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

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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.

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ICMeHeS 2024: International Conference on Medical and Health Sciences

Examination Stress and Salivary Cortisol Levels in Kazakh and Indian Medical Students

Anastasia Kononets
Nazarbayev University

Gulmira Zharmakhanova
West Kazakhstan Marat Ospanov Medical University

Victoria Kononets
West Kazakhstan Marat Ospanov Medical University

Abstract: Stress is an important component of modern life and has become a serious problem for the health of the population as a whole. Studying at a medical university is accompanied by significant psycho-emotional stress among students. Medical students experience acute stress during exams. One of the markers of stress is cortisol. **Objectives:** to evaluate the level of cortisol in Indian medical students and Kazakhstan medical students during the examination stress. **Methods:** The study involved 134 medical students aged from 18 to 23 years, of which 81 were men, 53 women, studying in the 1st, 2nd and 3rd years of the West Kazakhstan Medical University named after Marat Ospanov. Among them are 67 students from India (46 men and 21 women) and 67 students from Kazakhstan (35 men and 32 women). Groups for the study were formed by random sampling. Free cortisol concentrations in saliva were measured in a diagnostic laboratory using the standard kit "Cortisol Saliva ELISA" of the company "DBC". **Results:** No significant differences were found in the level of saliva cortisol under conditions outside exam stress (Cortisol 1) and saliva cortisol under exam stress conditions (Cortisol 2) between a group of Indian and Kazakhstan students without regard to year of study. The level of free saliva cortisol under conditions outside exam stress is significantly higher in the general group of women compared to the general group of men. Increased salivary cortisol levels under examination stress were observed in all groups and examined. **Conclusion:** Identification of a contingent of students with high levels of cortisol (i.e., more susceptible to stress) will allow us to develop and recommend a set of measures that will help reduce cortisol levels and neutralize its pathogenic effect on the functioning of the main systems of the body.

Keywords: Exam stress, Students, Cortisol, Saliva, ELISA

Introduction

Stress is an important component of modern life. This has become a serious problem for the health. The transition from school to university education is a process that induces significant mental and emotional stress (McEwen, 2007; Conley et al., 2015; Inostroza et al., 2024). The transition from school to university education is a process that requires significant mental and emotional stress. Exam stress is a special form of emotional stress. The process of studying at the university can be considered as chronic stress, the severity of which depends on a number of factors (including social factors - living conditions, material status, family situation, as well as individual characteristics). Education at a medical university is accompanied by significant psycho-emotional stress among students (Al-Ayadhi, 2005; Daya & Hearn, 2018; Wang et al., 2024).

Students of the medical university experience acute stress during the exams, which adversely affects their performance, and can also lead to the development of pathological conditions (Daya & Hearn, 2018; Kulsoom & Afsar, 2015; Fawzy & Hamed, 2017).

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Depression leads to increase in the secretion of ACTH and activation of the hypothalamic pituitary adrenal axis (HPA), which increases the level of cortisol. Activation of HPA is an adaptive mechanism that allows the human body to maintain physiological stability in response to signals of general stress (Schulkin, 2011; An et al., 2016; Basilicata et al., 2023).

The change in forms and places of training is usually accompanied by the development of stress (Parent et al., 2019). It is known that the change of place of residence, and long journeys over long distances are stress factors and are accompanied by an increase in cortisol concentration in saliva (Stevens et al., 2018). Foreign students who come from a region with different climatic and geographic characteristics experience additional stress factors in comparison with students studying in their home country.

The aim of this study was to evaluate the level of cortisol in Indian and Kazakh medical students during the examination stress. To solve the aim, we staged the following tasks:

1. To identify differences in cortisol levels between groups of students from Kazakhstan and India in normal and exam-stress conditions.
2. To compare cortisol levels among first-, second-, and third-year students under normal conditions and during exam stress.
3. To explore the relationship between cortisol levels and gender.

Method

Study Participants

The study involved 134 medical students from the age of 18 to 23 years (81 men, average age 19.84 ± 1.65 years); 53 women (average age 19.68 ± 1.53 years old) enrolled in 1, 2, and 3 courses at the West Kazakhstan Medical University, among them 67 Indian students (46 men and 21 women) and 67 Kazakh students (35 men and 32 women). Groups for the study were formed by random sampling. According to the results of the conclusions of the doctors, all the subjects at the time of the examination did not have acute diseases and chronic neurological, psychiatric and somatic pathology. Exclusion criteria were also smoking, the use of psychoactive substances and drugs, pregnancy.

Methods

Considering a number of difficulties associated with the determination of serum cortisol — invasiveness, soreness, stressful conditions of the blood sampling procedure, preference was given to the method of analysis of salivary cortisol. Saliva was used as a material for the study on the determination of cortisol concentration (indicator "free cortisol of saliva" (ng/ml). Saliva samples were collected in Eppendorf-type tubes from chemically inert material. Sampling for cortisol was performed twice - one month before the exam (Cortisol 1 indicator) and during the exam (Cortisol 2 indicator). The time of the survey was from 9 to 10 a.m. The survey was performed on an empty stomach. Eating, drinking, chewing gum and brushing teeth 3 hour before sampling were excluded. Saliva samples were frozen and stored at -25°C until analysis. Determination of the concentration of free cortisol in saliva was performed in the diagnostic laboratory using the standard set "Cortisol Saliva ELISA" of the company "DBC".

Statistical Analysis

Statistical processing of the results was carried out using descriptive statistics methods. While analyzing intergroup differences for independent samples, we used the non-parametric Mann-Whitney test (U-test), the Wilcoxon test, the Kruskal-Wallis test implemented in the program Statistica 10.0.

Results and Discussion

The data in Table 1 indicate an absence of significant differences between groups of Indian and Kazakh students (without gender and year of study) in the level of saliva cortisol under normal conditions and examination stress conditions.

Table 1. Salivary free cortisol levels in Kazakh and Indian students

Indicators	Indian students (n = 67)	Kazakh students (n = 67)	p
Cortisol 1 (one month before the exam), ng / ml	59.36±25.09	61.99±23.76	0.59
Cortisol 2 (during the exam), ng / ml	71.48±21.62	74.29±20.96	0.29

The level of free salivary cortisol under normal conditions outside exam stress (Cortisol 1) was significantly higher in the general group of women (Figure 1), in the general group of second-year students (Figure 2), and also in a group of Indian first-year students in comparison with a group of Kazakhstan's first-year students (Table 2).

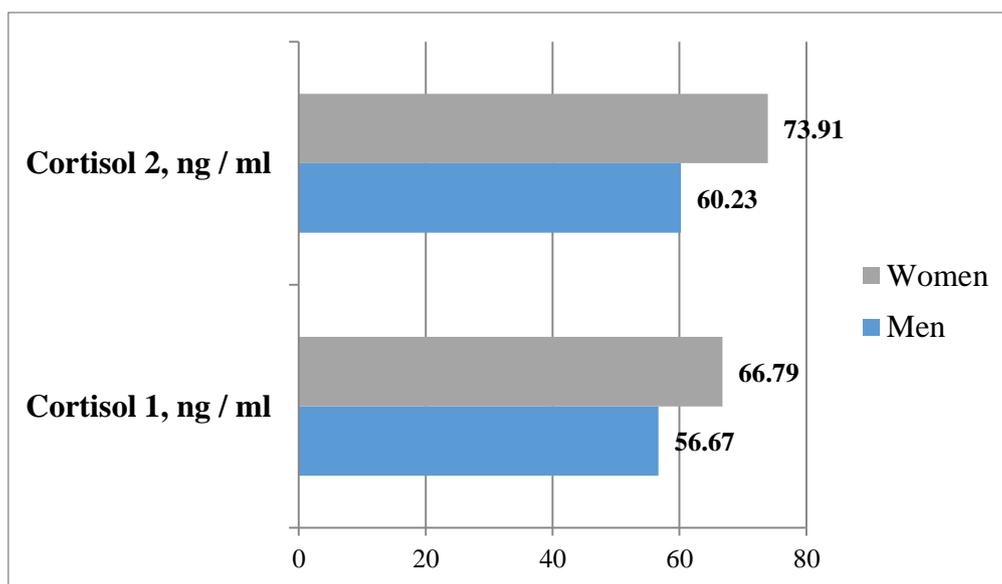


Figure 1. The level of free salivary cortisol in groups of male and female students

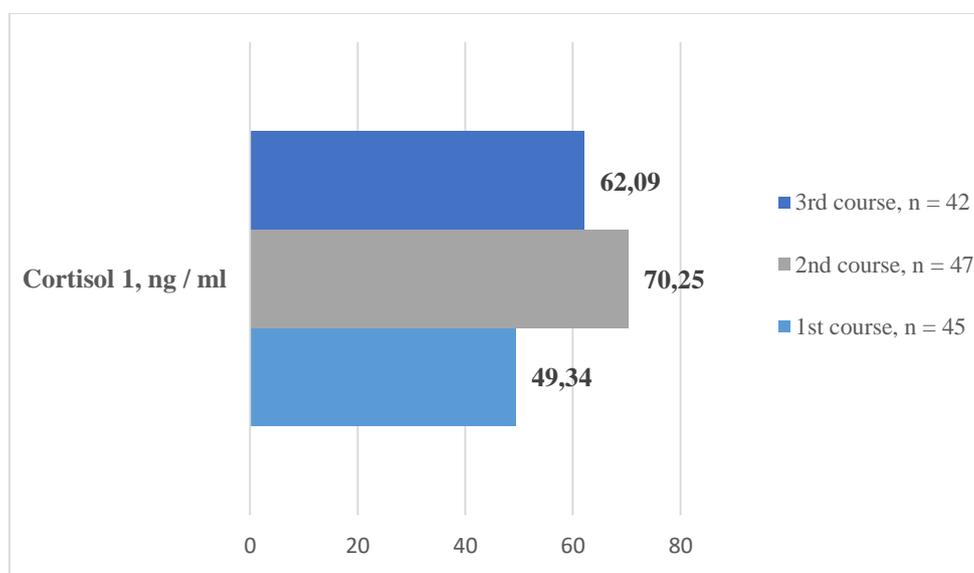


Figure 2. The level of free salivary cortisol in groups of students 1-3 courses

Attention is drawn to the significantly lower level of Cortisol 1 in Kazakhstan first-year students in comparison with Indian first-year students (Table 2).

Table 2. The level of free salivary cortisol in groups of Kazakhstan and Indian students of 1-3 courses

Indicators	Indian students			Kazakh students		
	1 st course (n = 24)	2 nd course (n = 23)	3 rd course (n = 20)	1 st course (n = 21)	2 nd course (n = 24)	3 rd course (n = 22)
	1	2	3	4	5	6
Cortisol 1 (one month before the exam), ng / ml	58.65 ±27.27	65.70 ±20.61	52,90 ±26.48	38.70 ±18.29	74.62 ±16.18	70.45 ±19.22
	p 1-4 = 0,006**		p 3-6 = 0,023*	p 4-5 = 0,000***	p 4-6 = 0,000***	
Cortisol 2 (during the exam), ng / ml	70.34 ±23.01	67.82 ±21.15	77.07 ±20.35	67.08 ±24.48	81.80 ±14.93	72.98 ±21.15
	p 2-5 =0,0041**					

Note: p - statistically significant differences between groups (* p <0.05; ** p <0.01; *** p <0.001;)

A significant increase in the level of salivary cortisol under exam stress (Cortisol 2) was observed in all groups of subjects, with the exception of a group of second-year students (Figure 3).

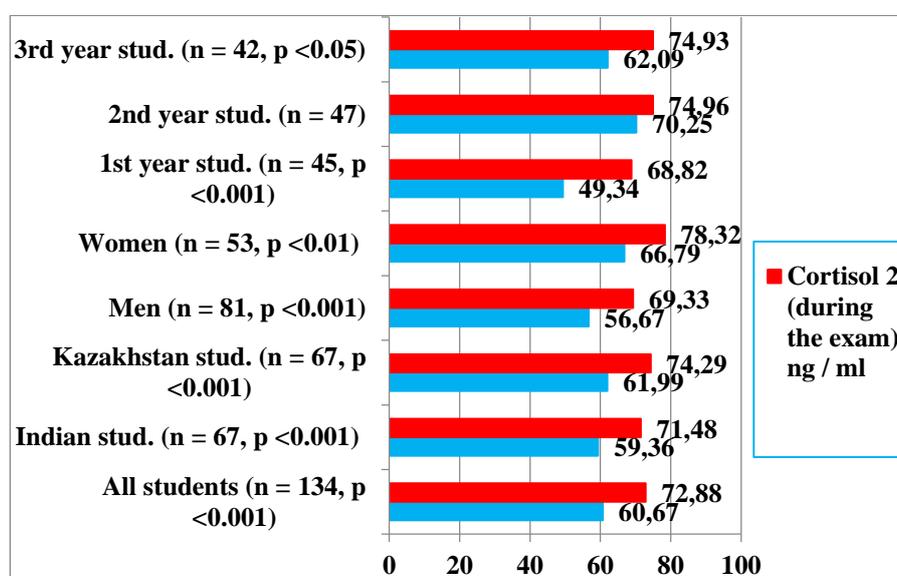


Figure 3. The level of cortisol outside exam stress and during exam stress in different groups of students

Analysis of data taking into account gender indicates a significantly higher rise in Cortisol 2 in the total group of women compared to the group of men (Figure 1). A higher level of cortisol in the group of women compared with the group of men, obviously, may be associated with the influence of sex hormones. These data are consistent with research data reporting a higher concentration of cortisol in groups of women compared with men (Welker et al., 2014; Labad et al., 2015). Given the differences between the group of women and men in terms of Cortisol 1, the increase in Cortisol 2 in the group of women compared to the group of men is quite natural.

