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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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ICMeHeLS 2024: International Conference on Medical, Health and Life Sciences

A Multiplex PCR Assay for Identification of Major Mastitis Causing Pathogens in Buffalo's Raw Milk and Evaluation of Their Sensitivity

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Abstract: Mastitis is a prevalent issue in dairy herds, particularly among buffaloes, leading to significant economic losses. This study developed a sensitive, specific, and cost-effective multiplex PCR assay for the identification of major pathogens responsible for mastitis in raw buffalo milk. A total of 200 milk samples were collected from various areas of District Multan and tested for the presence of pathogens. Additionally, the antibiotic susceptibility of drug-resistant bacteria was evaluated. The multiplex PCR assay demonstrated 100% specificity and high sensitivity (0.01ng/ul detection limit), identifying multiple pathogens simultaneously.

Keywords: Mastitis, Buffalo milk, Multiplex PCR, Pathogens, Antibiotic susceptibility, Sensitivity, Specificity.

Introduction

Mastitis is one of the most economically significant diseases affecting dairy herds worldwide, particularly in buffaloes, which are a major source of milk in many regions, including South Asia (Bari et al., 2022). It is characterized by the inflammation of the udder, mastitis not only affects milk production but also degrades milk quality, often resulting in contamination and rendering the milk unsuitable for consumption or further dairy processing (Reshi et al., 2015). The condition manifest in two forms: clinical and subclinical. Clinical mastitis shows obvious symptoms such as swelling, redness, and reduced milk yield, whereas subclinical mastitis, which often goes undetected, causes long-term damage to the udder and continuous bacterial shedding into the milk (Ruegg & Adkins, 2024).

Buffalo milk, known for its high nutritional content and versatility in producing dairy products like butter, yogurt, and cheese, is crucial to human diets, particularly in regions like Pakistan. However, its potential benefits are compromised when mastitis-causing pathogens contaminate the milk (Javeid et al., 2020; Saleem et al.). Several pathogens are implicated in mastitis, with the most common being *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Escherichia coli (Javeid et al., 2020; Saleem et al.)*. These pathogens vary in their persistence and impact on the milk supply, often causing severe economic losses to dairy farmers due to reduced milk yield, increased veterinary costs, and, in extreme cases, culling of affected animals (Parveen et al., 2021).

The traditional methods for detecting mastitis pathogens, such as bacterial culturing and biochemical tests, are time-consuming and lack the sensitivity needed for early detection, especially in cases of subclinical mastitis (Parveen et al., 2021; Reshi et al., 2015). In recent years, molecular techniques such as Polymerase Chain Reaction (PCR) have gained prominence due to their rapid, sensitive, and specific detection of pathogens. PCR-based methods can detect pathogens at low levels, even in asymptomatic cases, and are invaluable for early intervention. However, conventional PCR typically targets a single pathogen per assay, making it less efficient for diagnosing multifactorial infections like mastitis, which often involves multiple pathogens (Javeid et al., 2020; Parveen et al., 2021; Saleem et al., 2019).

To address this limitation, multiplex PCR assays have been developed to detect several pathogens simultaneously in a single reaction. This study focuses on the development of a sensitive, specific, and cost-effective multiplex PCR assay for the identification of major mastitis-causing pathogens in raw buffalo milk (Safdar & Abasıyanık, 2013). Additionally, the study evaluates the antibiotic sensitivity of these pathogens to assess the presence of drug-resistant strains, which is critical for the management and treatment of mastitis (Awandkar et al., 2022). By integrating multiplex PCR into routine diagnostics, the detection of pathogens is significantly expedited, allowing for timely and more targeted treatment interventions. The information gained from this study will not only improve the understanding of pathogen prevalence in buffalo herds but also guide effective antibiotic use, which is crucial in the fight against antimicrobial resistance in dairy farming.

Material and Methods

Sample Collection

A total of 200 buffalo milk samples were collected from four regions in District Multan: Multan Saddar, Multan City, Jalalpur Pirwala, and Shujabad. The samples were collected from January 2022 to February 2024, stored at +4°C, and transported to the Genetic Lab for bacterial culturing and DNA extraction.

Bacterial Isolation and Identification

Milk samples were diluted and cultured on various agar media. The isolated bacteria were subjected to DNA extraction, and specific primers were designed for the identification of pathogenic bacteria via simplex and multiplex PCR assays.

Multiplex PCR Assay

The multiplex PCR assay was optimized to detect multiple pathogens in a single PCR tube. This assay was tested for both specificity and sensitivity, with various concentrations of bacterial DNA ranging from 50ng to 0.001ng.

Antibiotic Susceptibility Testing

Bacteria isolated from the samples were tested for antibiotic susceptibility using antibiotics such as Ciprofloxacin, Azithromycin, Gentamicin, Amoxicillin, Streptomycin, Erythromycin, and Norfloxacin. The effectiveness of these antibiotics was evaluated to determine resistance patterns.

Results

Specificity and Sensitivity of PCR

The multiplex PCR assay exhibited 100% specificity, accurately detecting all targeted pathogens, including *Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus parauberis*, *Staphylococcus aureus*, and *Escherichia coli* (Figure is not available). Sensitivity tests showed that the assay could detect bacterial DNA concentrations as low as 0.01ng (Figure is not available).

Pathogen Prevalence

Out of the 200 samples, the following pathogens were detected: *S. uberis* (21%), *S. parauberis* (30%), *S. agalactiae* (19%), *S. dysgalactiae* (20%), *S. aureus* (32%), and *E. coli* (34%) (Figure 1).

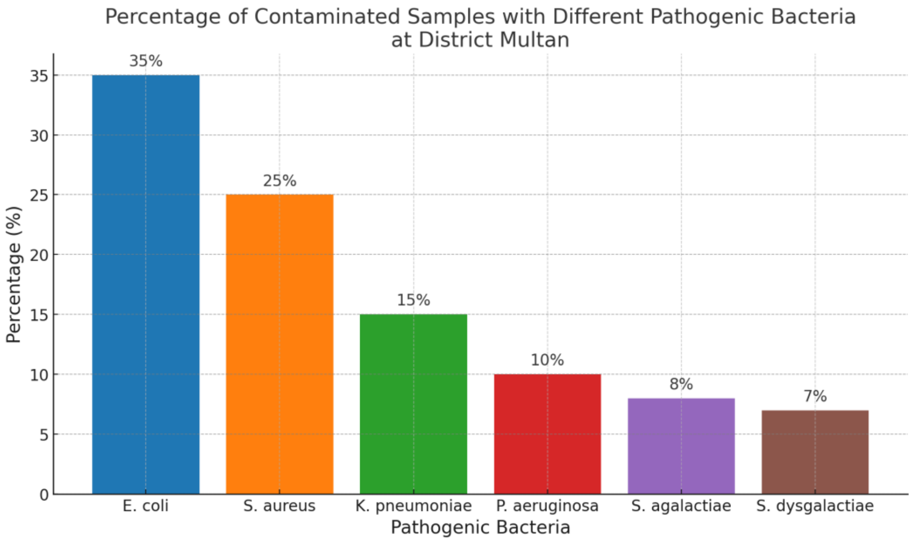

Figure 1. Percentage of contaminated samples with different pathogenic bacteria at district multan

Antibiotic Susceptibility

The antibiotic tests indicated varying levels of susceptibility among the pathogens. *E. coli*, for example, exhibited susceptibility to Ciprofloxacin and Azithromycin, while resistance was noted for Amoxicillin and Streptomycin (Figure 2).

