborn errors of metabolism and expanded newborn screening: Review and update. *Critical Reviews in Clinical Laboratory Sciences*, *50*(6), 142–162.

Manta-Vogli, P. D., Schulpis, K. H., Loukas, Y. L., & Dotsikas, Y. (2020). Birth weight related blood concentrations of the neurotransmission amino acids glutamine plus glutamate, phenylalanine and tyrosine in full-term breastfed infants perinatally. *Journal of Pediatric Endocrinology & Metabolism: JPEM*, *33*(3), 367–373.

Phipps, W. S., Jones, P. M., & Patel, K. (2019). Amino and organic acid analysis: Essential tools in the diagnosis of inborn errors of metabolism. *Advances in Clinical Chemistry*, *92*, 59–103.

Piraud, M., Ruet, S., Boyer, S., Acquaviva, C., Clerc-Renaud, P., Cheillan, D., & Vianey-Saban, C. (2011). Amino acid profiling for the diagnosis of inborn errors of metabolism. *Methods in Molecular Biology (Clifton, N.J.)*, *708*, 25–53.

Sarker, S. K., Islam, M. T., Biswas, A., Bhuyan, G. S., Sultana, R., Sultana, N., Rakhshanda, S., Begum, M. N., Rahat, A., Yeasmin, S., Khanam, M., Saha, A. K., Noor, F. A., Sajib, A. A., Islam, A. B. M. M. K., Qadri, S. K., Shahidullah, M., Mannan, M. A., Muraduzzaman, A. K. M., Shirin, T., … Mannoor, K. (2019). Age-specific cut-off values of amino acids and acylcarnitines for diagnosis of inborn errors of metabolism using liquid chromatography tandem mass spectrometry. *BioMed Research International*, 3460902.

Syrlybayeva, L., Zharmakhanova, G., & Kononets, V. (2023). Age-specific reference values for amino acid content in dried blood spots in children in western Kazakhstan, measured by tandem mass spectrometry. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 12*, 116-123.

Uaariyapanichkul, J., Chomtho, S., Suphapeetiporn, K., Shotelersuk, V., Punnahitananda, S., Chinjarernpan, P., & Suteerojntrakool, O. (2018). Age-related reference intervals for blood amino acids in thai pediatric population measured by liquid chromatography tandem mass spectrometry. *Journal of Nutrition and Metabolism*, *2018*, 5124035.

Yang, C. J., Wei, N., Li, M., Xie, K., Li, J. Q., Huang, C. G., Xiao, Y. S., Liu, W. H., & Chen, X. G. (2018). Diagnosis and therapeutic monitoring of inborn errors of metabolism in 100,077 newborns from Jining city in China. *BMC Pediatrics*, *18*(1), 110.

Yu, T., Han, Y., Pu, X., Duan, H., Zhi, L., Zhang, Y., Lei, L., Zhao, Y., Cao, Q., & Xu, H. (2018). Performance of LC/MS/MS in analyzing multiple amino acids and acylcarnitines in dried blood spot of very low birth weight, low birth weight, and normal weight neonates. *Clinical Laboratory*, *64*(9), 1333–1339.

Zharmakhanova, G., Kononets, V., & Syrlybayeva, L. (2023). Selective screening for inborn errors of metabolism using tandem mass spectrometry in newborns of the west Kazakhstan: Pilot study. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 12*, 37-43.

Author Information

Gulmira Zharmakhanova
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan
Contact e-mail: *gmzh@list.ru*

Victoria Kononets
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

Lyazzat Syrlybayeva
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

Zhanylsyn Gaisiyeva
West Kazakhstan Marat Ospanov Medical University
Maresyev Street 68, Aktobe 030019, Kazakhstan

To cite this article:

Zharmakhanova, G., Kononets, V., Syrlybayeva, L., & Gaisiyeva, Z. (2024). Essential and conditionally essential amino acid profile in west Kazakhstan children with suspected inborn errors of metabolism. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13*, 119-127.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 128-131

ICGeHeS 2024: International Conference on General Health Sciences

Determination of miRNA Expression Levels in Bladder Cancer

Mehmet Aydin Dagdeviren
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract:Bladder cancer is the ninth most common cancer worldwide, with the highest incidence rates observed in men in some countries in Southern and Western Europe, North America, North Africa, or Western Asia (Antoni, S. et al., 2017). Although gender differences vary greatly between countries, incidence rates are lower in men than in women. According to the data announced by the World Health Organization in 2022: In European and Asian comparisons; This rate is 21.1% and 5.6% per 100,000 people, respectively, and 22.6% in Turkey (Globocan, 2022). MicroRNAs (miRNAs) are non-protein-coding, single-stranded RNA molecules of 18-25 nucleotides in length and constitute a class of endogenous small RNAs (Celik et al., 2013). Studies have shown that microRNAs may also function as oncogenes or tumor suppressors in Bladder cancer. Although the expression levels of microRNA 22 5p and microRNA 337 have been determined in many cancers, including lung, prostate and colon cancer, no studies have been found on their expression levels in bladder cancer patients. In our study, miRNA 22 5p and miRNA 337 5p expression levels in bladder cancer patients will be calculated quantitatively using the Real-Time PCR method. In the first stage of the study, samples will be taken from the bladder using the TUR method, one from healthy tissue and the other from cancerous tissue. miRNA will be isolated from the tissue samples taken, cDNA will be synthesized from the miRNA samples and expression levels will be determined with the Real-Time PCR method using miRNA 22 5p, miRNA 337 5p specific primers and U6 primer as the reference gene. The data will be analyzed and interpreted with the SPSS package program.This study was planned to determine whether these two miRNAs can guide early diagnosis and diagnosis in bladder cancer patients and to provide clinicians with diagnosis and treatment planning.