The high level of Cortisol 1 among Indian first-year students in comparison with Kazakh first-year students is noteworthy. However, the level of Cortisol 1 in Indian first-year students did not significantly differ from the corresponding indicator in Indian students of the 2nd and 3rd courses, while the level of Cortisol 1 in Kazakh first-year students was significantly lower than this indicator of Kazakhstan students of the 2nd and 3rd courses.

It should be noted that the collection of samples under non-stress conditions was carried out during the 1st semester, before the first examination session. Undoubtedly, the examination session is a period of time during which students are exposed to a significant number of stress factors. First-year students had no experience in the examination session, which may explain their low Cortisol 1 in comparison with groups of students of 2nd and

3rd courses. Significantly high Cortisol 1 in Indian first-year students in comparison with a group of Kazakh first-year students can probably be explained by the influence of a combination of social and physical factors acting on them in a relatively unfamiliar environment. Education at a medical university is traditionally considered one of the most difficult, so it is only natural that the examination session provokes the formation of acute stress, which is manifested by an increase in Cortisol 2 in all groups of students surveyed.

Conclusion

No significant differences were found in Cortisol 1 and Cortisol 2 between a group of Indian and Kazakh students irrespective of year and gender. The level of Cortisol 1 and Cortisol 2 is significantly higher in the general group of women compared to the general group of men. The level of Cortisol 1 is significantly lower in the general group of first-year students, as well as in the group of Kazakhstan's first-year students in comparison with the group of Indian first-year students. Increased salivary cortisol levels under examination stress Cortisol 2 were observed in all groups.

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 Medical and Health Sciences (www.icmehes.net) held in Antalya/Turkey on November 14-17, 2024.

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Author Information

Anastasia Kononets

Nazarbayev University
Ave. Kabanbay Batyr 53, Astana 010000, Kazakhstan
Contact e-mail: anastassiya.kononets@nu.edu.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

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Epidemiological, Clinical and Therapeutic Profile of Rectal Adenocarcinomas Treated in the Radiotherapy Department of EHSO Emir Abdelkader

Bouhadiba Afaf

Ministry of Health, Population and Hospital Reform, Algeria

Abstract: Rectal cancer is classified among the most common cancers in the world, the second most common digestive cancer in Algeria; however its incidence in our country remains lower than that of western countries. The interest of this article is the retrospective analysis of the epidemiological, clinical and therapeutic aspects of malignant tumors of the rectum managed in the department of the EHS oncology Emir Abdelkader in Oran, Algeria. Concomitant preoperative radio chemotherapy is the standard treatment for locally advanced rectal adenocarcinomas; however it can be carried out postoperatively if the initial stage of the disease is underestimated. We conducted a retrospective epidemiological, clinical, anatomopathological, therapeutic and prognosis study at the radiotherapy department of the EHS oncology Emir Abdelkader in Oran over a period of 6 years from January 2017 to December 2019 involving 70 patients taken in charge during this period. This hypo fractionated irradiation regimen at a dose of 39 Gray could be a therapeutic alternative in the preoperative situation for locally advanced rectal tumors with promising results in terms of effectiveness and an acceptable toxicity profile.

Keywords: Cancer, Epidemiological, Anatomopathological, Radiotherapy, Therapeutic.

Introduction

Rectal cancer is a common disease. It reaches nearly 15,000 new people each year, slightly more men (53%) than women (47%). In more than 9 cases out of 10, it occurs after 50 years. Most of the time, rectal cancers develop from a benign tumor (non-cancerous) called an adenomatous polyp or adenoma. These benign tumors are very common and generally remain harmless. But 2 to 3% of them develop, grow and eventually turn into cancer. This transformation is slow, it takes on average more than 10 years. Evidence is accumulating that rectal cancer is distinct from colon cancer with different aetiologies and risk factors (Aleksandrova et al., 2014; Kirkegaard et al., 2010), possibly reflecting different environmental exposures.

High body mass index, body or abdominal fatness and diabetes type II are seen as risk factors. Longstanding ulcerative colitis and Crohn's disease affecting the rectum, excessive consumption of red or processed meat and tobacco as well as moderate/heavy alcohol use increase the risk. A healthy lifestyle and exercise can reduce the risk of developing rectal cancer (Murphy et al., 2012; Valentini et al., 20156). Consumption of garlic, milk, calcium and high dietary fibre are regarded as protective (Clinton et al., 2020). Although regular use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with reduced incidence, and there may be a protective effect of vitamin D via antitumour immunity, no formal guidelines for pharmacological primary prevention should be advised. Rectal cancer is classified among the most common cancers in the world, the second most common digestive cancer in Algeria; however, its incidence in our country remains higher than that of occidental countries.

The aim of this study is to evaluate the effectiveness and tolerance of preoperative hypo fractionated radiotherapy (associated or not with chemotherapy) in the treatment of locally advanced rectal cancer.

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Materials and Methods

Seventy patients received preoperative hypo fractionated radiotherapy from January 2017 to December 2019; fifty-three (75.7%) received induction chemotherapy followed by chemo radiotherapy, twelve (17.1%) received chemo radiotherapy and five (7.1%) received exclusive radiotherapy irradiation was three –dimensional conformation delivered at the dose of 39gray in 13fractions on the PTV.

Therapeutic

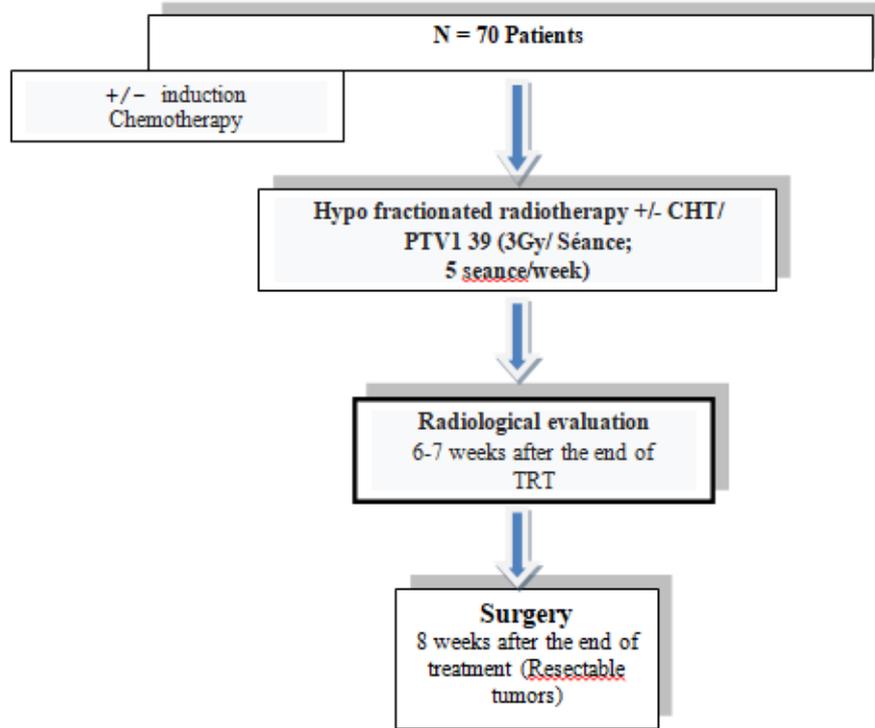


Figure 1. Therapeutic

Results and Discussion

The median age was 58 years (range23-85 years). The histology was dominated by adenocarcinoma with a rate of 98.6%.sixty-two patients (88.6%) were stage III, four (5.7%) stage II and four (5.7%) stage IV according to the American joint commission on cancer (AJCC). Forty-one patients (58.6%) were operated on the histological results of the surgical specimens were in favor of a complete reponse in 17.1% and stages I, II, III in 46.3%, 12.2% and 24.4% respectively with a median follow-up of 27 months (range 6-40 months). During treatment, grade 1 and 2 radio dermatitis according to RTOG was observed in thirty-three patients (47.1%), grade 1 and 2 radiation anitis in twenty-seven (38.6%), digestive complications grade 1 in fifteen patients (21.4%). Lately, digestive discorders were noted in six patients (8.6%), sexual in four (5.7%) and urinary in four patients as well (5.7%). The survival rate without loco regional recurrence at 24 months was 93.1% .the overall survival rate at 24 months was 87.7%.

Median follow-up: **26.3 months**



2 Loco regional relapses
14 Metastatic relapses
19 deaths

Table 1. Patient characteristics.

	n (%)
Total Number of patients (2017-2019)	70 (100)
Age (years): Median	58
Interval	23-85
Sex: Female	25 (35.7)
Male	45 (64.3)
Seat(IRM):	
Down	21 (30)
Medium	7 (10)
Low-medium	20 (28.6)
Medium-high	20 (28.6)
Low-medium-high	2 (2.9)
Stadiums(AJCC2017)	
I	4 (5.7)
III	62 (88.6)
IV	4 (5.7)
Induction chemotherapy:	
Yes	53 (75.5)
No	17 (24.3)
Surgery:	
Yes	41 (58.6)
No	29 (41.4)
Histology of the surgical specimen:	
Stage 0	7 (17.1)
Stage I	19 (46.3)
StageII	5 (12.2)
Stage III	10 (24.4)

Table 2. Acute toxicity observed

Grade 1-2	n (%)
Radiodermatitis	33(47.1)
Anite	27(38.6)
Ileitis	27(38.6)
Cystitis	15(21.4)

Table 3. Late toxicity observed

Grade 1-2	n (%)
Digestive disorders	6 (8.6)
Sexual complications	4 (5.7)
Urinary complications	4(5.7)

Table 4. Survival rate

	Rate at 18 months	Rate at 24 months	Rate at 36 months
Survival without loco regional recurrences	96.7% (+/-0.2%)	93.1% (+/-0.3%)	93.1% (+/-0.3%)
Overall survival	89.8%(+/-0.3)	87.7% (+/-0.4%)	55.7% (+/-0.9%)

Conclusion

This treatment with hypo fractionation at 39gray may be a preoperative therapeutic alternative for locally advanced rectal tumors with promising results in terms of efficacy and an acceptable toxicity profile.

Scientific Ethics Declaration

The author declares 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 Medical and Health Sciences (www.icmehes.net) held in Antalya/Turkey on November 14-17, 2024.

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Author Information

Bouhadiba Afaf

Ministry of Health, Population and Hospital Reform,
Algeria
Contact e-mail: afafbouhadiba@gmail.com

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Janus Nanoparticles Loaded 3D Bio Printed Scaffold as a Dual Drug Delivery System

Mahinour Elmowafy

Egypt-Japan University for Science and Technology
Alexandria University

Samir El-Mashtoly

Leibniz Institute of Photonic Technology

Ahmed Khalil

Fayoum University
Egypt-Japan University of Science and Technology

Amr Nassrallah

Egypt-Japan University of Science and Technology

Abstract: Herein, a new type of controlled-release system is fabricated using polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles for the creation preparation study. They include doxorubicin (DOX), a standard chemical for chemotherapy, and curcumin, part of the plant turmeric. The material is produced as a dual-loaded nanoparticle system, where the widely used chemotherapeutic drug Doxorubicin (DOX) and the commonly known anti-inflammatory/antioxidant Curcumin are encapsulated in their respective phases, PCL and PLGA. Next, the Janus NPs were loaded into a 3D bioprinted support matrix composed solely of Carbopol. The design of this scaffold was engineered to provide a biocompatible and supportive environment for the controlled release of medication over an extended period. The biocompatibility, kinetics of drug release, and biomechanical properties were also analyzed in the 3D-printed scaffold. The structural integrity of the scaffold was strengthened, and DOX as well as curcumin can be continuously released for long periods by using Janus nanoparticles. Moreover, dual drug-loaded nanoparticles provided a sustained release plan that could be an innovative technique for improved anticancer activity in synergy therapy; the scaffold supported particle stability as indicated by favorable results of preliminary research. This system might offer a more potent and target-oriented strategy in cancer therapy through the sustained release of curcumin and DOX, effectively reducing cancer cell viability compared to conventional drug delivery approaches.

Keywords: Janus nanoparticles, 3D bioprinting, Drug delivery

Introduction

Each year millions of new cases are identified, making cancer one of the most significant global health issues. Although significant strides have been made in the scientific understanding of cancer, treating it remains a daunting proposition fraught with the enervating attributes of conventional therapeutic modalities. Chemotherapy, one of the more common cancer treatments, has been effective in controlling or curing some cancers. However, its application might lead to severe side effects, including non-specific targeting, systemic toxicity, and resistance occurrence, which reduce the therapeutic effectiveness of this drug and increase patient morbidity. These challenges highlight an imminent need for more sophisticated precision and targeted drug delivery strategies to perpetuate treatment efficacy and ameliorate side effects. In the modern era, nanotechnology can offer solutions to these problems through the development of nanoparticle-based drug

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delivery systems. Not only does modifying nanoparticles improve a drug's solubility, stability, and bioavailability, but it also better directs the medication to cancerous regions. Janus NPs have one of the most unique biphasic structures, making them an interesting subset among other NP types. These nanoparticles have opposite top and bottom surfaces to which different functionalities can be added. Named after the two-faced Roman god Janus, their structural flexibility provides an attractive option for combinational cancer therapy by carrying multiple therapeutic drugs with diverse physicochemical properties and modes of action.