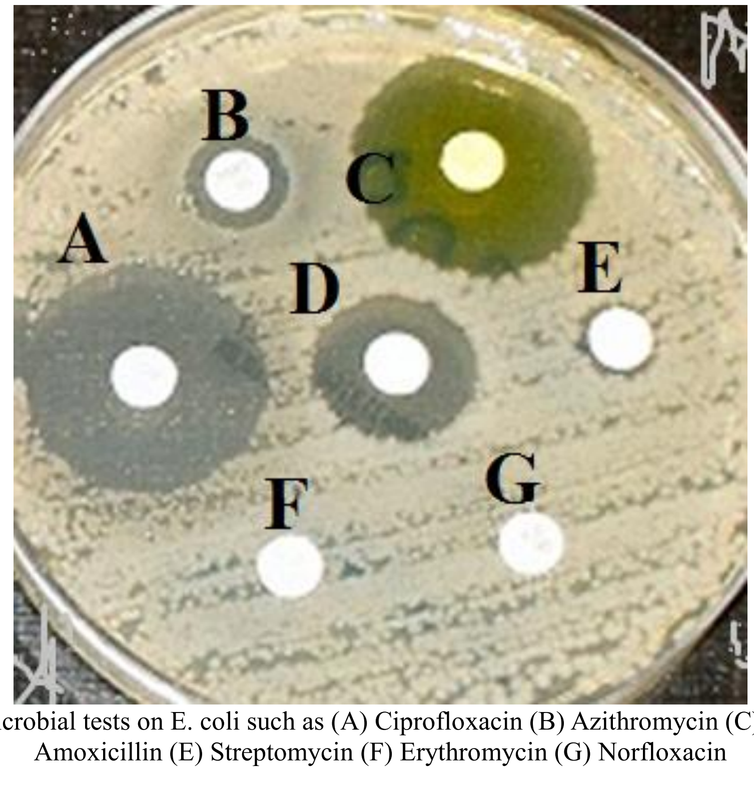

Figure 2. Antimicrobial tests on E. coli such as (A) Ciprofloxacin (B) Azithromycin (C) Gentamicin (D) Amoxicillin (E) Streptomycin (F) Erythromycin (G) Norfloxacin

Discussion

The development and validation of a multiplex PCR assay for the identification of major mastitis-causing pathogens in buffalo milk represents a significant advancement in the rapid diagnosis and management of this economically critical disease (Parveen et al., 2021; Safdar & Abasıyanık, 2013). This study successfully demonstrated the multiplex PCR assay's high specificity and sensitivity, making it an invaluable tool for the simultaneous detection of multiple pathogens in a single reaction. The ability to identify pathogens such as Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, and Escherichia coli with 100% accuracy offers a clear advantage over traditional methods like bacterial culturing, which are laborious, time-consuming, and often less sensitive.

One of the key findings of this study is the assay's sensitivity, which can detect as little as 0.01ng/ul of bacterial DNA. This sensitivity is crucial for diagnosing subclinical mastitis, a form of the disease where infection persists without visible symptoms, leading to prolonged economic losses due to decreased milk production and quality (Safdar & Junejo, 2015; Safdar & Ozaslan, 2022; Safdar et al., 2023; Zaheer & Safdar, 2020). Early detection through multiplex PCR enables timely intervention, reducing the spread of infection within herds and minimizing the need for costly treatments or culling.

Furthermore, the study's results revealed a high prevalence of mastitis pathogens in buffalo herds in the Multan region, with E. coli and Staphylococcus aureus being the most frequently isolated bacteria (Zaheer & Safdar, 2020). These findings underscore the need for enhanced mastitis control programs in the region, including better hygiene practices, routine screening, and targeted antibiotic therapy. The study also evaluated the antibiotic susceptibility of the identified pathogens, revealing varying levels of resistance, particularly in E. coli isolates. This highlights the growing challenge of antimicrobial resistance (AMR) in dairy farming and the importance of responsible antibiotic use (Parveen et al., 2021; Zaheer & Safdar, 2020).

The development of a rapid, cost-effective diagnostic tool like multiplex PCR not only aids in better management of mastitis but also helps in minimizing the use of antibiotics, reducing the risk of AMR. As the assay is highly adaptable, it can be expanded to include additional pathogens or modified for use in other livestock species, making it a versatile tool for veterinary diagnostics.

Conclusion

The multiplex PCR assay developed in this study offers a rapid and reliable tool for the simultaneous detection of multiple mastitis-causing pathogens in buffalo milk. Its high specificity and sensitivity make it an ideal method for early diagnosis, which is crucial for effective management of mastitis in dairy herds. The study also highlights the importance of monitoring antibiotic resistance to improve treatment outcomes for mastitis-affected animals.

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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Robotic Stretcher for Spinal Muscular Atrophy Patient: Test of User Controllability with Operating Device and Monitoring System

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Abstract: Diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy (SMA) often cause motor disabilities through the progressive loss of muscle power. Also, many SMA patients must lie horizontally and thus cannot operate a wheelchair. The aim of this study was to develop a robotic stretcher for an SMA patient with severe motor disabilities to enable her to maneuver independently inside a building. The concept underlying the stretcher is that the user should be able to drive the stretcher using a suitable operating device while watching a display feed from cameras mounted on the stretcher. We have developed new devices with an operating device and its algorithm, monitoring system, mechanical frame, and control system tailored to the user's limited abilities (motion in only one finger). We have verified their functions through tests of a prototype machine operated by the target user. The tests were conducted with the machine positioned on the ground to simulate a shopping mall environment. The subject was able to control the machine effectively, aided by the monitoring system that provided visibility of the stretcher's position within the environment. Remarkably, even without specific guidance, the subject could operate the stretcher efficiently. To enhance the stretcher's operability, we considered focusing on controlling the straight motion performance of the mechanical body, which includes two independently motor-driven wheels and casters.

Keywords: Welfare robotics, Service robotics, Medical robotics

Introduction

Diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) often cause motor disabilities with progressive loss of muscle power. To provide solutions for their mobility needs, we developed a

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

robotic stretcher for an SMA patient in a joint project. The user of the stretcher aims to operate it independently, initially inside buildings and eventually outdoors. Conventional electric wheelchairs offer a variety of operating devices. Joysticks and head-joysticks have been developed for smooth wheelchair locomotion (Rofer, 2009), as well as controls that can detect the user's breath. Furthermore, a switch system that controls a self-aided manipulator system has been developed for bed-ridden patients (Hanafusa et al., 2009). However, due to the severe limitation of the current user's motor abilities, it was not feasible to apply any of these devices directly to a stretcher.

With an operating system that senses the bio-signals of the user's body such as by electromyography or electroencephalography, the user can drive the mechanism by activating his/her target muscle (Sankai et al., 2000; Zang & Nakamura, 2006). The combination of electroencephalogram (EEG) and functional electrical stimulation (FES) has been researched for rehabilitation systems (Takahashi et al., 2009). Also, there are some vision-based methods for controlling machines. For example, an upper extremity-supporting robot was developed using a system that could detect gaze motion (Sakaki, 2007, 2009). Visually based assistive robots were developed for holding daily items for bedridden patients. (Kim et al., 2009).

In addition, a multi-sensor system including a camera for monitoring a patient's physiological status was developed (Peng, 2009). However, we could not apply these methods to the target system due to the difficulty of simultaneously both watching environment around the user through a monitor and operating the stretcher by means of the user's bio-signals such as her gaze motion. In addition, the user's field of view is strictly limited by her lying position on the bed. On the other hand, brain-machine interface (BCI) technology has the potential to induce robot motion in association with the user's intentions by connecting the brain directly to the robot (Branner et al., 2004). However, the risk of infection in the user's brain during the surgical operation remains.

Goal of the Robotic Stretcher

The goal of this project was to develop a robotic stretcher for an SMA patient (Fig. 1(a)) to enable her to maneuver independently, first, inside a building and, ultimately, outdoors. Figure 1 (b) shows the concept of the stretcher. The user can drive the stretcher using the operating device despite her disabilities by watching the display feed from the cameras on board the stretcher. Four technical areas needed to be addressed: the operating device and its algorithm, the monitoring system composed with the display and the cameras, and the body frame mechanism supporting the user's body. Also, it was necessary to verify the safety and easy operability of the total system comprising these devices. First, the operating device needed to be operable even with the user's very limited abilities. The subject was able to move her index finger, but in a small range of motion and with little force. Also, the finger could not move in any direction outside the plane of flexion and extension. Therefore, a conventional manual device such as a joystick was not applicable.

(a)

(b)

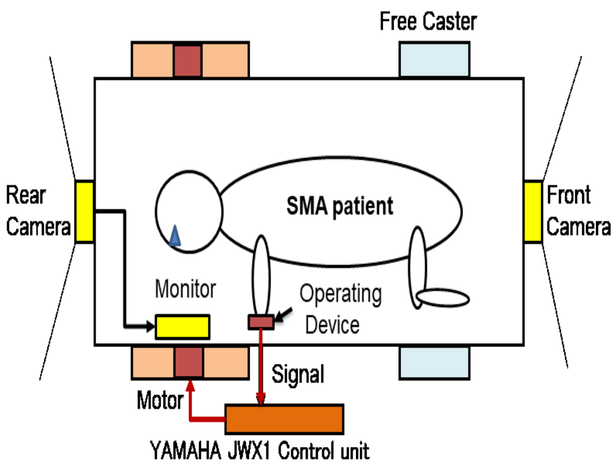

Figure 1. (a) Target user of robotic stretcher lying on the conventional stretcher driven manually by a care-giver, and (b) concept of robotic stretcher for SMA patient for independent operation by the user.