Keywords: Bladder cancer, miRNA, Real-time PCR, Expression

Introduction

Cancer is a disease that occurs at the cellular level. Clinically, cancer; It is defined as a condition that covers nearly a hundred complex diseases that behave differently depending on the cell type from which it originates. Cancer types differ depending on the patient's age at onset, cancer's growth rate, spread, stage, and response to treatment. However, all cancer types have common features at the molecular level, and these common features group them into a single category (Klug et al., 2011).

Cancer is one of the deaths with known causes both in the world and in our country, and it is an important public health problem as it is the second cause of death after heart and circulatory system diseases. It is also a chronic disease that is increasing worldwide and causing significant material, spiritual, social and economic losses in societies. Cancer causes the death of 8.2 million people in the world every year and affects all people by infecting 14 million people (Ergin et al., 2019).

Bladder cancer is a global health problem with incidence and prognosis that vary by gender. This type of cancer has a number of different molecular subtypes depending on whether the disease is non-muscle invasive or

muscle invasive. It is known that the mutation burden is higher in muscle-invasive disease than in non-muscle-invasive disease. Genes such as TERT, FGFR3, TP53, PIK3CA, STAG2, as well as genes involved in chromatin modification, are among those frequently mutated (Dyrskjøt, 2023). Bladder cancer can be divided into two categories based on genetic instability at the nucleotide and chromosomal levels. In order to understand the basic mechanisms of cancer development, it is necessary to know the DNA missmatch repair (MMT) system and its correlation with other systemic pathways. Zigeuner et al. (2006), they stated that cancer suppressor genes, namely the retinoblastoma (Rb) gene on chromosome 13q and the p53 gene on chromosome 17p, play an important role in the formation and progression of bladder cancer. In a study on bladder cancer, the finding of a deletion on chromosome 9 suggested that there were other cancer suppressor genes effective on chromosomes 3, 4, 8, 11, and 14 (Edge et al., 2010).

Two different groups of bladder cancer have been identified in the molecular pathogenesis of the disease. First group; They are localized, papillary and low-grade cancers resulting from urothelial hyperplasia. These cancers also exhibit one or more mutations along with the fibroblast growth factor receptor 3 (FGFR3) gene mutation. This histological type generally has a better prognosis; There is a relationship between mutant FGFR3 gene expression and lack of muscle invasion. The second group is; Bladder cancer consists of non-papillary and high-grade cancers (Ojea et al., 2007). The second group is; They defined bladder cancer as non-papillary and high-grade cancers.

This group of cancers develop on the basis of severe dysplasia and carcinoma in situ. They often exhibit multiple chromosomal anomalies, such as genetic alterations in the p53 pathway and loss of heterozygosity on chromosome 9. The long-term survival of these cancers is low due to the high rate of progression and muscle invasion (Johar et al., 2013). The main treatment for non-muscle invasive bladder cancer is transurethral resection; As standard treatment, radical cystectomy treatment is performed together with neoadjuvant chemotherapy. Accordingly, immune checkpoint inhibitors have been shown to help treat metastatic bladder cancer in both groups (Dyrskjøtet al., 2023).

The incidence of bladder cancer in men is 5 times higher than in women, and the average age of diagnosis is 65 years. More than 80% of these do not spread to the muscles. The remaining 20% of tumors are muscle-invasive tumors and have a less favorable prognosis. 5-year survival is 50%. When diagnosed early, the 5-year survival rate of bladder cancer is approximately 94%, so timely intervention can significantly increase the patient's possıblty of survival (Goodison et al., 2013).

While radical surgery is required for muscle-invasive bladder cancer, non-muscle-invasive bladder cancer can be treated more conservatively with transurethral resection of the tumor. However, more than 70% of bladder cancer patients experience recurrence of the disease within the first two years after diagnosis. If left untreated, this initially non-muscle-invasive bladder cancer may turn into muscle-invasive (Goodison et al., 2013). The possibility of recurrence of non-muscle-invasive bladder cancer makes it one of the most common cancers worldwide. Once treated, bladder cancer patients need to be under constant surveillance with routine cystoscopy examinations and cytology for early detection of new cancer development. The gold standard for the initial clinical diagnosis of bladder cancer is cystoscopic examination of the bladder. Cystoscopy is an invasive procedure that may require anesthetizing the patient and then performing a biopsy for histopathological diagnosis and staging (Trivedi, 2009). Voiding urinary cytology (VUC) currently remains the preferred method for non-invasive evaluation of patients other than cystoscopy (Trivedi, 2009). VUC is based on microscopic visualization of cancer cells shed in voided urine. Low-grade tumors and lower-stage tumors shed fewer cancer cells into the urine, and therefore the sensitivity of detecting these early-stage tumors with VUC ranges from 20% to 40% (Tetu, 2009). However, in this method, results cannot be obtained quickly because there may be differences between microscopic observers and diagnosis is difficult and relatively expensive.