A novel drug delivery system is developed, fabricated, and described in this study, using polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles. Due to their biocompatibility, tunable degradation rates, and ability to encapsulate a broad range of therapeutic agents, PCL and PLGA have been extensively studied for drug delivery applications. In this system, the Janus nanoparticles have dual therapeutic components with different and complementary anticancer activities: curcumin (CUR) and doxorubicin (DOX). The turmeric plant produces a polyphenol known as curcumin, which has long been notable for its anti-inflammatory, antioxidant, and antimicrobial effects. It has been shown to induce apoptosis, inhibit cancer cell proliferation, and enhance the effects of other chemotherapeutic drugs. However, the application of curcumin has been limited due to its poor solubility in water and susceptibility to degradation. In contrast, doxorubicin (DOX) is a widely used chemotherapeutic drug effective against various malignancies but is associated with severe adverse effects, including cardiotoxicity. To address the limitations of each therapeutic agent, DOX is encapsulated into PLGA, and curcumin is loaded onto PCL within the Janus nanoparticles, creating a dual-agent system with different mechanisms of action. This system is further enhanced by integrating these Janus nanoparticles into a 3D bioprinted scaffold matrix composed solely of Carbopol, which provides a biocompatible and supportive environment for the controlled release of encapsulated drugs. Carbopol, a synthetic polymer, is well-known for its stability and gel-forming properties, making it an ideal material for this scaffold. The present study details the mechanical behavior, biocompatibility, and drug release kinetics of this 3D bioprinted scaffold. Due to the higher stability of the scaffold combined with Janus nanoparticles, curcumin, and DOX can diffuse out steadily onto a solid substrate. The drug release profile was sequential, with DOX following curcumin. This sequential release is beneficial for synergistic cancer therapy since curcumin can sensitize the effect of DOX in killing cancer cells, potentially contributing to a more substantial therapeutic outcome. The study suggests that the scaffold enabled a controlled and prolonged release of curcumin as well as DOX. Furthermore, the results of this study suggest that an approach using a Janus-nanoparticle-based dual payload on 3D bio-printed scaffolds may lead to a long-term reduction in cancer cell viability by releasing drugs over extended periods, potentially offering a more targeted and efficient strategy compared with traditional drug delivery methods. The findings of this study provide insights into how this innovative drug delivery method may help overcome limitations associated with existing cancer therapies, bringing researchers closer to more personalized and definitive clinical strategies. This study offers a comprehensive overview of the original combination of Janus nanoparticles and 3D-printed drug delivery systems, highlighting its potential to enhance treatment efficacy and improve the quality of life for cancer patients by overcoming major challenges in anticancer therapy, including drug solubilization, protection from degradation before entering blood circulation, and specific targeting."

Method

Preparation of Janus Particles

Preparation of the First Emulsion (W1/O):

An aqueous phase (W1) was obtained by dissolving a hydrophilic drug (curcumin) in deionized water. In the second step, to produce organic phase (O) PLGA and PCL were each dissolved separately in dichloromethane (DCM). Then, to form primary emulsions (W1/O), the aqueous phase (W1) was dropwise added into the organic phase under stirring at 4000 rpm. The liquid was also emulsified with ultrasonication to form fine droplets. Following these steps, the sonication process will take two to five minutes depending on the desired droplet size.

Preparation of the Second Emulsion (W1/O/W2):

The external aqueous phase (W2) was prepared by dissolving polyvinyl alcohol (PVA) as an aqueous solution in deionized water. Thereafter, the first emulsion (W1/O) was added to the PVA solution while stirring at a high rate. The second emulsion (W1/O/W2) is composed of an organic phase containing PCL and PLGA spread in

the external aqueous solution forming. The second emulsion was prepared similarly using an ultrasonicator, yet again to ensure a uniform and regular (stable) final product. This sonication step enhances the encapsulation efficiency of drugs and decreases particle size further.

Solvent Evaporation:

The double emulsion (W1/O/W2) was then stirred at room temperature for several hours (4–6 h, typically), allowing the DCM to evaporate. Since the organic solvent evaporated, Solid Janus particles of PLGA/PCL are formed. Stirring was continued until the smell of DCM evaporating completely disappeared.

Particle Collection and Washing:

Janus particles produced were collected by centrifugation at 10,000 rpm for 10 min. Particles were washed to free the suspension of any remaining PVA and unincorporated drug after centrifuging particles at 2000 rpm for 10 min, ejecting out supernatant. Great care was taken to remove all contaminants, and the washing process involved three ‘washes.

Particle Freeze-Drying:

This was followed by drying of washed PLGA/PCL Janus particles in a small amount of deionized water (placed again under vacuum) and subsequent freeze-drying. Most of the time, you can expect it to take 24-48 hrs depending on how much suspension is being dried and the load configuration for your freeze dryer.

Characterization of the Janus Particles:

Janus particle drug loading efficiency and release kinetics after preparation were assessed. The method should provide a complete guide for preparing PLGA/PCL Janus particles using the double emulsion technique, which is capable of encapsulating both hydrophilic and hydrophobic drugs.

Preparation of Bioink for 3D Bioprinting

Formulation of Bioink:

For bioink preparation, Carbopol powder was dissolved in deionized water to produce a 0.5% (w/v) Carbopol solution. As a viscosity enhancer to ensure the homogeneity of the bioink and to achieve the desired consistency for the 3D bioprinting process, Carbopol alone was used, ensuring a smooth and consistent gel formation.

Nanoparticle Incorporation:

PLGA/PCL Janus nanoparticles loaded with CUR & DOX were distributed in the bioink and continuously stirred to create a homogeneous suspension.

3D Bioprinting Process

A 3D bioprinter INKREDIBLE+ was used with an extrusion-based printhead on a syringe platform. The bioink was then syringed into a chamber and extruded through silicon-based nozzles to construct 3D structures layer by layer. The printing parameters, such as pressure, speed, and layer height, were optimized for resolution and structural integrity. These 3D structures were subsequently crosslinked by submerging them in a 100 mM calcium chloride solution post-printing to ensure the mechanical stability of the printed construct

Evaluation of Drug Release and Biocompatibility

In Vitro Drug Release Studies

At 37°C, the printed structures were submerged in phosphate-buffered saline (PBS). With the use of UV-Vis spectrophotometry, the release of doxorubicin and curcumin was tracked throughout time.

Data Analysis

Statistical Analysis

Every experiment was carried out three times, and the results were shown as mean \pm standard deviation (SD). One-way ANOVA with post-hoc analysis was used to establish statistical significance ($p < 0.05$ was deemed significant).

Results and Discussion

Characterization of PLGA/PCL Janus Nanoparticles

Zeta Potential and Morphology

In the inverted microscope image, two hemispherical patches are shown corresponding to PLGA and PCL suggesting effective synthesis (Fig.1). The zeta potential of Janus nanoparticles was -25 mV, suggesting a moderate surface charge (Fig.2) This negative charge is useful, as in the end it stabilizes colloidal particles there and does not aggregate in the bioink matrix.

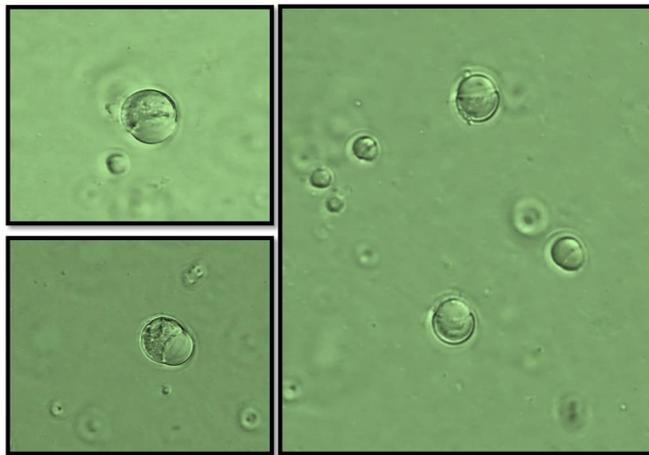


Figure 1. Image for PLGA/PCL Janus nanoparticles loaded with DOX and CUR

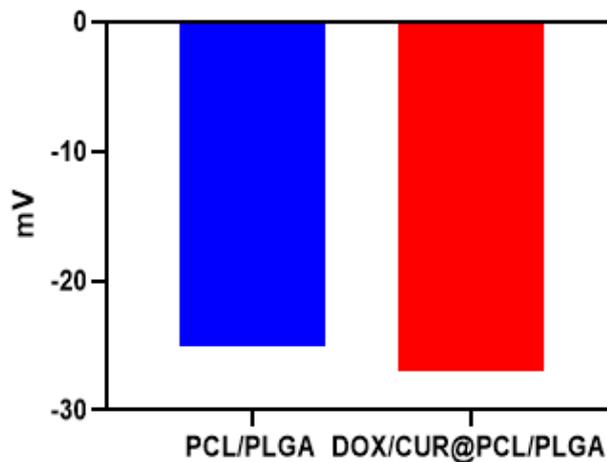


Figure 2. Zeta potential for PCL/PLGA Janus nanoparticles

Drug Encapsulation Efficiency

The enhanced bioink enabled high resolution and fidelity 3D bioprinting of different geometries such as grid, and circular/cylindrical shapes. The structures were crosslinked post-printing in a 0.6M calcium chloride solution to add mechanical strength, thus safeguarding the shape and integrity of objects after they had dissolved away all the PVA.

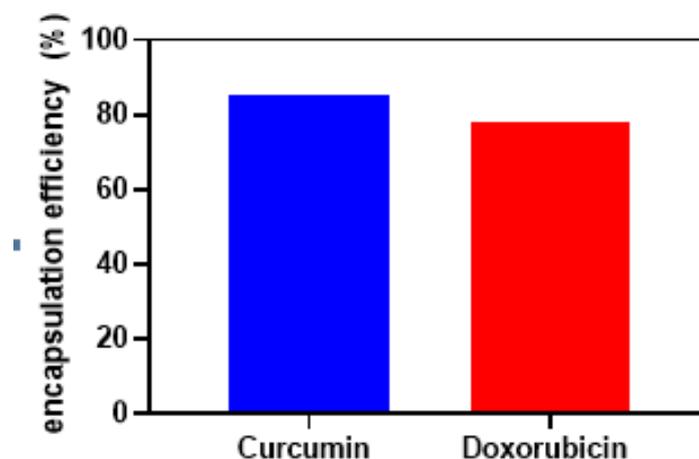


Figure 3. Encapsulation efficiency of CUR and DOX @ PLGA/PCL

Characterization of 3D Bioprinting of Constructs

Printability and Structural Integrity

The aqueous printable matrix was designed to print high-resolution and fidelity arbitrary geometries, like grids or cylinders (which imitate organoids) in 3D. Mechanical stiffness was enforced after printing with additional post-processing crosslinking in a calcium chloride solution that preserved the construction geometry.

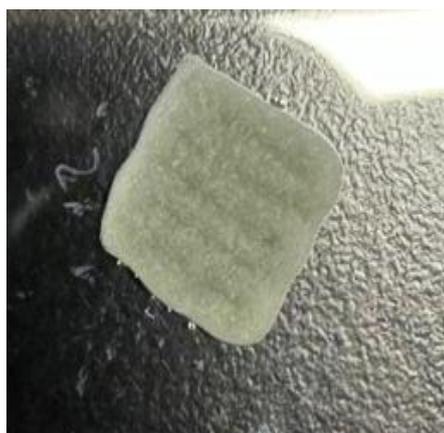


Figure 4. Picture for carbopol scaffold loaded with PCL/PLGA JNPS loaded with DOX and CUR.

In Vitro Drug Release Study

Cumulative Drug Release:

Biphasic release patterns for doxorubicin and curcumin were observed in the 3D-printed structures. A near zero-order release was found over 14 days, with an initial burst of surface-bound drug released in the first 24-48 hours. Such biphasic release assists in building a medicinal effect in no time, on the other hand, it provides an overextended existence of the drug. The PCL phase, in contrast with the PLGA phase, was more hydrophobic (and positively) toluene compatible which resulted in a slow release of Curcumin and doxorubicin released

faster because of its more aqueous solubility. This unique release profile demonstrates that Janus nanoparticles can alter drug-release kinetics, which could potentially be used to tailor treatment regimens (Fig.5).

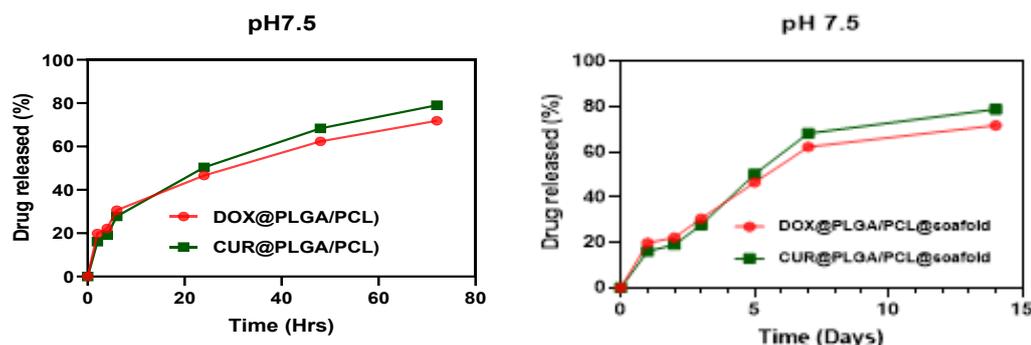


Figure 5. In vitro release of DOX and CUR from PLGA/PCL JNPS and PLGA/PCL JNPS@3d bio-printed scaffold

Conclusion

In the present study, a new multi-drug delivery system using 3D bioprinting was developed simply by mixing PLGA/PCL Janus particles with doxorubicin and curcumin in a Carbopol bioink. The Janus structure of the nanoparticles was able to package and release both hydrophilic drugs (DOX) and hydrophobic drugs (CUR), making it an even more flexible platform for combination therapy. By tuning the bioink's rheological properties for 3D bioprinting, we achieved higher levels of structural integrity and resolution. It was found that nanoparticles did not affect the mechanical properties of the bio-ink, nor the printing results, compared to non-nanoparticle formulations—a finding that supports this technique as an effective method for generating complex 3D structures. The system demonstrated a biphasic release profile, i.e., an initial burst followed by a sustained release in *in-vitro* drug release tests. This profile is key for maintaining therapeutic drug levels throughout a dosing interval. The cytotoxicity study on cancer cells demonstrated an enhanced apoptotic response from the two drug-loaded constructs, indicating that the combination therapy of doxorubicin and curcumin exhibited a synergistic effect. In conclusion, this study offers a blueprint for developing nanoparticles as an ultra-flexible and rapid medication delivery system using cutting-edge nanoparticle technology along with 3D bioprinting. These positive *in vitro* results provide a foundation for more thoroughly studying the therapeutic efficacy and safety of this approach in subsequent studies using relevant animal models. This method is an applicable strategy for targeted drug delivery and personalized medicine, where it could be extended to different medication combinations or therapeutic fields.