A new device had to be developed to translate a wide range of signals into precise operation, even with the subject's weak finger force and with less freedom of finger motion than in a less-disabled user. Secondly, the monitoring system needs to cover a wide "dead angle" because of the patient's body position; specifically, she was always lying down on the right side of her body in a bed. Therefore, she has a severe restriction to recognize the views toward her head, feet and back. Thirdly, the mechanism supporting the user's body had to be designed to be equipped with the new operating devices, the driving unit, and the monitoring system. Fourthly, some results from our risk assessment of the prototype machine are explained. Also, we show the experimental results for the subject's use of the prototype machine with the new operating device, control system and mechanism.

Operating Device

Motor Ability of Target User's Finger

Because of the paralysis of the user's whole body except for her right index finger, the motor abilities of the finger were researched in relation to the possible operation of the stretcher (Sakaki et al., 2010). The range of motion of the right index finger and the available force exerted by the finger were measured. Figure 2(a) shows the position angles from the most extended to the most flexed. The angle of the finger was defined as the angle between two straight lines from the MP joint to the tip of the finger. The range of motion was 45.0 degrees. The maximum forces in the flexion and extension directions were 4.1*10-4 N and 8.2*10-4 N, respectively. In addition, the maximum frequencies of the flexing and extending motions in the neutral and full ranges of motion were 2.0 Hz and 0.3 Hz, respectively. Based on these results, the motor ability of the target index finger does not seem to be inferior to that of a normal subject in terms of the response and precision of positioning of the finger. However, the range of motion and force of the finger are considerably inferior to those of a normal subject. We considered these conditions in the development of the device.

Switch Circuit for Target User's Finger with Control Hardware

The operating system developed for the prototype model comprises 1) a touch switch circuit and probe, 2) a supporting mechanism to adjust to the shape and posture of the user's finger so it can touch the probe, 3) a controller with microcomputers, 4) a control algorithm installed in the controller to generate the commands to motor drivers from a touch switch circuit, and 5) a display showing the state of the current command.

We have investigated several devices (Kim et al., 2009). However, many sensors present difficulties such as setting the sensor on the user's hand, high costs, and issues with noisy signals and the certainty of executing the STOP command. The subject's finger, being tilted and slightly flexed, cannot evenly touch switches mounted on a flat sheet. Due to her weak finger force, she cannot securely press a switch, resulting in unstable or noisy output signals. Initially, we applied a conductive-type touch switch (Kim et al., 2009; Peng et al., 2009; Sakaki et al., 2011). It could sense a light touch and adapt to the finger's position and shape. However, recent experiments revealed that the user was unable to operate the capacitive touch sensor for unknown reasons, likely due to insufficient change in fingertip capacitance.

Consequently, we replaced it with a mechanical push-button switch. This switch system has been validated through experiments using the switch to control the stretcher motions (Fig. 2(b)). Similarly, the STOP command can be reliably executed by releasing the finger from the probe. The device with the mechanical switch is connected to microcomputers (Arduino Uno) (Fig. 3(a)), producing low-noise output signals. The circuit's output is linked to the Arduino, which calculates the velocity command for the motors to the driver unit JWX-1 (YAMAHA Co.). The command is selected in a round-robin format, changing with each switch activation. The selected command is executed by maintaining contact with the probe for over one second (Fig. 3(b)).

We developed the switch system with the sensor probe and the supporting mechanism. Attaching the operating device to the user's finger took time due to the flexible tube's short length, which hindered switch position adjustment to the index finger. The large sensing base size caused interference with finger operation. Additionally, touching the sensor's back in an extended posture caused user fatigue. Mounting the sensor probe on a long arm's tip facilitated device position adjustment to the index finger. The sensor device, attached directly to the arm, allowed easy finger contact with the probe. We tested the old and new devices, comparing the setting time, command completion time, and command accuracy. The new mechanical switch showed shorter setting and command completion times with the same accuracy level.

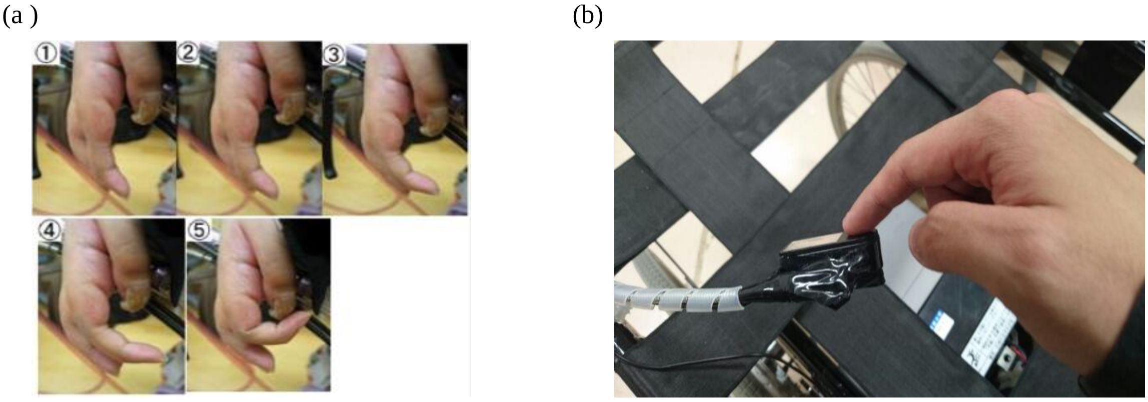

Figure 2. (a) Positions of the target user's index finger. (1) Maximum extension, (2) Light extension, (3) Neutral, (4) Light flexion, (5) Maximum flexion. (b) Mechanical light touch switch suitable for the target use's finger.

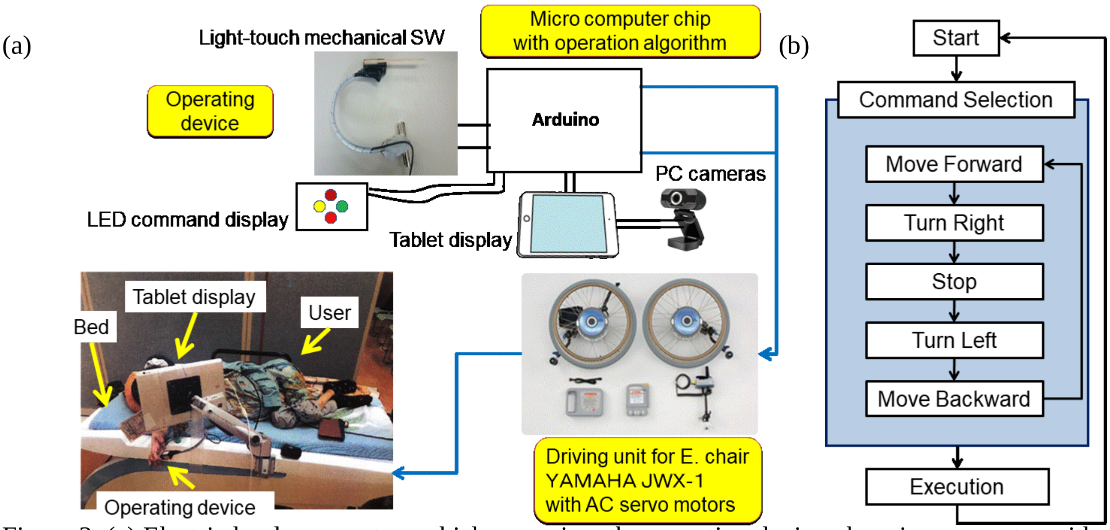

Figure 3. (a) Electric hardware system which comprises the operating device, the microcomputer with operation algorithm and the driving unit YAMAHA JWX-1. (b) The command is selected in a round-robin format.