What is miRNA? and its relationship with Bladder Cancer

miRNAs were first identified as genes that play a regulatory role in developmental timing events in a model organism, C. elegans (Kato & Slack, 2008). miRNAs are small non protein coding RNA molecules approximately 18-25 nucleotides long. These molecules affect many biological processes, including cellular. It is involved in proliferation, differentiation and apoptosis and plays important roles in normal development, physiology and disease (Celik et al., 2013; Chandrasekaran et al., 2019). Outside of these features, it has been determined that more than half of miRNA molecules are located in cancer-related gene regions or fragile regions in the human genome. In cancer development, miRNAs can act as oncogenes or tumor suppressors

depending on the mRNAs they target, so miRNAs appear to be regulators of tumor progression, metastasis, and invasion. (Celik et al., 2013; Saydam et al., 2011).

Uncovering new mechanisms and relationships will also contribute to the development of diagnosis and treatment methods. Urinary bladder cancer is the fourth most common cancer in the Western world. This cancer is initially limited to the mucosa or submucosa in approximately 75% of cases, and these cases are grouped as non-muscular invasive bladder cancer (NMIBC), while approximately 25% are referred to as muscle-invasive bladder cancer (MIBC). Standard initial diagnostic and prognostic evaluation of bladder cancer patients includes cystoscopy and histopathological analysis of biopsy samples. However, current prognostic markers such as tumor grade, stage, and size may not accurately reflect the clinical outcome. Therefore, new biomarkers need to be identified to improve the diagnosis and prognosis of different types of bladder cancer. One of these biomarkers is miRNA. miRNAs are small non-protein coding RNAs consisting of 19 to 24 nucleotides that regulate gene expression by degrading mRNAs post-transcriptionally or impairing translation abilities. The involvement of miRNAs in gene regulatory processes and their role in many diseases, including cancer, make them very attractive for diagnosis, prognosis and treatment in clinical practice. The increasing number of studies investigating bladder cancer specific miRNA expression profiles indicates increasing interest in searching for specific miRNAs to serve as diagnostic or prognostic biomarkers. All miRNA studies were based on analysis of miRNA expression and comparisons with reference genes. In bladder cancer profiling studies, reference genes RNU6B and RNU48 were used. In our study, RNU6B will be used as the reference gene. The RNU6B reference gene has been used and validated in most studies. Selection of validated reference miRNAs is important to obtain reliable miRNA expression data. The levels of miRNA 22 5p in mesenchymal stem cells and myocardial infarction, and the levels of miRNA 337 5p in gastric cancer and breast cancer were investigated. For this reason, considering the relationship of the two miRNAs with other types of cancer, it has been observed that their levels in bladder cancer have not been investigated. For this purpose, we aim to determine the expression levels of miRNA 22 5p and miRNA 337 5p in Bladder Cancer patients by Real-Time PCR method.

Conclusion

This study will be carried out to determine whether these two miRNAs can guide early diagnosis and diagnosis in Bladder Cancer patients and will provide preliminary information by providing preliminary information to clinicians and contributing to the literature on this subject.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

*This study was supported by Gaziantep University Scientific Research Project Unit. Project number: FEF.DT.22.27

References

Antoni, S., Ferlay, J., Soerjomataram, I., Znaor, A., Jemal, A., & Bray, F. (2017). Bladder cancer incidence and mortality: A global overview and recent trends. *European Urology*, *71*(1), 96-108.

Celik, D. A., Kosar, P. A., & Ozcelik, N. (2013). MikroRNA lar ve kanser ile iliskisi. *SDU Tıp Fakultesi Dergisi*, *20*(3), 121-127.

Chandrasekaran, A. R., MacIsaac, M., Dey, P., Levchenko, O., Zhou, L., Andres, M., & Halvorsen, K. (2019). Cellular microRNA detection with miRacles: microRNA-activated conditional looping of engineered switches. *Science Advances*, *5*(3), eaau9443.

Dyrskjøt, L., Hansel, D. E., Efstathiou, J. A., Knowles, M. A., Galsky, M. D., Teoh, J., & Theodorescu, D. (2023). Bladder cancer. *Nature Reviews Disease Primers*, *9*(1), 58.

Edge, S. E., Bryd, D. R.,. & Trittz, A. G.(2010). *AJCC cancer staging manual* (pp.347-376). New York, NY: Springer.

Ergin, A., Ozdilek, R., & Dutucu, N. (2019). 2012-2017 yılları arasında kadınlarda gorulen kanser turleri ve dağılımları: Bir universite hastanesi ornegi. *Kadın Saglıgı Hemsireligi Dergisi*, *5*(1), 1-21.

Goodison, S., Rosser, C. J., & Urquidi, V. (2013). Bladder cancer detection and monitoring: Assessment of urine and blood based marker tests. *Molecular Diagnosis & Therapy*, *17*, 71-84.