Recommendations

The study's conclusions lead to the following suggestions for further investigation and advancement in the area of 3D bio-printed medication delivery systems: Even though the *in vitro* results are encouraging, more *in vivo* research is necessary to assess the safety, biodistribution, and therapeutic efficacy of the PLGA/PCL Janus nanoparticles loaded with doxorubicin and curcumin. The goals of these investigations ought to be to ascertain the ideal dosage schedule and comprehend the pharmacokinetics and pharmacodynamics of the dual-drug system in a living being.

Scientific Ethics Declaration

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

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Author Information

Mahinour Elmowafy

Biotechnology Program, Egypt-Japan University of Science and Technology (E-JUST), New Borg El-Arab City, Alexandria 21934, Egypt.
Institute of Graduate Studies and Research, Alexandria University
Contact e-mail: Mahinour.saieed@ejust.edu.eg

Amr Nasrallah

Biotechnology Program, Egypt-Japan University of Science and Technology (E-JUST), New Borg El-Arab City, Alexandria 21934, Egypt.

Samir Elmashtoly

Leibniz Institute of Photonic Technology
Albert-Einstein-Straße 907745 Jena, Germany

Ahmed Khalil

Institute of Basic and Applied Sciences, Egypt-Japan University of Science and Technology (E-JUST), New Borg El-Arab City, Alexandria 21934, Egypt.
Environmental and Smart Technology Group, Faculty of Science, Fayoum University, Fayoum 63514, Egypt.

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Fabrication of a 2D-Surfactant Exfoliated Hexagonal Boron Nitride Nanoparticles as Efficient pH-Sensitive Drug Delivery System for Lung Cancer Therapy

Tartisio Olimpio De-Kenyi Hamuha

Egypt-Japan University of Science and Technology

Amr Abdelmotagaly NassrallahEgypt-Japan University of Science and Technology
Cairo University**Samir El-Mashtoly**

Leibniz Institute of Photonic Technology

Ahmed Saad Gomaa KhalilEgypt-Japan University of Science and Technology
University Fayoum University

Abstract: Lung cancer is one of the most prevalent cancers and it is also the primary cause of cancer-related mortality globally. However, due to variations in tobacco use patterns, exposure to environmental risk factors, and genetics, lung cancer incidence and mortality rates vary significantly worldwide. Since smoking is the primary risk factor for lung cancer, developing more effective therapeutic strategies and innovative drug delivery systems may help to raise the disease's survival rates. In this study, a novel pH-sensitive nanocarrier based on a composite of a two-dimensional hexagonal boron nitride (2D-hBN) with unique properties was synthesized to deliver Doxorubicin (DOX). Firstly, bulk hBN powder was exfoliated with a sodium cholate salt, sonicated, and centrifuged to obtain the as-prepared 2D-hBN nanocarriers, and finally, DOX was entrapped for targeted drug delivery and tumor therapy. High DOX loading and entrapment efficiency (LE% and EE%, respectively) were obtained. The EE% and LE% for sodium cholate exfoliated 2D-hBN obtained were 84.50% and 25.48%, respectively. *In vitro* drug release experiments demonstrated a pH-sensitive non-Fickian release profile with a release percentage of 73.5%. Preliminary *in-vitro* cytotoxicity was done via MTT assay, using the human lung adenocarcinoma cell line (A549), and the 2D-hBN@DOX nanocomposites were shown to drastically reduce the viability of the cancer cells compared to 2D-hBN, indicating the great efficacy of the former nanocomposites in hindering the proliferation of cancer cells.

Keywords: Boron nitride, Cancer treatment, Doxorubicin, Lung cancer, Sodium cholate

Introduction

Cancer is the second most common cause of disease-related death in humans globally, and it is predicted that 13.1 million people will die from cancer by the year 2030 (Siegel et al., 2024; Lu et al., 2018). Chemotherapy is a common therapeutic option, yet its effectiveness for other cancer types is limited (Fusser et al., 2019). This fundamental cancer treatment has serious side effects and damages normal, non-cancerous cells (Liao et al., 2014; Su et al., 2011). Drug delivery systems (DDS) are designed to reduce unwanted side effects (Prabhakar et al., 2013; Rapoport 2007; Malekimusav et al., 2019).

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DDSs are developed in attempts to regulate drug release in an effort to reduce cellular toxicity while maintaining therapeutic efficacy (Prabhakar et al., 2013; Rapoport 2007; Malekimusav et al., 2019; Amini-Fazl et al., 2019). The ingenious strategy uses pH differences between healthy and tumorous tissues to deliver the anti-cancer drug to the desired location while minimizing damage to surrounding cells (Amini-Fazl et al., 2019). Enhancing permeability and retention (EPR), which causes an increasing buildup of the anti-cancer medication in malignant tissue, is the foundation of DDS (Fusser et al., 2019; Liao et al., 2014; Su et al., 2011; Prabhakar et al., 2013; Rapoport 2007; Malekimusavi et al., 2019; Amini-Fazl et al., 2019; MacEwan et al., 2010) The EPR effect tacitly confers macromolecules to penetrate the interstitial spaces in cancer tissue, thus enhancing the overall efficacy of chemotherapeutic substances (Shi et al., 2017).

To develop DDSs, nanoscale anti-cancer drug carriers can be synthesized to facilitate regulated and prolonged release of anti-tumor drugs and enhance drug distribution (Shi et al., 2017; Arruebo et al., 2007; Merisko-Liversidge et al., 2011; Zhen et al., 2013; Azizi et al., 2018). Therefore, applying cutting-edge and effective methods to cancer therapy, such as nanoparticles-based DDS, would be advantageous in a number of ways (Sarani et al., 2024). Hexagonal boron nitride is an inorganic layered material with a single atom thin with alternating arrangement of B and N similar to graphene and black phosphorus (BP). Among nanoparticles, two-dimensional hexagonal boron nitride (2D-hBN) nanomaterials have several intriguing biological and chemical properties such as biocompatibility, antibacterial properties, chemical inertness, thermal stability, and surface functionalization that make them promising materials for diverse biomedical applications (Gautam et al., 2021; Santos et al., 2021). Because of these advantages, scientists and medical professionals may be interested in using such materials in nano-formulations to deliver therapeutics to malignant tumor sites (Feng et al., 2021).

Method

Fabrication of 2D-hBN Nanocomposites

Boron nitride powder (CAS 10043-11-5) ~1 μm , 98% was purchased from Sigma Aldrich and cholic acid sodium salt (CAS 206986-87-0) was purchased from Thermo Fisher (Kandel) GmbH, Germany. The 2D-hBN utilized herein was fabricated using a liquid exfoliation, sonication, and centrifugation technique based on surfactants, as in (Kurapati et al., 2016) with a few modifications.

Briefly, 0.05 g bulk boron nitride powder and 0.01 g sodium cholate salt were weighed and mixed with 50 mL aqueous solution of deionized (DI) water (pH 7.6) in a glass beaker to induce liquid phase exfoliation. The resulting dispersion was sonicated with a Daihan Scientific Ultrasonic Cleaner (60% Amplitude) for 1 h before centrifugation by Sigma 2-16P centrifuge at 5000 rpm for 90 mins. Subsequently, the dispersion's supernatant liquid was pipetted off and the remaining sediment was reagitated in a fresh aqueous solution of sodium cholate salt and DI water, 0.003 g in 50 mL, respectively. The reagitated sediment was then sonicated at the same previous settings for 5 h more. After the sonication treatment was finished, the solution was divided into aliquots and centrifuged for 90 minutes at 2000 rpm. The sediment that included the unexfoliated hBN was subsequently disposed of, and the leftover supernatant was centrifuged again for 90 minutes at 5000 rpm. Finally, upon removal of the forthcoming supernatant, the nanocomposites utilized herein were found to be surfactant-exfoliated 2D-hBN.

Drug Loading

Doxorubicin hydrochloride (DOX) 50 mg (EDA Reg. 24995/2007) was purchased from Hikma Specialized Pharmaceuticals, Badr City, Cairo, Egypt. The DOX loading experiment was done by dispersing 5 mg of the 2D-hBN nanosheets in DI water (pH 7.0) and sonicated at 60% Amplitude for 5 mins. Next, the dispersion and 5 mL (0.2 mg/mL) DOX solution in phosphate-buffered saline (PBS (pH 7.4)) were blended in a 1:1 ratio, and the mixture was magnetically agitated for 24 hours at room temperature in a dark cabinet. The obtained solution was dialyzed to remove unbound DOX molecules by centrifuging at 8000 rpm for 30 mins and resuspended in PBS (pH 7.4).

To determine the DOX entrapment efficiency (EE%) and the loading efficiency (LE%), the total amount of unbound DOX was ascertained by measuring the absorbance at 480 nm relative to the DOX standard calibration curve (Fig. 1 (c)) recorded under similar conditions. The EE% and LE% were computed according to the equations (1) and (2) below (Zavareh et al., 2020).

$$EE(\%) = \frac{(\text{Total amount of DOX}) - (\text{Free amount of DOX})}{(\text{Total amount of DOX})} \times 100 \quad (1)$$

$$LE(\%) = \frac{(\text{Total amount of DOX}) - (\text{Free amount of DOX})}{(\text{Total amount of 2D-hBN})} \times 100 \quad (2)$$

Drug Release

The *in vitro* release experiments were studied in a comparable way to that as in (Espinoza et al., 2022) at the absorbance of the collected buffer solution (PBS) at a wavelength of 480 nm. Briefly, the 2D-hBN@DOX pellets from the drug loading experiment dispersed in 5 mL PBS (pH 7.4) were centrifuged at 8000 rpm for 20 mins. Then, the supernatant from the solution was decanted by careful pipetting and a 1 mL sediment of the 2D-hBN@DOX was loaded into two different dialysis bags (having a molecular weight cut-off equals to 3.5 kDa) corresponding to the two pH 7.4 and 5.0. And the two bags were dialyzed in a 50 mL PBS buffer solution at 37 °C and agitated gently at 100 rpm in a dark cabinet. At predetermined intervals (0, 1, 2, 4, 6, 12, 24, and 48 h), 6 mL aliquots from each media were extracted from the reservoirs for analysis and replenished with an equal volume of fresh PBS medium, respectively. Equation (3) was utilized to determine the percentage of DOX released.

$$\text{Drug Release (\%)} = \frac{\text{DOX in dialysis medium}}{\text{Total DOX in the system}} \times 100 \quad (3)$$

Cytotoxicity Assay

Using the MTT assay, the capacity of fabricated 2D-hBN nanocomposites, such as 2D-hBN@DOX, to limit the development of lung cancer cells was investigated on A549 cells following treatment with 0 $\mu\text{g mL}^{-1}$, 65 $\mu\text{g mL}^{-1}$, 125 $\mu\text{g mL}^{-1}$, and 250 $\mu\text{g mL}^{-1}$ doses. This rapid quantitative assay measures cellular metabolic activity by evaluating the reduction of MTT to purple formazan crystals primarily by mitochondrial dehydrogenase with the aid of a solubilization solution dimethyl sulfoxide (DMSO), an acidified ethanol solution. This enzyme in living cells is responsible for converting the yellow tetrazole to insoluble purple formazan crystals (Fotouhi et al., 2021). This test was run at 24 and 48 hours after treatment times.

In detail, the fabricated specimens were initially made by dissolving them in a tissue culture media that contained serum. The samples were then sterilized at pH 7.4 with 0.2 μm filtration. Conversely, 5000 cells/100 μL of A549 cells were individually seeded in each well of 96-well plates and allowed to attach to the wells during the course of a 24-hour incubation. Subsequently, the culture media was extracted, 100 μL of four specimen concentrations were added to each well, and the plates were cultured for 24 h and 48 h. Following this procedure, 20 μL of sterile-filtered MTT salt made in PBS pH 7.4 (5 mg/mL) was applied to the wells of the plate, and the plates were incubated for 4 hours after the medium was replaced with 200 μL Roswell Park Memorial Institute (RPMI) with no serum.

The following step involved pipetting off the well suspension liquid, and 100 μL DMSO added to each well, and then the plates were incubated for 20 minutes. The optical density (OD), also known as color intensity, of the wells was then measured at 590 nm ($n = 1$), and the cell viability values were computed as the difference in OD values between the treated and non-treated cells.

Results and Discussion

The UV-Vis absorption spectra of the fabricated 2D-hBN product, investigated by Agilent Carry 60 spectrophotometer (Figure 1.(a)), revealed the characteristic peak of the 2D-hBN at 204 nm.

Drug Loading and Release experiments

Drug Loading

The successful loading of DOX onto the 2D-*h*BN was affirmed by also using Agilent Carry 60 spectrophotometer. The 2D-*h*BN@DOX absorption spectra were compared with that of the markers (2D-*h*BN and free DOX). The absorption spectra of the 2D-*h*BN@DOX nanocomposites showed a DOX-related absorbance peak at 481 nm, as shown in Figure 1. (a), which confirmed the successful loading of DOX onto the 2D-*h*BN. Using a DOX Standard Calibration Curve (Figure 1. (c)), the EE% and LE% of the 2D-*h*BN@DOX nanocomposites were calculated. The findings were $84 \pm 0.50\%$ and $25 \pm 0.48\%$, respectively.