Monitoring System of Surroundings of Stretcher

The monitoring system surroundings of the stretcher needs to cover a wide "dead angle" because of the patient's body position and posture; specifically, she was always lying down on the right side of her body in a bed (Fig. 4 (a)). The system helps the user to recognize the environment around the stretcher body. The user cannot recognize the surrounding environment on the back, head, and feet. To solve this problem, several web cameras were installed on the stretcher and displayed on the tablet mounted on the stretcher body (Fig. 4 (b)).

As an initial trial, we arranged four cameras around the stretcher to operate using the images. The usability was verified through experiments to determine if the user could recognize the surroundings and operate the stretcher smoothly. The operation experiments with four cameras revealed issues like blind spots and difficulty in distance recognition for walls or other obstacles. Thus, we installed six cameras to six and reconsidered their position and posture. We also added an auxiliary line to the image to indicate distance. However, six or more cameras made the display images small and hard to recognize. We experimentally examined the camera position and posture, obstacle visibility, image delay, continuity, and distance perception (Fig. 4(b)).

We compared the blind spot areas within a 1.5-meter radius half-circle on the screen (Fig. 4(c)). Additionally, we considered risks like obstacle collisions when operating through the camera image and devised countermeasures. Camera position ② had less image overlap than position ①, with a front blind spot width of less than 7 cm. Position ② was installed (Fig. 4(b)). Video continuity was confirmed by checking if the surroundings appeared continuously when a person moved around the device. Distance perception was evaluated by bringing the stretcher closer to the wall, referring to the auxiliary line image, and judging the collision verge distance. We conducted similar experiments with the camera mounting posture tilted downward by 0, 5, 10, and 15 degrees. Image delay did not affect operation, and image continuity was confirmed. However, distance perception remained unchanged with or without the auxiliary line (Fig. 4(d)). Tilting the camera downward improved distance understanding but hindered distant object recognition. Therefore, we decided not to tilt the forward-facing camera.

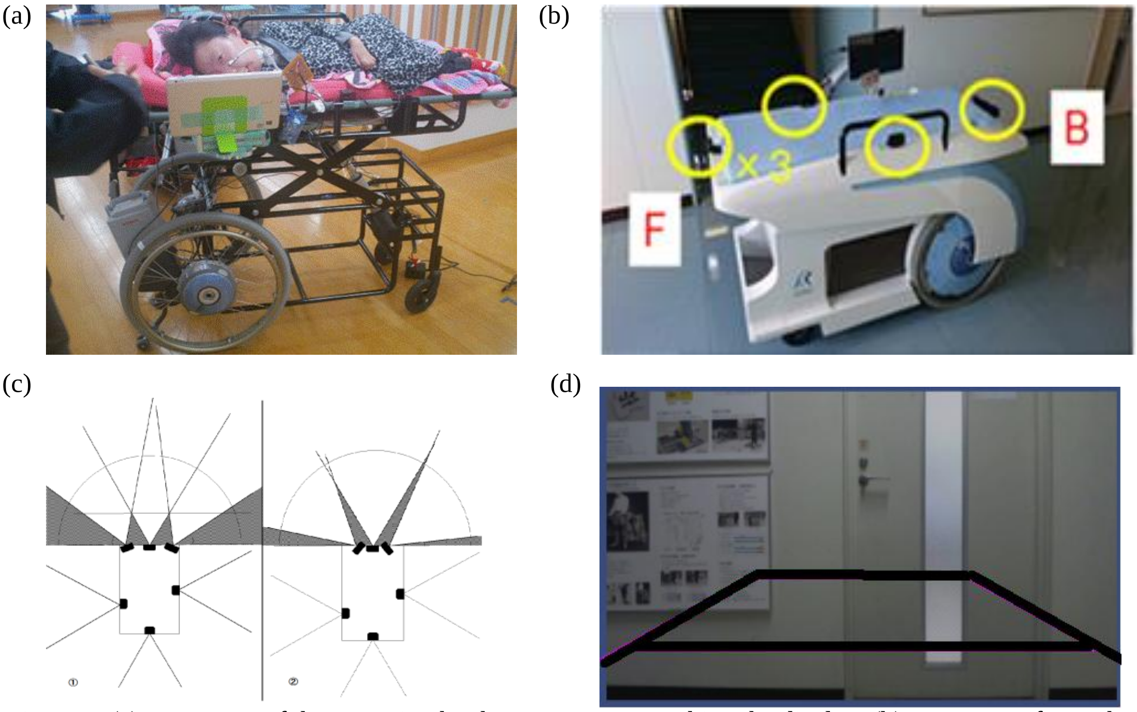

Figure 4. (a) Test scene of the operation by the target user, watching the display. (b) Prototype of stretcher body with exterior and camera positions. F is front side and B is back side. (c) Test results of the area of the blind spots of camera images. (d) The auxiliary line installed in the camera image to get the sense of depth to the wall.

The monitoring system, which utilizes several cameras and displays, will reduce positioning and turning errors by helping the user recognize the environment around the stretcher. To verify the effectiveness of the monitoring system in suppressing errors for straight-line movement, the stretcher was tested to move straight and stop so that the auxiliary line of the side image of the stretcher was aligned with the center of the red mark on the wall in the test room. The stopping errors were measured five times. For turning experiment, the stretcher was rotated 90 degrees to the left while viewing the image in front of the stretcher and stopped so that the image auxiliary line matched the mark in the test room. As a result of the stop accuracy test during a 2-meter straight run, the average error without a camera image was 0.13 m with a standard deviation of 0.04 m. With a camera image, the average error was 0.05 m with a standard deviation of 0.03 m, indicating an improvement in stop accuracy. Additionally, during the stop accuracy test for a 90-degree turn, the average error without a camera image was 10.0 degrees with a standard deviation of 2.2 degrees. With a camera image, the average error was 4.0 degrees with a standard deviation of 0.8 degrees, indicating an improvement in stop accuracy.

Stretcher Body Structure and Risk Assessment

We have developed several stretcher body-frames. The first prototype, made of aluminum alloy pipes (A5052, 3 mm in diameter), was designed to be identical in size to the stretcher the target user currently uses. In designing the frame mechanism, we used the commercial nonlinear finite element analysis, MSC Marc (MSC Software Corporation), to analyze the mechanical performance. Axial force and a bending moment were applied to the frame pipes. The maximum stress inside the frame calculated by FEM (Finite Element Method) was less than the allowable stress for the aluminum alloy, 100 MPa. The results of the stress analysis demonstrated the sufficiency of the frame structure, 600 mm W * 1200 mm L * 700 mm H. The driving unit for an electric chair, namely JWX-1, was mounted on the body frame, including AC servo motors and a motor driver. However, the straightness is still not sufficient. That is, when the machine was moved straight for 5 meters and the deviation to the side was measured 5 times, the deviation to the left was outside the allowable range. It is believed that the battery mounting position is biased to the left and is causing interference with the caster direction. The distortion-free vehicle body described in the experiment results was constructed using steel pipes with a diameter of 3 mm. The dimensions of this vehicle body remain consistent with the previous model, featuring measurements of the same frame structure: 600 mm in width, 1200 mm in length, and 700 mm in height. Notably, the strain issue has been successfully resolved.

We have assessed the risks of the full-size stretcher to improve the vehicle's safety. We have categorized the risks in terms of the risk of operator injury, other risks to the operator's health and the risk to other people or to the operating environment. Based on the results of the preliminary risk assessment, we have improved the intrinsic safety of the stretcher, particularly by adding protective guard rails. In addition, we have developed the camera-based monitoring system so that a user who is lying sideways on the bed can monitor obstacles around the stretcher. According to the standard of risk management (ISO 14121), we have assessed the risks such as the identification of reasonably foreseeable misuse, the hazard identification, the risk evaluation, and the assessment of risk level. Until reaching to the verification of the tolerable situations, the reasonable risk reductions were considered.

Test of Prototype Machine by Target User

The environment for the tests of the stretcher simulated maneuvering around a shop in a shopping mall as shown in Fig. 5(a). First, the stretcher was operated to stop in front of the shop on the reference line, which was the center line of the corridor in the shop. Secondly, the stretcher turned around at 0.5 π to head for the corridor. Thirdly, the machine went straight down the corridor, which was 2 meters wide, for 10 meters into the simulated shop. The margins of the width, which were +/- 700 mm derived from the 600 mm width of the machine at the center of the corridor are the target positioning error at the terminal of the corridor. The performance of the stretcher in positioning on the line, turning around to head for the corridor and moving forward was evaluated.