Johar, R.S., Hayn, M.H.,…& Stegemann, A.P. (2013). Compli- cations after robot assisted radical cystectomy: Results from the International Robotic Cystectomy Consortium. *European Urology*,*64*(1), 52-57.

Kato, M., & Slack, F. J. (2008). microRNAs: small molecules with big roles - C. elegans to human cancer. *Biology of The Cell*, *100*(2), 71–81.

Kaufman, D.S., Shipley, W.U., Feldman, A.S.(2009). Bladder cancer. *Lancet, 374*(9685), 239–249

Klug, W. S., Cummings, M. R., & Spencer, C.A. (2011). *Genetik kavramlar*. Ankara: Palme Yayıncılık.

Kumar, A., Kumar, R., Gupta, N.P.(2006). Comparison of NMP22 Bladder Chek test and urine cytology for the detection of recurrent bladder cancer. *Japanese Journal of Clinical Oncology, 36*(3),172–175.

Millan-Rodriguez F., Chechile-Toniolo G., Salvador-Bayarri J., Palou J,. Algaba F., Vicente-Rodriguez, J. (2000). Primary superficial bladder cancer risk groups according to progression, mortality and recurrence. *Journal of Urology, 164*(3), 680–684.

Nakamura, K., Kasraeian, A., Iczkowski, K. A., Chang, M., Pendleton, J., Anai, S., & Rosser, C. J. (2009). Utility of serial urinary cytology in the initial evaluation of the patient with microscopic hematuria. *BMC Urology*, *9*, 1-6.

Ojea, A., Nogueira, J. L., Solsona, E., Flores, N., Gómez, J. M. F., Molina, J. R., ... & CUETO Group. (2007). A multicentre, randomised prospective trial comparing three intravesical adjuvant therapies for intermediate-risk superficial bladder cancer: low-dose bacillus Calmette-Guerin (27 mg) versus very low-dose bacillus Calmette-Guerin (13.5 mg) versus mitomycin C. *European Urology*, *52*(5), 1398-1406.

Têtu, B. (2009). Diagnosis of urothelial carcinoma from urine. *Modern Pathology*, *22*(2), 53-59.

Trivedi D., & Messing, E.M.(2009) Commentary: the role of cytologic analysis of voided urine in the work-up of asymptomatic microhematuria. *BMC Urology*, *9*,13.

Zigeuner, R. E., Hutterer, G., Chromecki, T., Rehak, P., & Langner, C. (2006). Bladder tumour development after urothelial carcinoma of the upper urinary tract is related to primary tumour location. *BJU International*, *98*(6), 1181-1186.

Author Information

Mehmet Aydin Dagdeviren
University of Gaziantep, Department of Biology, 27310 Gaziantep, Turkiye
Contact e-mail: *aydin_dagdeviren@hotmail.com*

Mehmet Ozaslan
University of Gaziantep, Department of Biology, 27310 Gaziantep, Turkiye

To cite this article:

Dagdeviren, M.A., & Ozaslan, M. (2024). Determination of miRNA expression levels in bladder cancer *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS),*128-131

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2024

Volume 13, Pages 132-136

ICGeHeS 2024: International Conference on General Health Sciences

Prevention of Venous Congestion in Superficial Temporal Artery Pedicled Scalp Flaps Using Deep Temporal Fascia

Aydin Turan
Gaziosmanpasa University

Abstract: Background: Venous congestion is an important complication in superficial temporal artery pedicled scalp flaps. In this study, we added deep temporal fascia, including the middle temporal vein, to the flap pedicle to prevent venous congestion in these flaps. Methods: A horn-shaped superficial temporal artery pedicled scalp flap was used to repair scalp and facial defects in 16 patients with a mean age of 52 years. The flaps were raised together with the superficial and deep temporal fascia at the base of the pedicle. The flap sizes ranged from 9×4 cm to 23×7 cm, and the mean follow-up period was seven months. Results: Venous congestion did not develop in any of the flaps. Abnormal hair distribution developed in three patients and hematoma in one patient. In all of the patients, the donor site was closed primarily and there was no flap loss. Conclusions: In scalp flaps with a superficial temporal artery pedicle, venous congestion can be prevented, and elevated more safely by adding a deep temporal fascia to the flap pedicle.

Keywords: Scalp flap, Venous congestion, Horn-shaped skin paddle, Deep temporal vein, Deep temporal fascia

Introduction

Superficial temporal artery (STA) pedicle scalp flaps are important surgical tools for scalp and face repairs. The hairless areas on the upper face (such as the frontal area) and hairy areas on the scalp are elevated over the STA or its branches as scalp flaps (Ozdemir et al.,2002; Hiremath et al.,2022). The most important disadvantage of STA pedicle scalp flaps is that they often cause venous congestion (Ozdemir et al.,2002; Ausen et al.,2011; Tenna et al.,2013; Loh et al.,2019). This may occur since the superficial temporal vein (STV) can not be included in the flap pedicle because of its greater variation (Ausen et al.,2011; Loh et al.,2019). When the STV can not be included in the flap pedicle, the venous return of the flap is provided by the thin concomitant veins that run on both sides of the STA and its branches (Beheiry et al.,2007; Imanishi et al.,2002), thus, venous congestion is inevitable in large flaps. Therefore, the deep temporal fascia (DTF), including the middle temporal vein, was added to the flap pedicle to increase venous outflow in the flaps.