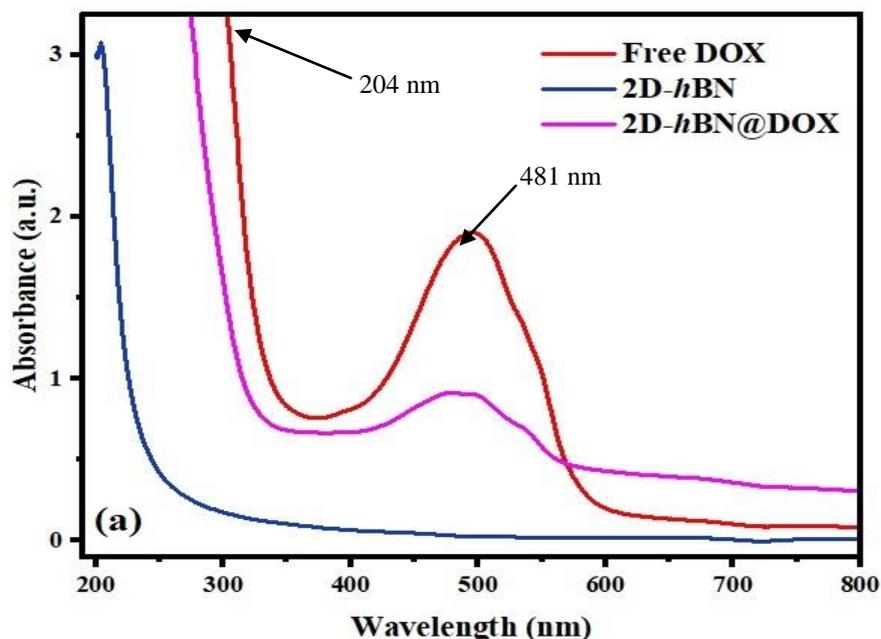


Figure 1. (a) UV-Vis spectra of free DOX, 2D-*h*BN, and 2D-*h*BN@DOX.

Drug Release

The *in vitro* drug release characteristics of the 2D-*h*BN@DOX were studied at physiological pH 7.4 and endosomal pH 5.0 of cancer cells (Figure 2. (b)). The findings demonstrated that in an acidic environment (i.e., pH 5.0), DOX was released more quickly during the first 24 hours and then steadily over the next 72 hours, reaching 73.5%.

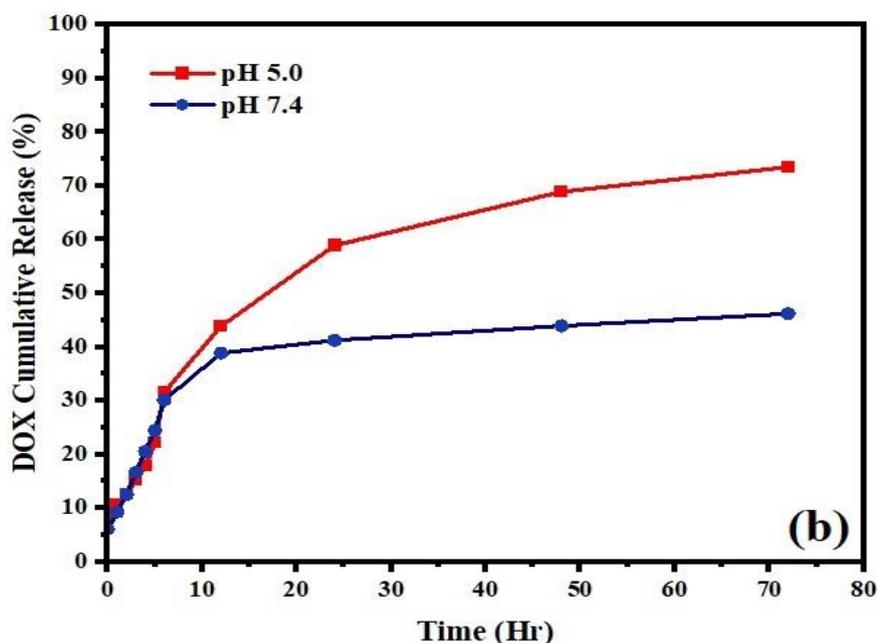


Figure 1. (b) Drug release profile of DOX from 2D-*h*BN@DOX nanocomposites.

According to reports, medications that are linked to or encased in cell membranes can be influenced by the environment's pH. The cell membrane can progressively lyse, or dissolve, in an acidic environment, allowing the drug to be released more easily. In contrast, after 72 hours, the DOX release rate was 46.2% under neutral conditions (pH = 7.4). Since tumor tissue grows best in an acidic environment, controlling the production of DOX in a way that is pH-responsive may be advantageous for the treatment of cancer. Moreover, DOX's adverse effects might be lessened in physiological settings due to a reduction in its circulatory leakage. The results suggest non-Fickian diffusion drug release mechanism which was noted by the rapid release at first then tailing off over time. This high release percentage proves the enhanced pH-responsiveness and controlled-release behavior of the 2D-*h*BN@DOX.

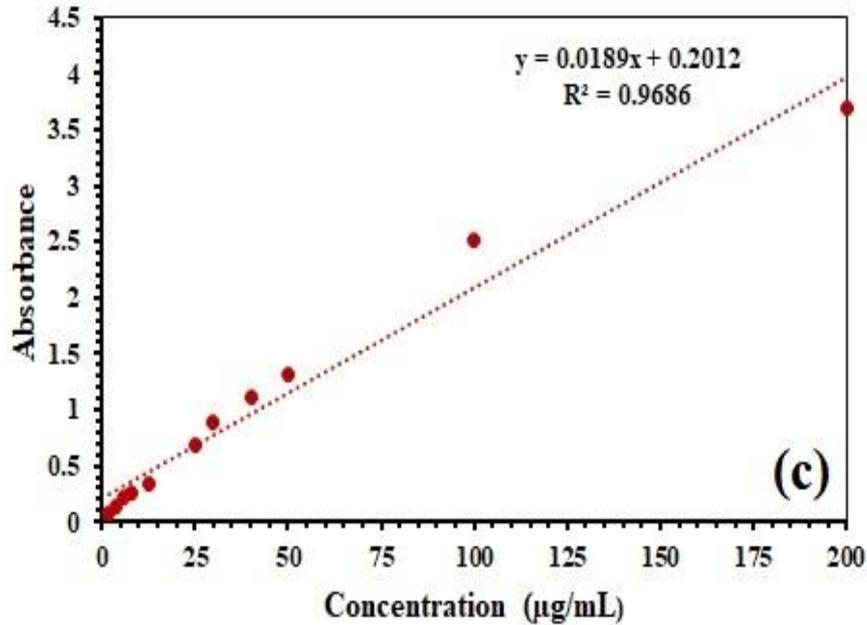


Figure 1. (c) DOX standard calibration curve.

Cytotoxicity Assay

To determine the potential of the fabricated 2D-*h*BN nanocomposites in suppressing the development of lung cancer cells, the MTT assay results showed no significant cytotoxicity on the A549 cell lines after treatment for 24 and 48 h with 2D-*h*BN, as shown in Figure 2. (a) and (b), affirming that the nanocomposites were non-toxic.

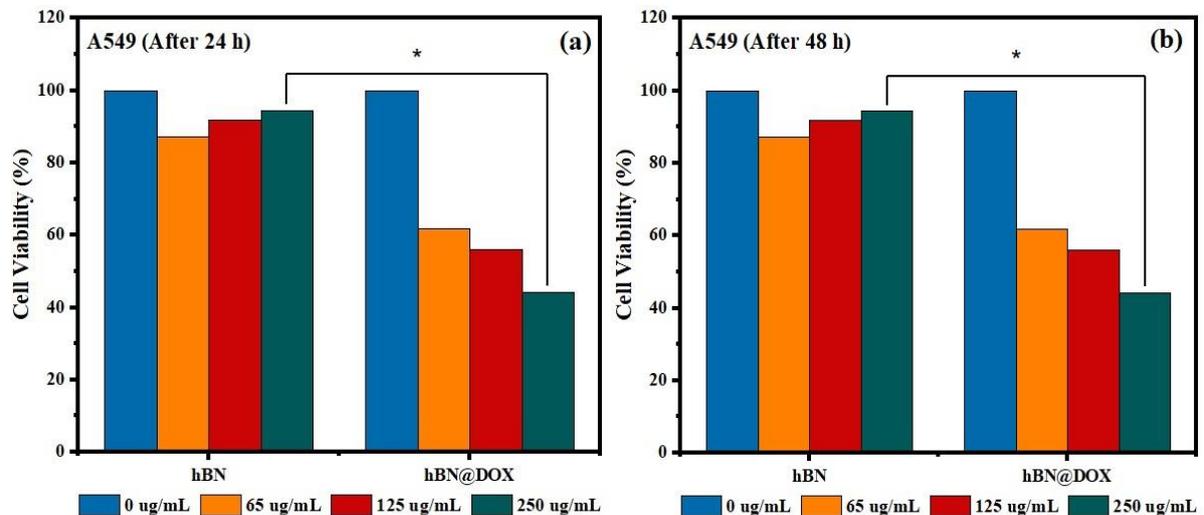


Figure 2. Cell viability results. * $P < 0.05$ is statistically significant.

There was a concentration-dependent cell viability feature for all samples as illustrated in Figure 2. (a) and (b). The cell viability increased as the 2D-*h*BN sample concentration was raised, indicating the nanocarrier's

biocompatibility, vice versa it is true regarding the 2D-*h*BN@DOX sample (Kumar et al., 2018; Khalef et al., 2024). Specifically, following 24 hours of treatment with 65 $\mu\text{g mL}^{-1}$ and 250 $\mu\text{g mL}^{-1}$ concentrations of 2D-*h*BN samples, 87% and 94% of A549 cells' viability were detected. These results indicate the nanocomposites' biocompatibility and further validate their safety for application in cancer drug delivery.

However, following a 24-hour treatment with the 2D-*h*BN@DOX nanocomposites, the viability of the cancer cells decreased to 61% and 44% at concentrations of 65 $\mu\text{g mL}^{-1}$ and 250 $\mu\text{g mL}^{-1}$, respectively. This indicates that the DOX-loaded 2D-*h*BN possesses cytotoxic properties. The A549 cell line's cell viability results after 48 hours of treatment were comparable to those after 24 hours. There is a high metabolic rate in cancer cells which makes them engulf every biocompatible substance in their surroundings through endocytosis without being cognizant of its cargo (DOX).

Conclusion

In conclusion, a 2D-*h*BN DDS exfoliated from bulk boron nitride powder with a surfactant sodium cholate salt was successfully fabricated in this study. Anti-cancer drug (DOX) was loaded and released from the 2D-*h*BN in a pH-dependent manner. Benefiting from the encapsulation of nanoparticles with DOX, the 2D-*h*BN@DOX exhibited enhanced performance in terms of biocompatibility and drug-loading capability. Furthermore, the high drug release percentage at acidic environment endowed the 2D-*h*BN@DOX with specific targeting capacity at tumor sites. Finally, preliminary MTT assay experiment with A549 cell lines also affirmed a tumor-inhibiting effect of the 2D-*h*BN@DOX nanocomposites.

Recommendations

There is a need to conduct further cellular uptake experiments to evaluate the effectiveness of the 2D-*h*BN nanoparticles loaded with different anti-tumor drug(s) in determining how well nanoparticles are internalized by cancer cells, which is essential for their therapeutic efficacy. Also, apoptosis assay, cell cycle arrest, and *in vivo* tumor therapy is required to help determine whether nanoparticles can induce programmed cell death (apoptosis) in cancer cells, to halt the division of cancer cells, thereby preventing tumor growth and proliferation, to evaluate of the efficacy of 2D-*h*BN@DOX nanocomposites for treating A549 cells-injected mice models, respectively.

Scientific Ethics Declaration

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

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Author Information

Tartisio Hamuha

Egypt-Japan University of Science and Technology
Biotechnology Program, Institute of Basic and Applied
Sciences, Egypt-Japan University of Science and
Technology (E-JUST), New Borg El Arab City, Alexandria
21934, Egypt

Samir El-Mashtoly

Leibniz Institute of Photonic Technology,
Albert-Einstein-Straße 9, Jena 07745, Germany

Amr Nassrallah

Egypt-Japan University of Science and Technology
Biotechnology Program, Institute of Basic and Applied
Sciences, Egypt-Japan University of Science and
Technology (E-JUST), New Borg El Arab City, Alexandria
21934, Egypt
Biochemistry Department, Faculty of Agriculture, Cairo
University, Giza 12613, Egypt.