In the test environment, she was able to operate the device to control the machine stably (Figure 5(b)). The machine moved through the simulated environment in three steps, 1) positioning after maneuvering straight along the wall, 2) taking a stance after turning, and 3) moving forward along the corridor. The trials were repeated five times by the user. The experimental results showed that these three positioning errors were as follows:1) the positioning error in the first step after walking straight was 50 mm +/- 33 mm as an average error with one standard deviation, 2) the turning error translated into the positioning error at the end of the corridor was 680 mm +/- 140 mm, 3) the positioning error at the end point of walking straight was measured in two cases depending on the posture of the casters. In the first case, the position of the casters, which had NOT been adjusted, was aligned with the turning movement. The error was 1260 mm +/- 910 mm. In the second case, the position of the casters was modified so that they were aligned with the forward direction. The error was 705 mm +/- 84 mm. This was the minimum error as the casters are straight forward. The overall operability of the motion in the model environment was evaluated using the Program Evaluation and Review Technique (PERT) [13]. The total positioning error through three motions was 1990 mm +/- 921 mm as the worst case with the maximum error in straight motion. On the other hand, the total positioning error through these three motions was 1435 mm +/- 167 mm as the best case with the minimum error of positioning error in straight motion. These two results are significantly larger than the target range of +/- 700 mm at the end of the corridor. The total error exceeds the target error given the offset and variance of the rotation error. (Fig. 5 (c)).

To suppress these errors, it is necessary to improve not only the operability but also the mobility of the vehicle. The robustness of the straight motion of the stretcher was to be achieved by adjusting the direction of motion. In the worst-case scenario, which produced the maximum errors, the casters were oriented either to the left or to the right with respect to the straight direction of the stretcher. In this situation, the mean of the total operation error deviated from the target (1990 mm) and the standard deviation was also large (921 mm). In the best-case scenario, which produced the smallest errors, the rollers were aligned in the straight direction. Although the mean of the total error differed from the target (1435 mm), the standard deviation was relatively small (700 mm). In this case, that smaller standard deviation means that if we can bring the mean of the actual data closer to the target, the values within that standard deviation are more likely to be within an acceptable range of deviation from the target. If the casters are aligned with the direction of travel, it is easy to achieve the target. However, if the caster orientation deviates from this position, the probability of success decreases. Therefore, we suggest measuring the starting orientation and adjusting the machine's position based on this measurement to achieve the target distance. To achieve this, adjustments should be made to align the rollers in a straight direction. However, after a 90-degree turn, the castors may still be oriented to the left or right. To align the casters with the straight direction, we should consider corrective action after the vehicle has completed the turn to align the casters.

(a) (b)

(c)

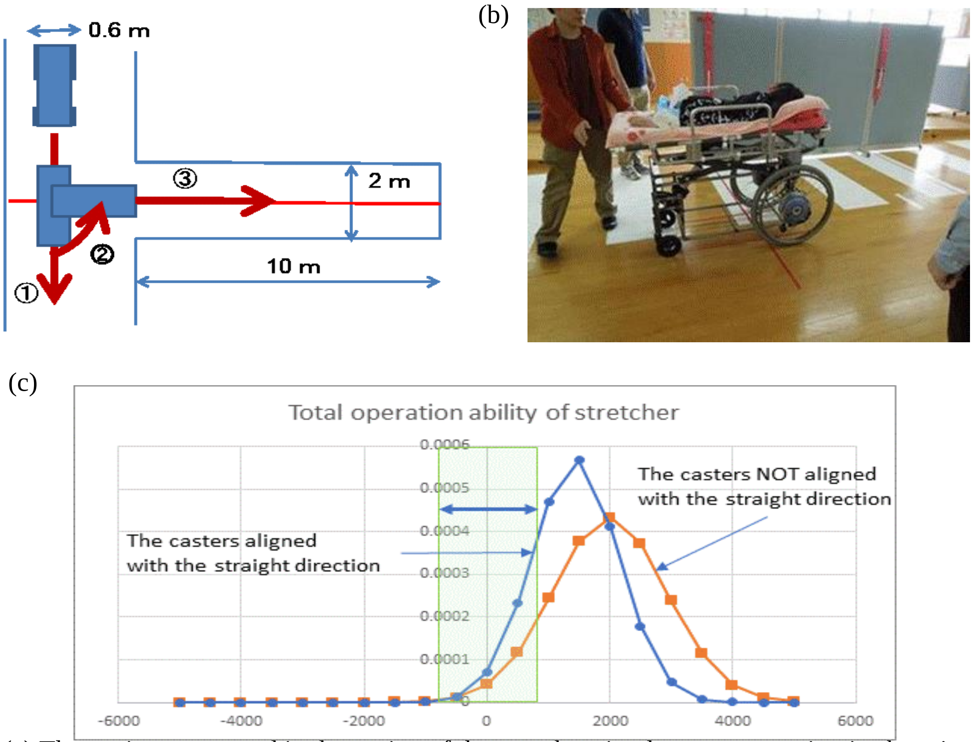

Figure 5.(a) The environment used in the testing of the stretcher simulates maneuvering in shopping mall. (b) Test scene by target user in the test environment. (c) The total positioning error through three motions in the two cases of the casters aligned (bule line) / NOT aligned (red line) with the straight direction in the corridor.

Conclusions and Future Topics

The goal of the project was to develop a robotic stretcher that would allow an SMA user with severe motor disabilities to maneuver independently inside a building. We developed new devices with an operating algorithm suited to the user's limited mobility (single-finger mobility) and verified the functioning of the stretcher through tests with a full-size prototype. In accordance with the mobility of the user's index finger, we developed an algorithm for operating the stretcher. Considering the reliability of the system against operation errors, the intuitiveness of the system, the responsiveness of the machine's motion, and the fatigue of the user, we have applied a conductive-type and mechanical push-button type switches. The mechanical type switches can be applied to the device even given the twisted position and weak force of the target user's finger. The several driving units have been fabricated with a full-size body frame made of aluminum alloy pipes and steel pipes. The target user was given opportunities to control the stretcher using the operating device. We concluded that the user could control the device stably. The controllability of the machine must be improved with more sophisticated interface and control methods that can give more precise directions for the unit to stop along walls and turn corners.

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

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Medical Chemistry and Its Influence on the Results of Treatment of Patients with Acute Bleeding from the Lower Compartments of the Gastrointestinal Tract

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Abstract: Emergency care for patients with gastrointestinal bleeding, despite the substantial progress in drug therapy and development of endoscopic and angiographic methods of hemostasis, remains a very important issue of modern medicine. Acute bleeding from the large intestine causes up to 12-15% of all gastrointestinal bleeding. In the general population it occurs in 0.03% of individuals. There is fibrinolysis inhibitor that had been widely used for last decade. It is Tranexamic acid. Administration of Tranexamic acid is included in national guidelines and a number of international clinical protocols that describes providing medical care to patients with bleeding. To assess efficiency of treatment strategy, that includes administration of Tranexamic acid in patients with bleeding from large intestine. Primary outcomes are number of rebleeding and surgeon interventions. There were 546 inpatients, that underwent treatment in proctology department of 6th Dnipro City Hospital from 2016 to 2023. Patients were between the ages of 35-89. The number of patients under the age of 44 was 34 (6%), 45-59 – 92 patients (17%), 60-74 – 224 patients (41%), over 75 – 196 patients (36%). Gender ratio is 234 males (42,8%) and 312 females (57,2%). After examination of patients, following diseases were determined as causes of bleeding from large intestine: Crohn's disease – 5,3 %; ulcerative colitis – 12,7 %; colonic diverticulitis – 36,5 %; colorectal cancer – 20,1 %; colonic polips - 1,3 %; hemorrhoids – 24,1%. Strategy including Tranexamic acid permitted to decrease amount of rebleeding and surgical interventions, performed due to failed hemostasis, from 25,6% to 12,9%. Total mortality decreased from 11,5% to 6,4%. Indications for surgical interventions in case of bleeding from the large intestine are failed medication and endoscopic hemostasis, as well as rebleeding. Rebleeding occurs more often in those who is suffered from diverticular disease of the sigmoid colon and colonic villous adenomas.

Keywords: Tranexamic acid, Patients, Bleeding.