Anatomy

The STA is one of the terminal branches of the external carotid artery and it passes 15.55 to 16.68 mm anterior to the tragus and ascends in the superficial temporal fascia (STF) (Pinar et al.,2006; Jean-Philippe et al.,2021). The STV does not have as predictable a course as the artery and generally does not follow the artery except in the most proximal part (Ausen et al.,2011; Loh et al.,2019; Delgova et al.,1991). This peculiarity of the STV can be main cause of the venous congestion observed in flaps raised over the branches of the STA with a narrow fascial pedicle. Thin parallel concomitant veins arise from the STV and run along both sides of the frontal and parietal branches of the STA(Beheiry et al.,2007; Imanishi et al.,2002) . These concomitant veins are the only structures that provide a venous circulation, especially in an axial pedicle flap raised over the branches of the STA; however, this circulation is sometimes insufficient. The middle temporal vein (MTV) has an average diameter of 1.88 mm and runs parallel to the frontal branch of the STA, between the superficial and deep layers

of the deep temporal fascia. Middle temporal vein, which forms a venous plexus with deep and superficial temporal veins in the temporal region and creates an alternative venous drainage system for the temporal region (Yano et al., 2014;Tansatit et al.,2015).

Patients and Methods

Between 2021- 2023, 16 scalp and facial defects were repaired using a horn-shaped STA pedicled scalp flap in 16 patients (15 male and 1 female). The mean patient age was 52 (16–88) years, and the mean follow-up period was seven (2–12) months. The flap sizes ranged from 9 × 4 to 23 × 7 cm.

Surgical Technique

Operations were performed under general anesthesia in all the patients. A horn-shaped skin paddle scalp flap was planned on the STA. The length of the flap skin paddle was planned to be at least twice the length of the defect area (Figure 1, left). Because the distal half was used to cover the donor area. The flap skin island was dissected in the subfascial (superficial temporal fascia) plane, starting from the upper edge. When the superior temporal line (STL) was reached at the lower edge of the flap skin island, the deep temporal fascia was cut and added to the flap pedicle (Figure 1, right). The flap pedicle was dissected down to the zygomatic arch and narrowed to approximately 3 cm from top to bottom (Figure 2, left and right).The flap was sutured to the defect area and donor area was closed primarily (Figure 3, left).

Results

Venous congestion did not develop in any flap and all flaps healed without loss. One patient developed a hematoma under the pedicle, and 3 patients developed abnormal hair distribution.

Case Reports

Case 1. A 88-year-old male with basal cell carcinoma in the right periorbital region was referred for treatment. The lesion was excised within safe surgical margins. A 23 x 7 cm horn-shaped scalp flap was planned (Figure 1, left). After the flap skin island was elevated in the subfascial plane (Figure 1, right), the flap pedicle was dissected at its base along with the STF and DTF (Figure 2, left and right).