Ahmed Khalil

Egypt-Japan University of Science and Technology
Institute of Basic and Applied Sciences, Egypt-Japan
University of Science and Technology (E-JUST), New Borg
El-Arab City, Alexandria 21934, Egypt,
Environmental and Smart Technology Group, Faculty of
Science, Fayoum University, Fayoum 63514, Egypt
Contact e-mail: ahmed.s.khalil@ejust.edu.eg

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Workplace Safety in Hospital Laboratories: Occupational Accidents and Work-Related Diseases

Moufida Gharbi

Badji Mokhtar University-Annaba

Sarra Chine

Badji Mokhtar University-Annaba

Nadia Tigha-Bouaziz

Badji Mokhtar University-Annaba

Meriem Khelifa

Badji Mokhtar University-Annaba

Djoumana Chaouch

Badji Mokhtar University-Annaba

Amira Segmane

Badji Mokhtar University-Annaba

Amani Sedira

Badji Mokhtar University-Annaba

Sarra Arouel

Badji Mokhtar University-Annaba

Chaima Boussebah

Badji Mokhtar University-Annaba

Abstract: Workplace safety in hospital laboratories is crucial for the health and well-being of employees. Due to the potentially hazardous nature of the chemicals handled, workers are exposed to a variety of risks, ranging from occupational accidents to work-related diseases. The main objective of our study was to identify work-related accidents and occupational diseases associated with exposure to occupational hazards in medical analysis laboratories and to determine preventive measures. *Materials and method* : The sample for our cross-sectional descriptive study consisted of 113 individuals working in the hemobiology, biochemistry, and anatomical pathology laboratories. A questionnaire was designed to collect data, providing a precise assessment of occupational accidents and the risks faced by the employees. *Results* :18.87% of participants reported having suffered a work-related accident, with no significant correlation with gender ($p = 0.955$) or age ($p = 0.133$). Among these accidents, 66.7% were of a mechanical nature, including 36.1% involving skin injuries and 21% involving eye injuries. Investigations into the causes revealed that 32.05% of the accidents resulted from a lack of protective measures, 50.76% were attributable to negligence, and 17.23 % were related to emergency situations. Furthermore, employees working in a fixed daily position are at an increased risk of accidents ($p = 0.003$). Additionally, occupational diseases have also been reported, with 32.29% of participants affected by occupational allergies and 33.22% by skin sensitizations. Moreover, approximately 14.81% of participants suffered from respiratory diseases, while 13.15% had eye injuries. Finally, about 6.48 % of participants were diagnosed with occupational cancer. *Conclusion* : Our study highlighted that occupational accidents have

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significant consequences on the health of employees, particularly concerning conditions related to exposure to chemical reagents. This underscores the urgent need to improve safety and prevention measures to ensure the protection of workers.

Keywords : Chemicals, Occupational accidents, Work-related diseases.

Introduction

Hospital laboratories are high-risk work environments. They combine, on one hand, the classic hazards of the workplace, such as occupational accidents, and, on the other hand, specific risks associated with their activities. Indeed, the daily handling of a wide variety of chemicals, some of which have potentially hazardous effects, poses a significant threat. These substances, available in various forms (liquids, powders, gases) and in varying quantities (from micrograms to kilograms), are used according to the needs of the analyses, which complicates risk management despite preventive efforts. (Cadet et al., 2024)

Workers are exposed to various hazards, ranging from immediate occupational accidents to work-related illnesses that may manifest after prolonged exposure. (INRS, 2017). Toxic, corrosive, flammable, or mutagenic chemicals can lead to acute poisoning through inhalation or direct contact. Some substances cause irritation or allergic reactions, while others, through accumulation, increase the risk of serious pathologies, such as cancers. (INRS, 2023b ; Roy et al., 2015). The effects of chemical exposure depend on several factors: the quantity and duration of exposure, the nature of the substance, the route of exposure, as well as the characteristics of the exposed individual (age, sex, medical history). (INRS, 2023b)

Understanding the various health effects resulting from exposure to chemicals allows for the identification and characterization of at-risk situations, the implementation of appropriate prevention and protection measures, and the prevention and detection of any poisoning following an incident or accident. (Cadet et al., 2024) Safety procedures and the provision of personal protective equipment (PPE) help to mitigate certain hazards; however, the complete elimination of risks remains challenging due to the unpredictable nature of certain incidents. (INRS, 2023a).

The primary objective of our study was to identify occupational accidents and work-related illnesses associated with exposure to occupational hazards in medical analysis laboratories and to determine preventive measures.

Materials and Method

This cross-sectional descriptive study was conducted among 113 employees working in three laboratories of a hospital-university center in eastern Algeria. The hematology laboratory accounted for 61% of the respondents, while the biochemistry and pathology-anatomy-cytology (PAC) laboratories contributed 14.2% and 24.8%, respectively. The vast majority of the staff voluntarily agreed to participate in the survey, resulting in a participation rate of 94.17%, thus ensuring a representative sample.

The data were collected during individual interviews using a previously developed and validated questionnaire to provide an accurate assessment of risks. This questionnaire was structured into four distinct sections, totaling 29 questions. Particular attention was given to the clarity and simplicity of the questions to facilitate understanding and ensure the reliability of the responses obtained. We opted primarily for closed-ended questions.

Questionnaire Structure

The first section collected personal data (age, sex, rank, level of education, marital status) and professional information (job position, work schedule, etc.), as well as medical history and toxic habits (tobacco, alcohol). The second section focused on occupational accidents and work-related illnesses. It facilitated the collection of data on the frequency and nature of accidents occurring in the workplace, as well as illnesses potentially related to short-term (acute poisoning) or prolonged exposure to chemical substances. The third section explored the use of individual and collective preventive measures, including the availability of protective equipment (gloves, masks, goggles, fume hoods, etc.), its actual use by staff, and perceptions of its effectiveness in risk prevention.

The fourth section addressed training in hygiene and safety. It aimed to evaluate the quality and frequency of training sessions provided and awareness-raising activities for staff regarding preventive measures and risk management.

Data Collection and Analysis

The data were entered and analyzed using SPSS version 25 software. Descriptive analyses were conducted to present the results, highlighting frequencies and percentages. Various statistical tests, including correlation and association tests (Chi-square test and Fisher's exact test), were used to explore potential relationships between the studied variables. For all analyses, a p-value of less than 0.05 was considered statistically significant.

Results and Discussion

Population Characteristics

The study involved a total of 113 workers. The population was predominantly female (87.61%). Males represented 12.39% of the participants, resulting in a sex ratio of 0.14. Laboratory technicians and biologists constituted the most represented categories of workers, accounting for a total of 47.79% of the participants. Residents followed with 28.32%, indicating a significant presence of training staff. The average age of the participants was 32.55 ± 7.69 years, with extremes ranging from 21 to 57 years. The majority of workers (82.24%) were aged between 20 and 40 years, while 15.46% were in the 40 to 60 age range.

These observations are consistent with the results of the study by Tait et al., conducted in 108 medical laboratories in Kenya, which included 204 employees with an average age of 30.1 ± 7.1 years. However, the female predominance was less pronounced in this study (51.5% women compared to 48.5% men). (Tait et al., 2018). Similarly, a study involving 234 laboratories in a hospital-university center in Turkey reported a female majority of 69.2%, which is close to our study; however, the predominant age group was 40 to 49 years (40.9%), contrasting with our results.(Boyacı, 2021). The data from our study indicated that 41.41% of the women in the three laboratories were married. A small percentage of 2.65% of the participants reported having toxic habits related to tobacco and/or alcohol.

Table 1. Characteristics of the study population

Characteristics	Frequency (%)	
Age group	[20-40[years	82,24 %
	[40-60[years	15,46 %
	Over 60 years	20.3 %
Gender	Female	87,61 %
	Male	12,39 %
Level of Education	University	84.1 %
	Secondary	11.50 %
	Primary	4.42 %
	Assistant Professors/Assistants	5.31 %
Grade	Residents	28.32 %
	Laboratory Technicians and Biologists	47.79 %
	Administrative Secretaries	11.50 %
	Cleaning Staff	4.42 %
	Toxic Habits:	Tobacco and/or Alcohol

Occupational Accidents (OA)

18.87% of workers reported having been victims of at least one occupational accident. This prevalence is lower than that reported in the study conducted by Appiagyei et al. in a public hospital in Ghana, where it was 29.7% over a period of 12 months. The annual incidence was estimated at 1.63 injuries per person, highlighting the increased exposure to occupational hazards for healthcare staff in these environments. (Appiagyei et al., 2021). Another study conducted in hospitals in Jeddah, Saudi Arabia, involving 387 participants, revealed that the overall prevalence of work-related accidents reached 52%. (Alameer & Noor Elahi, 2023)

These figures underscore the severity and recurrence of occupational risks in the healthcare sector, particularly in medical analysis laboratories, where the handling of chemical or biological products increases staff vulnerability. However, the statistical analysis conducted in our study did not show a significant correlation between work accidents and the sex ($r = 0.006$, $p = 0.955$) or age ($r = 0.162$, $p = 0.133$) of the respondents, suggesting that these characteristics are not major predictors of accidents in our sample. Regarding the nature of the accidents reported in the laboratories of our study, approximately 66.7% were mechanical in nature, 36.1% involved skin injuries, and 21% involved eye injuries. These types of accidents may result from improper handling of equipment or chemicals, highlighting the importance of increased vigilance. Blood exposure accidents, accounting for 32.5%, were observed in the biochemistry and hemobiology laboratories. This type of accident involved risks associated with exposure to infectious agents, often through needle stick injuries or contact with biological fluids.

A Lebanese study involving 220 participants reported a rate of 40.5% of mechanical accidents and 8.1% of biological accidents. These rates, which are lower than our results, may be explained by the availability of protective measures for 86.5% of the participants and by training and awareness initiatives, endorsed by 96.6% of the participants in that study. Regarding laboratory workers, it is noteworthy that women (85.9%) were significantly more exposed than men (14.1%) ($p = 0.044$). Age was not significantly associated with exposure to accidents ($p = 0.364$). (Nasrallah et al., 2022). Moreover, a study conducted in Kenya concluded that biological accidents were the most frequent, with 80% of respondents reporting exposure. Regarding chemical risks, the handling of unlabeled and unmarked products was the most common, affecting 38.2% of participants in the medical laboratories of Kajiado. (Tait et al., 2018)

In our study, according to the reports of the surveyed workers, negligence was the predominant factor, responsible for 50.76% of reported incidents, followed by a lack of protective measures, which contributed to 32.05% of accidents. Lastly, emergency situations or workload overload accounted for 17.23% of the accidents. Furthermore, the type of workstation appears to be a determining factor, with a significant association between the type of position and the frequency of accidents ($p = 0.003$). Employees with a fixed daily position are more likely to be victims of occupational accidents than those working on a rotating shift system. This trend may be explained by cumulative fatigue, monotony, or prolonged exposure to risks.

According to the study conducted in Ghana, multivariable analysis identified several factors influencing the occurrence of work-related injuries. Work-related stress was associated with an increased risk of accidents (adjusted OR: 2.68; 95% CI: 1.26-5.71). Additionally, employees working in laboratories had a significantly higher likelihood of sustaining injuries (adjusted OR: 3.26; 95% CI: 1.02-10.50). (Appiagyei et al., 2021). A study conducted in hospitals in Jeddah, Saudi Arabia, revealed that splashes to the eyes or mouth (20.4%) were the most frequent injuries. These incidents were significantly correlated with extended work hours ($p = 0.0001$) and insufficient use of personal protective equipment (PPE) ($p = 0.010$). (Alameer & Noor Elahi, 2023)

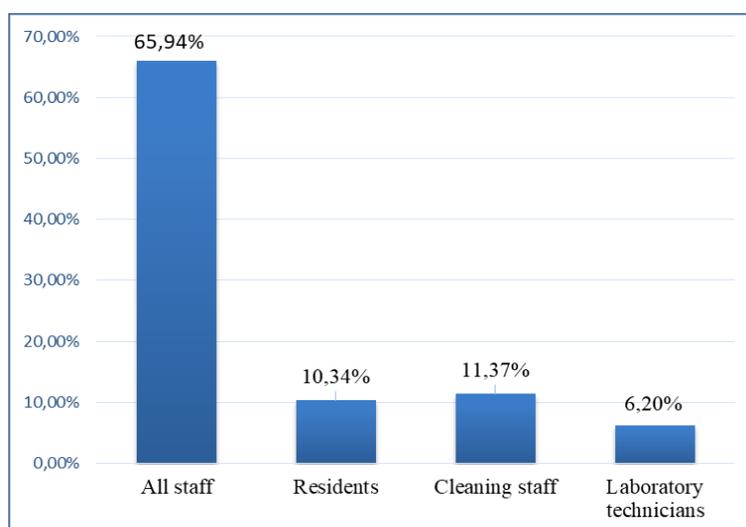


Figure 1. Distribution of occupational accidents by professional category in the three hospital laboratories

Similarly, the study conducted in Lebanon on 220 laboratory employees also highlighted concerning results. Full-time workers represented the majority of those exposed to occupational accidents (90.9%) and were significantly associated with the occurrence of accidents compared to part-time workers (9.1%) ($p <$

0.001).(Nasrallah et al., 2022). Occupational accidents in hospital laboratories disproportionately affect several categories of personnel. (Kouame et al, 2024). According to our data, 65.94% of the occupational accidents involved the entire hospital laboratory staff, encompassing various professional categories facing diverse risks.

Cleaning staff accounted for 10.34% of the cases. Their vulnerability primarily arose from their frequent exposure to chemicals and sharp objects, a situation exacerbated by often insufficient training in risk management. Residents, involved in 11.37% of the incidents, are particularly affected due to their direct participation in the handling of hazardous biological and chemical substances. Finally, laboratory technicians, who regularly handle biological samples and chemicals, were responsible for 6.20% of the reported accidents (Figure 1).

Presumed Work-Related Diseases (WRD)

Pathologies caused by chemicals can manifest several months or even years after exposure. (INRS, 2001). In our study, presumed work-related diseases were frequently reported, with 60.5% of cases attributed to prolonged contact and regular use of chemicals. The lack of preventive measures accounted for 31.6% of the cases. Furthermore, equipment-related issues were responsible for 7.1% of the diseases, while poor ventilation and excessive workload contributed marginally, accounting for 0.9% of the cases.

Dermatological Pathology

Prolonged exposure to chemicals is consistent with the reported symptoms. Indeed, 32.29% of workers suffered from occupational allergies, and 33.22% experienced skin irritations. Among them, 73.5% reported that the onset of these dermatological symptoms was closely related to the use of chemicals such as formaldehyde and xylene.

Respiratory Pathology

Furthermore, approximately 14.81% of participants reported respiratory diseases, with 55.67% attributing them to exposure to chemicals such as formaldehyde, xylene, and acetone.