Introduction

Bleeding from the lower part of the gastrointestinal tract (below the ligament of Treitz) in ≈20% of patients who are hospitalized for bleeding into the gastrointestinal tract. The most frequent cause of serious bleeding is diverticula of the large intestine, less often - inflammatory bowel diseases, hemorrhoids (varicose dilated hemorrhoidal veins), neoplasms and vascular malformations, and in childhood and adolescence - intussusception (based on polyps), inflammatory bowel diseases, inflammation of Meckel's diverticulum and polyps of the small or large intestine. Bleeding can also be a consequence of coagulopathy. To assess efficiency of treatment strategy, that includes administration of tranexamic acid in patients with bleeding from large intestine. Primary outcomes are number of rebleeding and surgeon interventions.

Emergency care for patients with gastrointestinal bleeding (GI), despite the significant progress in drug therapy and the development of endoscopic and angiographic methods of hemostasis, remains a very urgent issue of modern medicine (Ivashkin, 2003; Jensen, et al., Harris, 2002). The number of hospitalized patients does not tend to decrease, and the mortality rate in gastro-intestinal tract remains high. Contributes to this: insufficient provision of endoscopic equipment and specialist endoscopists working around the clock; centers for providing assistance to patients with gastro-intestinal tract bleeding. Uncorrected concomitant pathology significantly worsens the prognosis of patients with gastro-intestinal tract bleeding.

Bleeding from the lower parts of the gastrointestinal tract is bleeding from an area that is located distal to the ligament of Treitz. It can be acute and chronic, massive and hidden. The tactics of examination and treatment of such patients are not fully developed in comparison with bleeding from the upper gastrointestinal tract. Bleeding from the lower parts of the gastrointestinal tract makes up approximately 20% of all gastrointestinal bleeding. The overall mortality rate for such bleeding is not high, 3-4%, but the mortality rate in the elderly and senile, in the presence of concomitant pathology, can reach 10% and even 25% (Bohnacker et al., 2000; Colacchio et al., 1982; Harris, 2002; Terdiman, 2001)..

Elements of the diagnostic algorithm at gastro-intestinal tract bleeding are as follows: clinical assessment of the symptom complex of acute gastro-intestinal tract bleeding, determination of deficiency volume circulated blood and degree of blood loss, severity of the condition patient, determining the location of diagnostic and treatment measures. The final step of the diagnostic algorithm was establishing a clinical diagnosis and the choice of further treatment tactics.

Patients received conservative therapy (infusion-transfusion therapy, hemostatic, symptomatic, etc.) according to methodological recommendations, if necessary endoscopic hemostasis was performed. Due to the ineffectiveness of the above-mentioned measures, the question of performing a surgical procedure was decided intervention. First of all, when the patient was admitted, bleeding from the upper parts had to be ruled out Gastrointestinal tract, because in some cases with massive bleeding there may be the release of unchanged blood with fecal masses.

Bleeding that cannot be stopped by conservative treatment methods requires emergency surgery in 10% - 55% of patients. It is especially difficult to choose the scope of such interventions for non-localized sources of hemorrhage, in which the only rational method is forced subtotal resection or colectomy, which increase the lethality to 17.2% - 22%.

The fundamental question of treatment tactics has not been finally resolved: some surgeons believe that it is more expedient to first stop the bleeding, and therefore to apply special research methods to establish a nosological diagnosis, the second tend to operate on patients at the height of hemorrhage. Insufficiently developed differentiated indications for certain types of surgical intervention for this situation.

Views on the volume of operations for cancer - the most frequent cause of bleeding - are controversial. From the point of view of some surgeons, palliative operations should be used, others insist on the possibility of radical interventions. The issue of the possibility and expediency of adjuvant chemotherapy for patients with cancer complicated by bleeding was not adequately addressed.

As a result, the significant frequency of colorectal hemorrhages and the controversial views on their diagnosis and treatment methods put in a number of important and urgent problems of emergency abdominal surgery. For this, patients were administered nasogastric probe and aspirated stomach contents. Thus, 8 patients had blood impurities in the aspirated contents. Because these patients were admitted at night, it was the endoscopist on duty at home was called, who during urgent fibrogastroduodenoscopy, he diagnosed duodenal ulcer in 6 patients, gastric ulcer in 2, which were complicated by bleeding.

Method

There were 546 inpatients, that underwent treatment in proctology department of KI "6th Dnipro City Hospital DOR" from 2016 to 2023. It is clinical base of Department of Surgery №1 and Urology. Patients were between the ages of 35-89. The number of patients under the age of 44 was 34 (6%), 45-59 – 92 patients (17%), 60-74 – 224 patients (41%), over 75 – 196 patients (36%). Gender ratio is 234 males (42,8%) and 312 females (57,2%). After examination of patients, following diseases were determined as causes of bleeding from large intestine: Crohn's disease – 5,3 %; ulcerative colitis – 12,7 %; colonic diverticula – 36,5 %; colorectal cancer – 20,1 %; colonic polips - 1,3 %; hemorrhoids – 24,1%.

The nosological structure of the causes of colon bleeding was studied. The peculiarities of clinical symptoms and the course of colon bleeding depending on their cause have been clarified. For the first time, a formalized system for determining the localization of the source of bleeding, differential diagnosis of cancer and diverticulosis complicated by hemorrhage was proposed. The optimal diagnostic and therapeutic tactics for the treatment of patients with colon bleeding have been elaborated and substantiated. For the first time, a two-stage operation was used for cancer complicated by bleeding, in which the tumor-affected segment of the intestine is removed in the first stage to stop bleeding, and in the second stage, a regional lymphadenectomy is performed, if necessary, with additional removal of a segment of the intestine, the imposition of interintestinal anastomoses. The study of long-term results of cancer treatment complicated by bleeding, with the use of modern methods of statistical analysis, was further developed.

The results of the work improve the diagnosis of the causes and localization of the sources of colon bleeding, optimize the diagnostic and treatment tactics, expand the indications for surgical treatment of colorectal cancer. After admission of patients, detailed examination was performed in order to confirm the diagnosis. It was composed of clinical assessment, laboratory and instrumental investigations (colonoscopy, barium enema, US, CT, virtual colonoscopy etc.). Patients were prescribed non-surgical treatment. It included management of blood loss and taking hemostatic agents. 78 patients had been administered different hemostatic agents (ethamsylate, vicasol) by 2016. And 468 patients had been prescribed tranexamic acid (in patients without signs of decompensated cardiovascular failure, with low risk of thromboembolic complications) in dosage 10 mg/kg (but less than 1000 mg a day) since 2017.

General clinical symptoms revealed a number of features depending on the nature of the pathology that was the cause of bleeding. In particular, loss of appetite, rapid weight loss, bloating, defecation disorders in the form of constipation with diarrhea occurred more often in cancer than in other TC diseases ($p<0.05$). Patients with cancer, as well as with benign diseases of the anal area, complained of pain more often than with other diseases. Defecation disorders and the presence of mucus in feces were significantly more common in erosive-ulcerative diseases. Pain of various localization and nature was the most frequent symptom in cancer patients (66.9%). The pain was mostly constant (46%), less often spasm-like.

The latter concerns patients who had elements of intestinal obstruction, as well as patients in whom frequent imperative defecation with bloody masses came to the fore. Pain in the hypogastrium is especially common in cancer and diverticulosis. During an objective examination, reduced nutrition, palpation of a pathological tumor in the abdomen was found more often in cancer patients than in other types of pathology ($p<0.05$). Pain during digital examination of the rectum and palpation of a pathological lesion in the rectum is most typical for benign diseases of the anal area.

The cardinal sign of colon bleeding - blood discharge from the anus - was present in all patients, in particular, liquid blood without fecal masses - in 51.7% of patients, impurities in the fecal masses of practically unchanged blood - in 29.7%, changed blood - in 18 .6%. Two patients had melena, despite the verified absence of a source of bleeding in the upper parts of the alimentary canal. The first type was noted most often in non-neoplastic processes of the anal area, the second - in polyps (50%), fresh blood with admixture of mucus - in cancer patients.