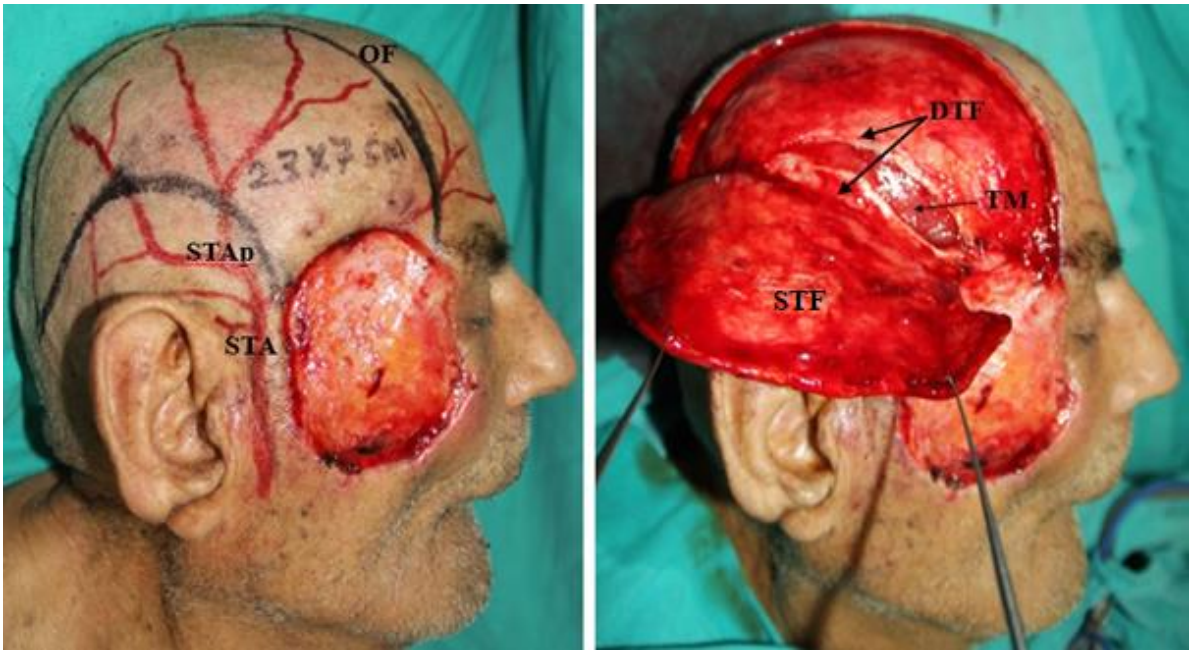


Figure 1. Intraoperative view of Case 1

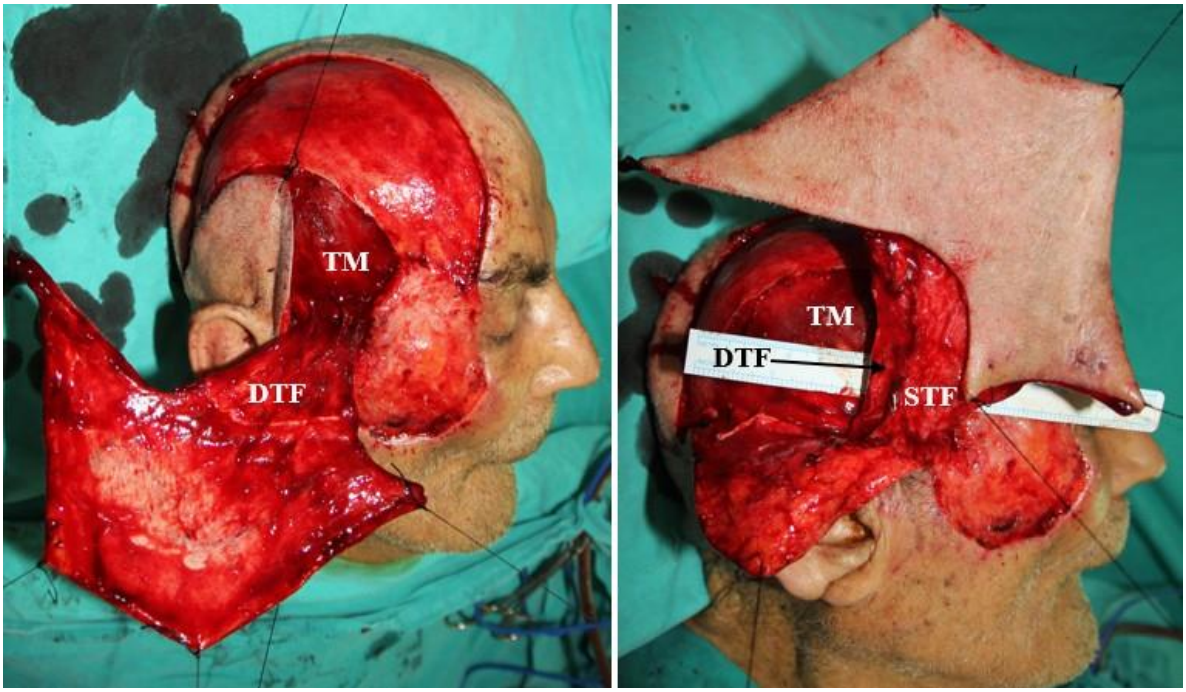


Figure 2. Intraoperative view of Case 1

The flap, whose elevation was completed, was sutured to the defect area and donor area was closed primarily (Figure 3.left). The flap healed without complications (Figure 3, right).