Ocular Pathology

In addition, 13.15% of workers reported ocular injuries, which were also associated with the use of chemicals, particularly formaldehyde and acetone. A Turkish study revealed that 22.5% of laboratory workers (N=9/40) had been diagnosed with an occupational disease. Furthermore, no significant differences were observed between sexes ($p=0.233$) or age ($p>0.05$) regarding the incidence of occupational diseases. (Taskingul et al., 2024). Similarly, a Lebanese study indicated that 57.6% of participants experienced health issues related to accidents in the laboratories. The primary routes of exposure among participants, in order of significance, were inhalation (45.4%), contact with the skin or eyes (40.5%), and injuries (13.1%).(Nasrallah et al., 2022).

CMR Pathologies (Carcinogenic, Mutagenic, and Reproductive Toxicity)

14.63% of women (6 out of 41) reported having experienced infertility. Regarding pregnancy outcomes, 17.14% of women who had been pregnant stated that they had experienced at least one miscarriage. Additionally, 8.57% reported having given birth to a stillborn child (Table 2). The results suggest a possible link between laboratory work and difficulties with fertility or pregnancy complications, although the small sample size and study protocol do not allow for formal confirmation.

In this context, a Finnish study (1990–2006) compared pregnancy outcomes between laboratory technicians and teachers, suggesting a potential reduction in fetal growth. (Halliday-Bell et al., 2010). However, results from the Danish national cohort (1997–2003) indicated that only female technicians handling radio-immuno-assays or radiolabeled markers exhibited an increased risk of preterm birth and major malformations, without suggesting a heightened risk of overall reproductive failure.(Zhu et al, 2006)

Finally, several studies have revealed an increased risk of spontaneous abortions, perinatal mortality, congenital malformations, as well as a higher incidence of chromosomal abnormalities among laboratory-exposed workers. (INRS, 2001). Additional studies are needed to explore the relationship between laboratory work and gynecological risks.

Table 2. Distribution of the frequency of occupational diseases among laboratory personnel by type of disease and chemical agents

Effect Type	Frequency	Chemical Products	
Dermatological Pathology	33.22 %	Formaldehyde	
		Xylene	
		Detergents	
		Latex	
		Staining reagents	
Respiratory Pathology	14,81 %	Formaldehyde	
		Xylene	
		Acetone	
		Antiseptics	
		Staining reagents	
Ocular Pathology	13.15 %	Formaldehyde	
		Acetone	
CMR pathologies	Sterility	14.63 %	Formaldehyde
	Abortion	17.14 %	Chloroform
	Stillborn	8.57 %	Basic Fuchsin
	Cancers	6.48%	ADVIA PEROX

Approximately 6.48% of workers were affected by cancer, although no direct link to chemical reagents has been demonstrated. However, these cancers may be associated with CMR (carcinogenic, mutagenic, and reproductive toxic) reagents, such as ADVIA Perox, chloroform, formaldehyde, and basic fuchsin. Occupational cancers may appear 10, 20, or even 40 years after exposure. The cancer risk for operators in laboratories is still not well understood. An international cohort study initiated in 1986 showed an increased risk for cancers such as leukemias, lymphomas, and cancers of the pancreas, brain, bones, thyroid, breast, and uterus, although the conclusions are limited by methodological biases and the diversity of exposures. (INRS, 2001)

A cohort study conducted in Sweden evaluated the overall incidence rate and standardized incidence ratio (SIR) of cancer among laboratory personnel. It showed that the overall incidence of occupational cancers in laboratories was 2.24%. Among male laboratory workers, the SIR for brain tumors was 1.69 (CI: 0.62–3.68). For women, the SIR for malignant melanoma was particularly high, reaching 3.51 (CI: 0.96–8.98). Additionally, the SIR for breast cancer among women working in laboratories was 1.62 (CI: 0.78–2.98). These types of cancers are associated with exposure to organic solvents and chemical reagents, such as formaldehyde, as well as CMR substances (carcinogenic, mutagenic, and reproductive toxic). (Wennborg et al., 2001). Another case-control study conducted in Sweden, integrated into the Malmö Diet and Cancer Study cohort, revealed that women exposed to chemicals in the workplace had an increased risk of breast cancer (OR = 1.59, 95% CI: 1.11–2.29). This risk was proportional to the duration of exposure. In particular, women exposed for more than 10 years to solvents had an odds ratio of 3.06 (95% CI: 1.18–7.96) (Videnros et al., 2020).

Compliance with Occupational Accident and Disease Prevention Measures

Among the surveyed personnel, 31.86% reported not adhering to prevention measures. This non-compliance was attributed to negligence in 22.68% of cases, a lack of preventive resources in 10.31%, and other reasons, such as absence of training, in 2.06% of cases. Workers also reported several gaps in protection, which directly influenced occupational accidents and diseases. Fume hoods were missing in 24.78% of cases, followed by windows (20.35%) and protective creams (23%). Additionally, protective eyewear and gloves were deemed insufficient in 18.58% and 10.61% of cases, respectively.

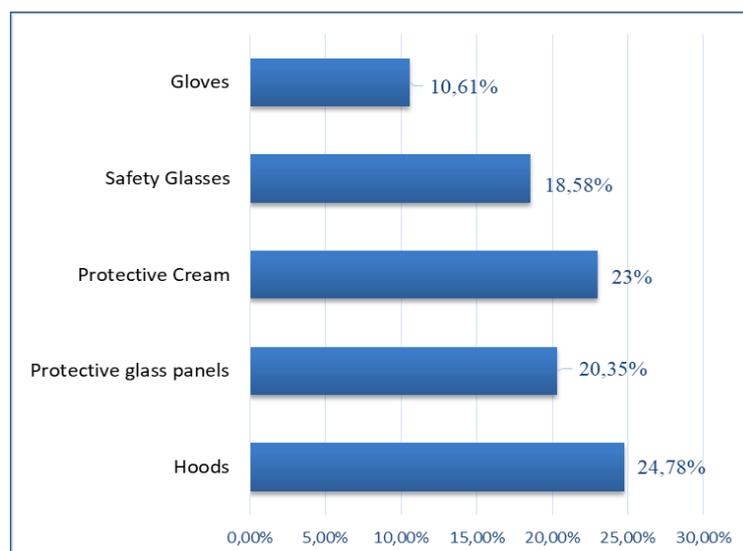


Figure 2. Distribution of insufficient protective measures reported by workers

Measures to Prevent and Reduce Occupational Accidents and Work-related Diseases

For 41.3% of the surveyed personnel, providing protective measures is considered the most effective solution for reducing the incidence of accidents. Approximately 30.2% believed that improving working conditions could also contribute to this reduction. Furthermore, 19% highlighted that raising awareness would play an important, though not decisive, role. Finally, only 9.5% of respondents viewed training as a relevant preventive measure, noting that it was not provided in 75.22% of cases.

Conclusion

This study highlighted the extent of the risks faced by hospital laboratory workers due to exposure to various chemicals. The results show a significant prevalence of occupational accidents and diseases, revealing gaps in prevention measures and the use of personal protective equipment. Factors such as negligence, lack of adequate training, and insufficient safety devices have been identified as key determinants in the occurrence of these incidents. This situation underscores the importance of proactive risk management and increased awareness of the dangers associated with handling chemical substances. The study thus emphasizes the need for ongoing attention to safety in these high-risk work environments to protect worker health and ensure a safer workplace.

Recommendations

- To enhance safety and reduce the risks of occupational diseases and work-related accidents, it is essential to:
- Strengthen personal protective equipment (PPE) by ensuring that all workers have access to adequate protection.
- Implement regular training programs to educate employees about the dangers of chemical products and best safety practices.
- Regularly assess and improve working conditions in laboratories to identify and rectify potential risks.
- Promote a safety culture where every employee feels responsible for their own safety and that of their colleagues, and where incidents can be reported without fear of retaliation.
- Establish clear emergency procedures for managing accidents or exposures, ensuring that all employees are trained in these protocols.
- Regularly monitor and evaluate health and safety incidents, as well as conduct frequent safety audits to ensure compliance with standards.
- Regularly assess the impact of chemicals used in laboratories..

Scientific Ethics Declaration

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

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Author Information

Moufida Gharbi

Faculty of Medicine, Badji Mokhtar University Annaba.
Algeria
Contact e-mail: moufida.gharbi@univ-annaba.dz

Chine Sara

Faculty of Medicine, Badji Mokhtar University Annaba.
Algeria

Tigha Bouaziz Nadia

Faculty of Medicine, Badji Mokhtar University Annaba.
Algeria

Meriem Khelifa

Faculty of Medicine, Badji Mokhtar University-Annaba,
Algeria

Djoumana Chaouch

Faculty of Medicine, Badji Mokhtar University-Annaba,
Algeria

Amira Segmane

Faculty of Medicine, Badji Mokhtar University-Annaba,
Algeria

Amani Sedira

Faculty of Medicine, Badji Mokhtar University-Annaba,
Algeria

Sarra Arouel

Faculty of Medicine, Badji Mokhtar University-Annaba,
Algeria

Chaima Boussebah

Faculty of Medicine, Badji Mokhtar University-Annaba,
Algeria

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Study of the Effect of Massage Therapy on Serum Cortisol Concentration in a Patient with Chronic Stress

Vildan Ahmed

NSA "Vassil Levski", Sofia

Denka Marinova

NSA "Vassil Levski", Sofia

Abstract: Stress is a condition that occurs when external or internal influences (stressors) are damaging or unusual in strength, which disrupt the homeostasis of the human organism. Cortisol is the glucocorticoid, which under the influence of stress factors increases its secretion more than 10 times. The aim of the study was to investigate the effect of massage therapy on serum cortisol concentration in patients with chronic stress. Material and methods: the study refers to a 3-month follow-up of a 32-year-old male patient with high serum cortisol levels. PSS – 10 (Perceived Stress scale) was used, serum cortisol was measured immunometrically with chemiluminescent labeling. Two courses of ten procedures were conducted with massage (classical or Chinese) therapy in the cervical-thoracic area. Results: Reduction of serum cortisol levels, reduction of pain symptoms, subjective and objective management of stressors. Conclusion: Massage therapy appears to be an effective method of managing stress and the pain symptoms associated with it.

Keywords: Stress, Massage therapy, Cortisol

Introduction

Daily responsibilities and lifestyle problems are the main cause of physical and psychological stress that deteriorates an individual's health. Prolonged exposure to stress activates the adrenocorticotrophic hormone (ACTH) system and causes the release of cortisol hormones from the adrenal cortex. Many other biomarkers are affected by stress, but cortisol is considered the most important and potentially clinically useful biomarker for stress assessment and monitoring. Accurate and timely detection of elevated cortisol levels can improve the diagnosis, treatment, and prevention of stress-related diseases such as anxiety disorders, metabolic dysregulation, and cardiovascular disease. (Mahdizadeh et al., 2019)

The brain is the central organ of stress and adaptation to stress because it perceives and determines what constitutes a threat, as well as the behavioral and physiological responses to the stressor that promote adaptation ("allostasis") but also contribute to pathophysiology ("allostatic load"/ overload). The adult, as well as the developing brain, possesses a remarkable capacity to exhibit structural and functional plasticity in response to stressful and other experiences, including neuronal replacement, dendritic remodeling, and synapse turnover. Stress can cause imbalances in the neural circuits underlying cognition, decision-making, anxiety, and mood, which can increase or decrease the expression of these behaviors and behavioral states. Furthermore, adverse early life experiences, interacting with alleles of certain genes, produce lasting effects on the brain and body through epigenetic mechanisms. Although prevention is key, brain plasticity offers hope for therapies that exploit brain-body interactions. (Iqbal et al., 2023)

Massage therapy is one of the socially preferred methods of alternative medicine. Although it is mostly applied to treat pain, recent studies recommend the application of this method to promote the development and growth

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of premature newborns, suppress depression and anxiety, stimulate attention and concentration, and improve the function of the immune system. (Broom et al., 2012). There is an increase in the use of complementary therapies of integrative medicine due to the high prevalence of pain and the unsatisfactory results of conventional therapy. In addition to physical relaxation, proponents of massage therapy claim that it promotes mental relaxation and affects the psycho-emotional aspects of patients' health. (Field, 2010), (Furlan et al., 2012), (Sagar et al., 2007) The present study is part of a doctoral thesis on the topic "Effect of massage therapy on stress levels and pain symptoms in the neck-shoulder area".

Objective

The study aims to follow the effect of massage therapy on serum cortisol concentration, subjective perception of stress, coping with challenges in professional life, pain symptoms in the neck, shoulder region, upper limbs, as well as to evaluate the general well-being following the applied therapy.

Material and Methodology

The study concerns a patient with an apparent age of 32 years old, corresponding to the actual one, who is the manager of an accounting firm. Complaints date back to 2016, starting gradually with dull pain and discomfort in the cervical region. Treatment was carried out with non-steroidal anti-inflammatory drugs prescribed by the personal physician. At his first visit, the patient complained of tension and stress at work, as well as pain in the neck-shoulder region with manifest stiffness and a feeling of restriction of movement. The patient reports pain radiating to the elbow and ankle joints, reporting episodes of acute symptoms in the ankle joints, up to a complete restriction of movement in them. From the examination, we conclude that he has a correct physique with developed musculature, without distortions in the sagittal and frontal planes, as well as other anomalies in the musculoskeletal system. By calculating the body mass index, the result is overweight of the first degree. The patient is active, reporting regular physical activity such as walking, boxing training, and swimming during the summer months.

To track the above markers we used the Perceived Stress Scale (PSS-10 scale), a test to determine the level of emotional burnout, the Visual Analog Scale (VAS), the Neck Disability Index (NDI) and a laboratory testing of serum cortisol. All studies were conducted before the start of the massage therapy, after the tenth procedure and three months after the end of the therapy.