Changed blood (cherry or raspberry color) occurred with approximately the same frequency in all types of pathology, except for non-neoplastic processes of the anal area. During a digital examination of the rectum in 13 patients, fresh blood with an admixture of mucus was found more often in cancer patients (8.1%) compared to other types of pathology. Fresh blood in the ampoule was determined in 44.4% of all cancer patients, which can be associated with the intermittent nature of the hemorrhage. With polyps, an admixture of fresh blood during a digital rectal examination was found in 4.5%, and according to the anamnesis - in 50% of patients, which was a sign of more massive, but short-term hemorrhages. It is with polyps that most often in the rectum we found normal fecal masses (22.7%). Determining the duration of actual bleeding causes significant difficulties. Bleeding can occur in several episodes; the time of the appearance of bloody secretions from the intestine is determined by the localization of the source and the intensity of the hemorrhage, the volume of the lumen and the functional characteristics of the TC. However, it is not always possible to rely on the general signs of bleeding. Taking into account these circumstances, we divided the patients into two groups, each of which was formed according to the most significant criteria that provided an opportunity to clarify this issue.

Results and Discussion

The analysis showed that the amount of operative interventions performed due to failed hemostatic treatment was 20 (25.6%) in 2016, whereas 59 patients underwent surgery from 2017 to 2022. Surgical activity in cause of ALI was 12.9%. Following surgical interventions were performed: Left hemicolectomy (diverticular bleeding) – 2 in 2016 and 5 between 2017-2022.

Right hemicolectomy (malignant tumors of the caecum) – 1 in 2016, and 4 between 2017-2022. Low anterior resection (rectal polyps) – 2 in 2016 and 5 between 2017-2022. Obstructive resection of sigmoid colon (diverticular bleeding) – 1 in 2016, 6 between 2017-2022. Laparotomy, polypectomy (polyps of sigmoid colon) – 2 in 2016, 5 between 2017-2022. Endoscopic polypectomy (colon polyps) – 4 in 2016, 9 between 2017-2022. Hartmann's operation (Crohn's disease, bleeding from the diverticula of sigmoid colon) – 3 in 2016, 9 between 2017-2022. Transanal removal of bleeding rectal polyps – 5 in 2016, 16 between 2017-2022. In addition, we analyzed the sources of bleeding, that required surgery treatment. The most frequent rebleeding was caused by diverticular disease of sigmoid colon as well as rectal and sigmoid colon polyps, that were histologically villous adenomas in 88% of cases. Outcomes assessment of suggested treatment strategy was performed by means of comparing surgical activity, postoperative and total mortality. Operative activity is 20 (25,6%) in 2016 and 59 (12,9%) between 2017-2022. Postoperative mortality is 1 (5%) in 2016 and 3 (0,7%) between 2017-2022. Total mortality is 11,5% in 2016 and 6,4% between 2017-2022.

This patient underwent endoscopic surgery hemostasis and after stabilization of the condition, the patients were transferred to the surgical department. To establish the source of bleeding except of physical examination methods with digital rectal examination, anoscopy, rectoromanoscopy, colonoscopy were used. If necessary, total colonoscopy performed 12-48 hours after the patient's admission to the hospital after stabilization of the patient's condition. Endofalk or Fortrans was used to prepare the colon for the study. In 2 patients with severe ongoing bleeding colonoscopy was performed without preparation.

Patients with mild and moderate bleeding were treated in the department of proctology, severe and extremely severe in division of reanimation. In most patients positive results were achieved using conservative therapy. But in 4 patients with non-specific ulcerative colitis it was not possible to achieve stable hemostasis, therefore 2 patients with total damage of the large intestine, a total colectomy was performed, in another 2nd patient to disconnect the affected part of the large intestine, taking into account the severe condition, ileostomies were removed. One is sick died after total colectomy. Bleeding from the lower parts of the gastrointestinal tract remains an important problem, help for such patients should be timely with the use of modern medical technologies, making and implementing quick decisions regarding diagnostic and treatment tactics. Thus, during the period of usage the suggested treatment strategy, the number of rebleeding and surgical interventions, associated with rebleeding, decreased from 25.6% to 12.9%. All-cause mortality rate decreased from 11.5% to 6.4%. There are 4 types of bleeding: 1) the bleeding stopped even before going to the clinic or on the first day of the hospital stay; 2) continued for 2-4 days, then stopped and did not recur; 3) stopped during the first day of stay in the hospital, then recovered; 4) the bleeding did not stop.

The first two types are qualified as stable, the third and fourth - as unstable hemostasis (considering continued bleeding as an extreme variant of unstable hemostasis). Unstable hemostasis prompts the use of special measures to stop bleeding, including surgery, and greatly complicates the full diagnosis of the nature and localization of the source of bleeding. Unstable hemostasis, noted in 12.7% of all patients, is most characteristic of colorectal cancer ($p<0.05$). "Bleeding in the anamnesis" was considered a hemorrhage that was separated from the actual one by a period of time of at least one week of normal defecation. 62 (20.7%) patients had a history of bleeding, especially often in patients with diverticulosis (41.7% of this pathology) and cancer (23.4%). These data, together with the course of bleeding in the hospital and catamnesis, give reason to believe that cancer and diverticulosis are the most prone to recurrence of bleeding. In patients with cancer, recurrences of bleeding occur within a relatively short period of time (usually half a year), in patients with diverticulosis - a much longer period of time *(Colacchio T.A).*

The source of bleeding in 137 (45.7%) patients was located in the rectum and anus; in 100 (33.3%) patients - in the left, in 36 (12%) - in the right sections of the colon. In 27 (9%) patients, the pathological process was located in several places or on a significant length of the intestine, which made it difficult to determine the localization of the source of bleeding (polyposis - in 1, diverticulosis - in 6, erosive-ulcerative processes - in 20 patients). On the basis of the identified significant differences ($p<0.05$) in the characteristics of hemorrhagic syndrome, a diagnostic index of localization of the source of hemorrhage was developed. The main ones are the age of the patients, the nature of discharge from the rectum, systolic blood pressure, the degree of anemia, the localization of pain, the volume of necessary blood transfusions. The sensitivity of the index was 82.3%, and the specificity was 78.9%. It was established that the severity of blood loss is dominated by hemorrhages from the right half of the colon.

Among various types of pathology, the most numerous group (124 out of 300 - 41.3%) is colon cancer, the share of which during the existence of the gastrointestinal bleeding center gradually increased from 32.8% to 53.5% ($p<0.05$). The most frequent cause of tumor bleeding was cancer of the rectum (50.0%) and sigmoid colon (19.3%). A significant part of patients (42.6%) had stage 4 of the disease, especially often patients with rectal cancer (57.6%). Contrary to the previously widespread opinion, bleeding also occurs in the early stages of the disease: the first and second stages of the tumor were found in 7 patients (6% of all cancer patients).

Reports in the literature that bleeding is often the first symptom of colorectal cancer requires clarification - it refers only to the first symptom that prompted patients to seek medical help (Ivashkin, 2003). A system of formalized differential diagnosis of colon cancer has been developed. For this purpose, symptoms were selected from the clinical characteristics of diseases complicated by bleeding, the frequency of which in cancer patients shows a statistically significant difference ($p<0.05$) in comparison with other types of pathology. The sum of points greater than 0 corresponds to the high significance of the diagnosis of cancer, less than 0 to the high significance of another cause of hemorrhage. The sensitivity of the identified diagnostic criteria is 58.9%, the specificity is 89.2%.

The given information makes it possible to establish a diagnosis of colon cancer with a high probability, but not with a significance that could be used as a basis for treatment tactics. Final verification belongs to direct diagnostic methods. The exception was 7 patients, in whom special research methods could not be applied, because ongoing hemorrhage required urgent surgical intervention. Non-neoplastic diseases of the anal area (57 patients - 19.0%) include hemorrhoids (54) and anal fissures (3). Patients of this group are the youngest ($p<0.05$), (average age 48,014.2 years), the majority are men (68.4%) ($p<0.05$). A typical symptom was pain in the area of the anal sphincter (56.1%), which arose or was aggravated by the act of defecation, tenesmus (52.6%).

Hemorrhagic syndrome was manifested by the release of fresh liquid blood from the anus, which in most patients (71.9%) was provoked by defecation. Rectal contents on digital examination also showed fresh liquid (42.1%) and admixture of unchanged blood (24.6%) in feces. The main methods of establishing a diagnosis in all patients were: examination of the anal area, digital examination and rectoromanoscopy. The group of erosive-ulcerative diseases includes erosive processes and non-specific ulcerative colitis. The diagnosis was established by the endoscopic method of research, if necessary with the addition of irrigoscopy, inspection ultrasonography of the abdomen and diagnostic laparoscopy.