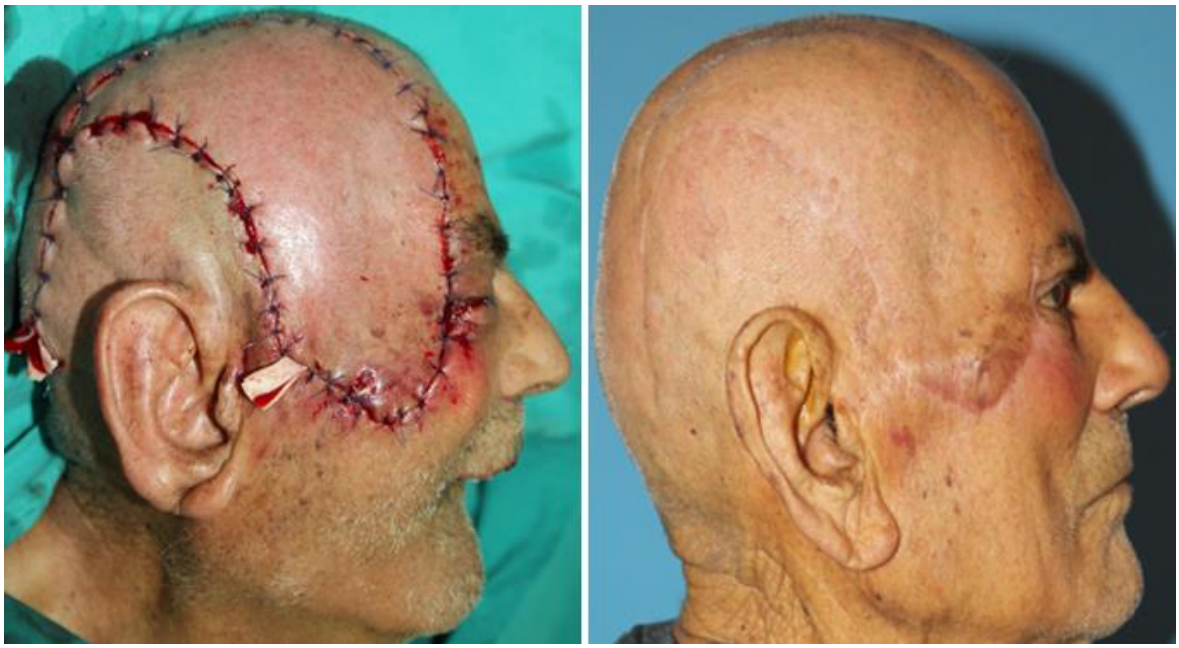


Figure 3. Intraoperative and postoperative view of Case1

Case 2. A 78-year-old male was referred for our clinic with basal cell carcinoma of the left posterior helix and posterior auricular region. After tumor excision, the defect area was closed with a 20 x 5.5 cm horn-shaped scalp flap. The defect and donor area healed without complications (Figure 4, left and right).

Discussion

STA pedicled scalp flaps are very useful for the reconstruction of the scalp and face. However, venous congestion is an important complication in these flaps. The insufficient venous drainage in STA pedicle flaps arises from the absence of the main collectors of the STV within the flap pedicle.

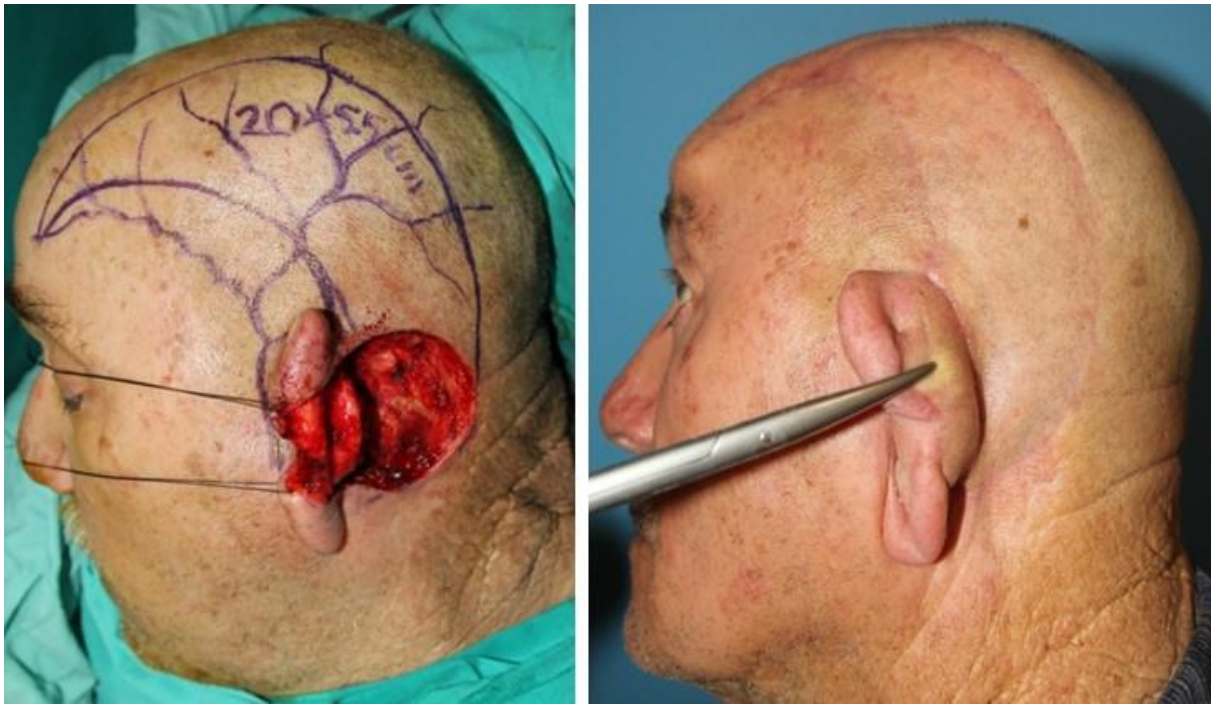


Figure 4. Intraoperative and postoperative view of Case 2

The primary reason for this is that typically, the branches of the STV run separately from the STA, except for the most proximal portion, particularly the frontal branch of the STA, which is not surrounded by a branch of the STV(Ausen et al.,2011; Loh et al.,2019;Imanishi et al.,2002; Delgova et al.,1991; Onishi et al., 2017). As a result, when raising flaps over the STA's frontal and parietal branches, the facial (STF) pedicle's width should be at least 2 cm (1 cm on each side of the artery) (Tenna et al.,2013). This ensures that the concomitant veins of these branches are included in the flap pedicle, which is necessary for proper venous circulation (Ausen et al.,2011;Tenna et al.,2013; Cao et al.,2021). However, these concomitant veins may not provide sufficient venous outflow in larger flaps.