The study was conducted from 20.05.2024 to 17.06.2024 in the Day Center for Children and Adults with Disabilities, Burgas, under the supervision of a personal physician. The laboratory tests were done in a certified laboratory - Diagnostic and consultation center II Burgas. Before starting the course of therapy, the patient filled out an informed consent form. For the realization of the set goal, we formulated the following tasks:

- Short-term tasks - Improving the range of motion, influencing tissue nutrition in the painful area, eliminating pain, relaxing the underlying muscles.
- Long-term tasks - Permanent tissue relaxation, improved activity between agonist and antagonist muscles, building a motor habit in static and dynamic work, sparing the musculoskeletal system and self-regulation in stress management.

Considering the patient's symptoms, the idea of a combined use of techniques from European massage therapy and techniques from Chinese traditional massage arose. We believe that the concepts of the two types of massage, in combination, could solve both local problems through the classical healing massage, as well as the overall impact of the body through the Chinese massage. The methodology was applied two or three times a week, and one course of treatment consisted of 10 procedures for 25 minutes per session. The massage was performed using natural almond oil with the addition of lavender, which prevented trauma to the underlying tissue and ensured smooth execution of the techniques. The starting position of the patient is a prone position, the massage procedure is carried out in a secluded and quiet working environment.

With the application of therapeutic massage, we aim to improve tissue nutrition in the painful areas, relax the muscles in a reflex way and ensure the release of humoral substances that suppress pain symptoms and improve the general condition. The principles of impact of Chinese massage are related to the concept of the free flow of Qi energy along the channels and collaterals, the hormone between Yin and Yang, as well as the regulation of the blood substance Xue and the proper function of the internal organs Zan. The techniques used in the classic

healing massage are squeezing, rubbing and impact grips. In the massage therapy, we included the techniques of Chinese massage - tui, an, dien, mo, zhou na, gong and kouji. Some of the techniques have been modified for greater impact and effectiveness, taking into account the underlying tissues and areas of impact. The active points used in the methodology are the following: Baihui (GV 20), Fengchi (GB 20), Fengfu (GV 16), Dazhui (GV 14), Jianjing (GB 21), Jianliao (TE 14), Feishu (BL 13), Quchi (LI 11), Waiguan (TE 5), Hegu (LI 4), Houxi (SL 3).

Massage Technique

1. Straight sliding rub "tui"- The technique is applied with the pad of the thumb from the base of the skull, along the trapezius muscle. It is applied 4-5 times per line, covering the entire area of the area.
2. Brush rubbing - Performed with the hand clenched into a fist, with the dorsal surface of the middle phalanges and the base of the palm in contact with the skin. The technique is dynamic, leads to local hyperemia. Short, straight movements are performed in all directions.
3. Filing- The technique is applied to the upper part of the trapezius muscle. The therapist places both hands parallel with the thumb up, with the hands making cross-directional movements, entraining a skin fold.
4. Squeeze- From the base of the occiput, the thenar and hypothenar embrace the underlying tissue and continue in a straight line to the top of the clavicle.
5. "Zhou" technique - Circular rubbing with the pads of the four fingers on the paravertebral muscles - from the points Fengchi (GB 20) to Feishu (BL 13). The technique is performed until the underlying tissue is warmed.
6. "Na" Technique on Jianjing Point (GB 21) - The thumb is placed on the point area, the other fingers are bent and the underlying tissue is pulled in sync.
7. Processing pressure points with Dien Technique - Starts with Baihui point (GV 20), then Fengchi (GB 20), Fengfu (GV 16), Dazhui (GV 14), Feishu (BL 13) and Jianliao (TE 14).
8. Technique "soft cutting" - Percussion technique with spread fingers, performed with the ulnar edge of the fifth and fourth fingers. It is performed on the trapezius and the upper part of the deltoid muscle.
9. "An" Palm Technique - Palm pressure is performed, lateral to the Dazhui point (GV 14) to the end of the blade of scapula.
10. "Tui" technique on the upper limb - The movement is performed from the proximal to the distal direction, with the therapist covering the entire inner part of the arm along the patient's limb. The technique is repeated 3-4 times.
11. Technique "On us" - Pulling and holding the underlying tissue, performed on the entire upper extremity musculature. 3-4 repetitions starting proximal to distal.
12. "Pie" technique - slapping blows - 3-4 repetitions.
13. "Gun" technique - Fingers slightly flexed, the therapist performs prono-supinatory movements from the ankle joint and at the same time moves in the distal direction along the patient's limb.
14. "Diene" technique point processing - Quchi (LI 11), Waiguan (TE 5), Hegu (LI 4), Houxi (SL 3). Each of the points is processed until the corresponding reaction is obtained.
15. Rubbing the neck with folded fingers while thumbs are fixed on the Fengchi points (GB 20). The cupping is carried out until hyperemia appears and the skin warms up.
16. "Pie" technique - The impact grip is performed over the entire area of the trapezius muscle. Movements are made from left to right and vice versa.
17. "Gun" technique - performed from left to right, 4-5 repetitions. Sensation of dissipating tension.
18. "Zhou mo" technique - from the tip of the blade of one scapula to the blade of other scapula, passing through the Jianjing (GB 21) and Dazhui (GV 14) points.

Results and Analysis

Before the start of the massage procedures, the patient was asked to fill out questionnaires selected by us according to the symptoms, in order to correctly track the impact of the therapy on the specific complaints. The reviewed available literature, in which the somatic manifestation of stress is increasingly mentioned, prompted us to examine the relationship between pain symptomatology, stress perception and cortisol values, in this case study. Testing was performed before manipulation, immediately after and at a later stage of about three months after treatment. The initial results of the completed questionnaires gave high values in all tests. The pain sensation in the neck, shoulder area and upper limbs was described as "strong" by the patient. We determined

the quality of life using the Neck Disability Index (NDI). A total score of 34 points is defined as "severe disability" on this scale (25-34). On the Perceived Stress Scale (PSS-10), "moderate stress" was reported, with a score of 23 approaching the high stress limit (14-26). The occupational burnout questionnaire defined by us also reported high numerical values, which correspond to developing phase (37-60) and formed phase (61 and more points) , and the patient calculated 57 points in the "tension" section, 119 "Resistance" points and 71 "Exhaustion" points. Cortisol testing was performed in a laboratory by a medical professional, with the patient instructed to attend the same lab for all samples. The initial value of cortisol (552.50) was above the accepted reference values (171.00-536.00), which is 16.50 units more than the maximum normal value.

After the massage procedure, the patient again filled out all the questionnaires and, the day after the last procedure, gave a serum cortisol sample. The pain symptomatology settled to "no pain", the Neck Disability Index was scored with a total score of 2, the feeling of perceived stress on the survey decreased by 15 points and the score was 8. The re-completion of the occupational burnout syndrome questionnaire reported significantly reduction of the summed points in all sections (57, 63, 44), which concludes that only the "resistance" phase is in the developing stage and the absence of burnout signals and symptoms in the other two. An interesting result was obtained for serum cortisol, with a decrease of as much as 344.9 units, which we believe is the ideal value for the particular patient, who shared that the month was one of the busiest in the annual plan of his professional direction. We believe that above all, personal satisfaction and self-assessment of the ability to control stressful situations in everyday life is a major marker to consider in medical practice.

A dynamic lifestyle and limited financial and time resources put therapists in a situation of quick, effective and long-term solving of health problems. For this reason, we tracked how well results persisted over time. After 82 days, all surveys were distributed again, and a serum sample was given again. The Visual Analog Scale was marked with "slight pain", the Neck Disability Index was assessed as a total of 4 points, which was interpreted as no impairment, the Perceived Stress Scale gave a result of 11 points (low stress). Burnout syndrome based on the answers given, it was calculated that the "tension" phase has increased by 19 points, the "resistance" phase by 9 points and the "exhaustion" phase by 1 point since the last filling. The serum cortisol sample came out with a value of 414.70 units.

There was an almost two-fold increase from the date of examination immediately after the treatment, but despite this, the levels of cortisol in the body were within the limits of the reference values and 137.8 units less than the initial sample, before the massage course. It should be taken into account that the patient complained of a cold in the period of the third sample, which to a certain extent may affect the cortisol profile, since it is known that inflammatory processes in the body indicate an influence of its increased synthesis.

We performed a repeated course of treatment from 7.10.2024 to 29.10.2024 to track its impact on the specific patient. The massage procedures were performed in the same way as from the first ten-procedure course. Post-session scores on pain questionnaires, neck impairment index, stress scale, burnout, and serum cortisol were reported to be relatively stable and did not show a drastic decrease in values as after the first treatment.

We believe that the initial clinical results regarding cortisol are related to the impact of the new treatment method, which results in the body responding with a reduction in cortisol levels. Repeated massage procedures again lead to a reduction of the values, but a relatively stable condition is reported, without drastic changes in the data.

Table 1. Conducted research by dates. Horizontally - studies, vertically - dates

Date	VAS	NDI	PSS-10	Burnout	Cortisol
17.05.2024	6	34	23	-57 -119 -71	552.50 171.00-536.00
19.06.2024	0	2	8	-0 -56 -27	207.6 171.00 - 536.00
10.09.2024	2	4	11	-19 -65 -28	414.70 171.00- 536
30.10.2024	0	2	9	-18 -52 -28	354.00 171.00- 536

Discussion

The obtained results stimulated us to search the available literature for similar studies and to compare the obtained data with the findings of other authors. Most reviewed literature sources on the subject prove the effectiveness of massage in overcoming pain symptoms, improving the quality of life and feeling satisfied. Studies monitoring serum cortisol were limited. In the review, we encountered articles describing salivary cortisol before and after massage sessions, in which a decrease was observed immediately after the procedure, but without a tendency to maintain the result. To date, there is a lack of large-scale studies on the long-term effects of this type of therapy, and we have not found a specific methodology, dosage, and application techniques.

In a study by Marinova, D. (2019) is investigated the effectiveness of traditional Chinese massage on range of motion and pain intensity in the cervical spine. Thirty -eight patients with chronic neck pain were randomly assigned to an experimental and a control group. The patients in the experimental group were given ten Chinese therapeutic massages and therapeutic exercises twice a week. The control group performed only controlled therapeutic exercises. Tracking the dynamics of pain symptoms showed a reduction in pain in both groups of patients, with a tendency towards a significant reduction in the experimental group.

A study that was conducted in North Macedonia (2024) evaluated the effectiveness of therapeutic massage for neck pain in different professional groups - bank employees, accountants, secretaries, textile workers, people in the business sector and others over a period of three years (2019-2022). The study involved 127 volunteers reporting an average pain intensity level of 7 on a numerical rating scale of 1 to 10. Each of them received massage treatments once or twice a month. Consistent massage therapy was found to significantly reduce neck pain, but missing a procedure for more than 4 months resulted in an escalation of pain intensity. The author recommends regular massage therapy to relieve pain symptoms. (Arsovski, 2024)

Field, T., (2014) concluded in their review that moderate pressure massage resulted in increased vagal activity and decreased cortisol levels. He summarizes that functional magnetic resonance imaging data show changes in several areas of the brain, including the amygdala, hypothalamus and anterior cingulate cortex, all of which are involved in stress and emotion regulation. The author recommends further research to identify the underlying neurophysiological and biochemical mechanisms associated with moderate pressure massage.

An interesting and large-scale study by Iranian scientists (2016) is looking for the relationship between stress levels, neck pain and burnout syndrome in nurses. The total number of participants was 1400. At the end of the study, it was found that neck pain was more common in nurses with high levels of stress. The authors claim that the sample size of their study was large enough to conclude that work-related stress can cause neck/shoulder pain among workers. (Bahrami-Ahmadi et al., 2016)

Silva et al. (2019) conducted an experiment with elderly people aged about 63 years, with depressive and anxiety symptoms, and concluded that an exercise program in an aquatic environment improves functional autonomy and reduces oxidative stress in old people. We believe that water rehabilitation would have a similar effect on young people, as spa and wellness methods would have a place in the complex improvement of the body - functionally and mentally.

Conclusion

The combined methodology performed on a patient with elevated levels of serum cortisol, subjected to chronic professional stress, and with pain symptoms, gave significant results, both in the early stage of treatment and after about three months of the procedures. The procedures performed reported significant improvements in pain perception, quality of life, perceived stress, as well as a reduction in cortisol by 344.9 units. In the long term, we can summarize that the described massage procedure most clearly affects the feeling of pain, quality of life and perceived stress. The second course with massage therapy in the specific case confirmed the improvements in all the indicators of the performed studies. Although the initial results in serum cholesterol levels were not observed, the repeated reduction of its values from 60.70 units, leads us to conclude that the values of serum cholesterol in the first stage of treatment are not random and that massage therapy leads to its reduction. The data obtained from the experiment lead us to prepare recommendations for the application of the methodology in patients with similar symptoms. Based on the obtained results, we believe that the treatment courses should be conducted at an interval of three months in order to maintain a high health status, expressed in musculoskeletal and psycho-emotional condition.

Recommendations

In order to maintain an optimal state of health, in such cases we recommend physical activity, meditations, and breathing exercises in addition to the prepared methodology. We believe that conducting treatment courses every three or four months would positively affect the condition of patients with chronic stress or the application of massage once a week as a prevention of the escalation of symptoms. We also believe that a large-scale study with more patients would strengthen our result and prove statistically the significance of the applied methodology. Also, the addition of acupuncture for long-standing symptoms would lead to a more effective and rapid response.

Scientific Ethics Declaration

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

Notes

* Special thanks to the patient who always responded to our requests and actively followed our every recommendation. We are grateful for his participation in this study, in which he further engaged by visiting the laboratory on days determined by us.

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Author Information

Vildan Ahmed

Ph.D

NSA "Vasil Levski", Kinesitherapy faculty
1700, "Acad. Stefan Mladenov" St. 21 Sofia, Bulgaria

Contact e-mail: vildan.ahmed941@gmail.com

Denka Marinova

Ph.D, Asst. Prof.

NSA "Vasil Levski", Kinesitherapy faculty
, 1700, "Acad. Stefan Mladenov" St. 21 Sofia, Bulgaria

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