To avoid the venous congestion seen in STA pedicled scalp flaps, we added deep temporal fascia to the flap pedicle. The addition of deep temporal fascia to the flap pedicle increased the venous outflow of the flap, thanks to the MTV it contains. Venous congestion did not develop in our flaps, because MTV serves as a potential alternative venous drainage system in the temporal region(Yano et al.,2014;Tansatit et al.,2015).

Conclucion

Large defects that are difficult to close with local flaps in a single session, can be closed with our flap in a single procedure.

Scientific Ethics Declaration

* The author declares that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the author.

* Gazi Osmanpasa University Faculty of Medicine Clinical Research Ethics Committee Decision No: 83116987-419, Project No: 23-KAEK-140, Meetin No: 2023/12, Meeting Date: 22.06.2023

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Alanya/Turkey on May, 02-05, 2024.

References

Ausen, K., & Pavlovic, I. (2011). Flaps pedicled on the superficial temporal artery and vein in facial reconstruction: A versatile option with a venous pitfall. *Journal of Plastic Surgery and Hand Surgery*, *45*(4-5), 178–187.

Beheiry, E. E., & Abdel-Hamid, F. A. M. (2007). An anatomical study of the temporal fascia and related temporal pads of fat. *Plastic and Reconstructive Surgery*, *119*(1), 136–144.

Cao, Z., Liu, L., Fan, J., Tian, J., Gan, C., Jiao, H., & Yang, Z. (2021). Aesthetic design of expanded bilateral superficial temporal vascular fascial pedicled flap for repairing maxillocervical scars. *Annals of Plastic Surgery*, *87*(5), 509–513.

Delgove, L., Lebeau, J., Raphaël, B., & Champetier, J. (1991). Drainage of the scalp by the superficial temporal vein: surgical implications. *Surgical and Radiologic Anatomy: SRA*, *13*(4), 277–282.

Hiremath, S., Boro, S. S., & Mathew, A. K. (2022). An experience of Scalp ısland flap based on the posterior branch of the superficial temporal artery for patients with mucormycosis and cancer. *Indian Journal of Otolaryngology and Head and Neck Surgery: Official Publication of the Association of Otolaryngologists of India*, *74*(Suppl 2), 3139–3144.

Imanishi, N., Nakajima, H., Minabe, T., Chang, H., & Aiso, S. (2002). Venous drainage architecture of the temporal and parietal regions: Anatomy of the superficial temporal artery and vein. *Plastic and Reconstructive Surgery*, *109*(7), 2197–2203.

Pinar, Y. A., & Govsa, F. (2006). Anatomy of the superficial temporal artery and its branches: Its importance for surgery. *Surgical and Radiologic Anatomy :SRA*, *28*(3), 248–253.

Jean-Philippe, H., Benoît, B., Francoise, K., & Michael, D. (2021). Anatomy and external landmarks of the superficial temporal artery using 3-dimensional computed tomography. *Surgical and Radiologic Anatomy: SRA*, *43*(2), 283–290.

Loh, C. Y. Y., Shanmugakrishnan, R. R., Nizamoglu, M., Tan, A., Duarte, M., Khan, W. U., & El-Muttardi, N. (2019). Venous congestion in pedicled frontal branch superficial temporal artery flaps reconstructions for head and neck defects: A review. *Annals of Plastic Surgery*, *82*(3), 330–336.

Onishi, S., Imanishi, N., Yoshimura, Y., Inoue, Y., Sakamoto, Y., Chang, H., & Okumoto, T. (2017). Venous drainage of the face. *Journal of Plastic, Reconstructive & Aesthetic Surgery: JPRAS*, *70*(4), 433–440.

Ozdemir, R., Sungur, N., Sensoz, O., Uysal, A. C., Ulusoy, M. G., Ortak, T., & Baran, C. N. (2002). Reconstruction of facial defects with superficial temporal artery island flaps: A donor site with various alternatives. *Plastic and Reconstructive Surgery*, *109*(5), 1528–1535

Tansatit, T., Apinuntrum, P., & Phetudom, T. (2015). An anatomical study of the middle temporal vein and the drainage vascular networks to assess the potential complications and the preventive maneuver during temporal augmentation using both anterograde and retrograde ınjections. *Aesthetic Plastic Surgery*, *39*(5), 791–799.

Tenna, S., Brunetti, B., Aveta, A., Poccia, I., & Persichetti, P. (2013). Scalp reconstruction with superficial temporal artery island flap: clinical experience on 30 consecutive cases. *Journal Of Plastic, Reconstructive & Aesthetic Surgery : JPRAS*, *66*(5), 660–666.

Yano, T., Okazaki, M., Yamaguchi, K., & Akita, K. (2014). Anatomy of the middle temporal vein: implications for skull-base and craniofacial reconstruction using free flaps. *Plastic and Reconstructive Surgery*, *134*(1), 92e–101e.

Author Information

Aydın Turan
Department of Plastic, Reconstructive and Aesthetic
Surgery, Gaziosmanpaşa University Medical School,
Muhittin Fisunoğlu street, campus
of the Ali Şevki Erek, 60100, Tokat, Türkiye
Contact e-mail: *aturanprs@yahoo.com*

To cite this article:

Turan, A (2024). Prevention of venous congestion in superficial temporal artery pedicled scalp flaps using deep temporal fascia. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 13,* 132-136