Diverticulosis was diagnosed in 36 patients. The average age of the patients was 67.2 ± 13.9 years and was the highest. Hemorrhage was a complication of both diverticulosis (23 patients) and diverticulitis (13), which was distinguished by the presence of a pain syndrome before the appearance of bleeding; inflammatory changes in peripheral blood, the presence of an infiltrate during palpation of the abdomen, a typical endoscopic picture - hyperemia around the eyes of diverticula and contact bleeding during instrumental palpation of the inflamed mucous membrane. Diverticulosis is characterized by particularly great difficulties in diagnosis during bleeding, more pronounced hemorrhagic syndrome, compared to other non-neoplastic diseases (often recurrent course, massive blood loss) (Jensen et al., 2000). A number of symptoms (age, local abdominal pain, presence of bleeding in the anamnesis) were identified, which statistically significantly differed from other types of pathology in terms of their frequency of presence or intensity. A differential diagnostic index of diverticulosis was developed based on clinical symptoms; sensitivity of the method is 83.3%, specificity - 65%. However, the diagnosis is finally verified by special research methods, mainly endoscopic or X-ray *(Bohnacker et al.., 2000)*.

Polyps (22 patients - 7.3%) were characterized by poor general clinical symptoms, but a pronounced hemorrhagic syndrome, which in sum increases diagnostic difficulties and prompts active treatment. Endoscopic research methods are of primary importance in diagnosis. Other diseases are diagnosed endoscopically, by intraoperative revision; hematological diseases were verified by examination of peripheral blood, bone marrow punctate.

The goals of treatment are: 1) stopping bleeding; 2) correction of homeostasis; 3) treatment of the disease that caused the bleeding; 4) treatment of accompanying diseases, which often worsen and decompensate in patients with bleeding. The most important vital task is to stop the bleeding. In the majority of patients, the most appropriate diagnostic and treatment program, according to which conservative methods first stop the bleeding, then special research methods are used, and based on the established diagnosis, the question of the nature of treatment - conservative, endoscopic or surgical - is decided.

The expediency of such tactics is based on the relatively benign course of colon bleeding, in most patients it is moderate and stops after the use of conservative hemostatic therapy. Hemorrhage in the lumen of the colon sharply complicates the resolution of diagnostic issues, primarily the localization and nature of the source of bleeding. Operations at the height of bleeding are associated with high mortality.

Such a tactic, however, is not always possible. Changes are made to this scheme if it is necessary to perform urgent surgical interventions, the indications for which are: 1) bleeding that does not stop, despite the use of all possible methods of hemostasis; 2) high probability of recurrence of bleeding due to unstable hemostasis; 3) combination of hemorrhage with other complications that require surgical treatment (perforation, purulent process, etc.).

The treatment of our patients began with an assessment of the amount of blood loss and its rapid and adequate supplementation in cases of necessity. The main indirect criterion for the massiveness of bleeding was the level of systolic and diastolic blood pressure at the time of bleeding, pulse characteristics, globular indicators (hemoglobin level, number of erythrocytes, hematocrit) *(Terdiman JP., 2002).*

Circulating blood volume was restored by infusion of crystalloid (lactosol, Ringer-Locke solution, 5% glucose solution, physiological solution, etc.) and colloidal (fresh-frozen plasma, albumin solution) solutions. Central venous pressure above 50 mmHg, systolic arterial pressure - above 100 mmHg, diuresis - more than 30 - 40 ml/h were criteria for recovery of Circulating blood volume.

General hemostatic therapy was used - etamsylate, 5% aminocaproic acid, fresh frozen plasma, 5% - 20% p-n albumin. The need for transfusion of blood products arose in 135 (45.0%) patients with colon bleeding, who were transfused with an average of 929.5 627.8 (ranging from 150 to 3350) ml of erythrocyte mass. Patients with cancer, hematological diseases, Randu-Osler-Weber disease and angiodysplasia required the largest number of transfusions.

We prescribed broad-spectrum antibiotics in combination with metronidazole to patients with massive blood loss, leukocytosis, and pain syndrome for therapeutic and preventive purposes. In the treatment of bleeding caused by inflammatory diseases, sulfasalazine, salofalk or their analogues were used. A theoretical generalization and a new solution to the scientific problem are given, which is manifested in the creation of formalized systems for determining the localization of the source of colorectal hemorrhage, diagnosis of cancer and diverticulosis of colon, development of optimal diagnostic and treatment tactics in patients with colonic bleeding (Harris, 2002).

- Colonic bleedings account for 8.7% of all hemorrhages in the lumen of the gastrointestinal tract. The most common causes are: cancer (41.3% of all colon), non-neoplastic diseases of the anal area (19.0%), erosive-ulcer diseases (14.3%), diverticulosis (12.0%), colon polyps (7.3 %).
- Hemorrhagic syndrome is characterized by a number of differences in various diseases. Bleeding occurs with the greatest blood loss in diverticulosis cancer, polyps, most prone to recurrence - in cancer and diverticulosis.
- The optimal diagnostic and therapeutic tactic in most patients consists in stopping bleeding with conservative measures, hence in diagnosing the cause of bleeding and determining the final method of treatment.
- In determining the indications for surgical treatment, the purpose of the operation, the essence of the pathology, the phase of the hemorrhagic process and the state of compensation for homeostasis disturbances are taken into account. It is appropriate to distinguish between emergency operations, with the aim of stopping bleeding, preventive - to prevent its recurrence in case of unstable hemostasis, planned - after restitution of the main indicators of homeostasis for the treatment of the disease that was the cause of bleeding.
- Diagnosis is based on direct research methods (mainly fibrocolonoscopy). Proposed and developed formalized systems for determining the location of the source of bleeding (sensitivity - 82.3%, specificity - 78.9%), diagnosis of colorectal cancer (sensitivity - 58.9%, specificity - 89.2%) and diverticulosis (sensitivity - 83, 3%,

specificity - 65%) can be considered as a basis for the final choice of treatment tactics only if urgent surgery is necessary in patients.
- Bleeding occurs in all stages of cancer. In 52.4% of cancer patients, bleeding is the first symptom that prompts patients to seek medical help.
- To prevent recurrence of bleeding, it is advisable to eliminate the source of bleeding from the alimentary canal. During surgical interventions for cancer of the fourth stage, bleeding is a significant indication for performing palliative operations, which corresponds to the main requirement of surgery - to stop bleeding.
- In cases in which, for one reason or another, full-fledged oncological and surgical treatment is impossible, the operation is carried out in two stages. The first stage most often consists in the removal of the segment of the TC affected by the tumor, which ensures hemostasis, the second - in regional lymphadenectomy, if necessary - additional removal of certain segments of the intestine, imposition of an interintestinal anastomosis. In most patients who underwent radical surgery for cancer, bleeding is not a contraindication to the use of adjuvant chemotherapy.
- Patients with polyps and non-cancerous diseases of the anal area are subject to planned operations among patients with other diseases that are complicated by colon bleeding. Patients with diverticulosis and erosive-ulcer diseases are indicated for emergency surgery in cases of continued bleeding when conservative therapy is ineffective.

Conclusion

Formalized systems for determining the localization of the source of colonic bleeding, diagnosing colorectal cancer and diverticulosis are proposed. The optimal diagnostic and therapeutic tactics for patients with colorectal hemorrhage and a new type of staged radical surgical interventions for colon cancer complicated by bleeding have been developed. The frequency of complications and postoperative mortality were evaluated. The long-term results of treatment were studied. Peculiarities of the course of colorectal bleeding are studied. Hemorrhagic syndrome is characterized by a number of differences in various diseases. Hemorrhages induce the most significant blood loss in cancer, diverticulosis and polyposis, they also are highly prone to relapses in the first two conditions.

Complaints, histories, clinical symptomatology as well as methods of diagnostics and treatment are analyzed. Direct methods of investigation (fibrocolonoscopy) constituted the basis of diagnostics. Formalized systems, designed for localizing the source of colorectal bleeding (sensitivity - 82,3%; specificity - 78,9%) as well as for diagnostics of colorectal cancer (sensitivity - 58,9%; specificity - 89,2%) and diverticulosis (sensitivity - 83,3%; specificity - 65%), are proposed and elaborated. The diagnostic systems serve mainly as approximative means; the may only be considered as a basis for the leading treatment option to be chosen, when an urgent surgical intervention in patients with colonic bleeding is required. An optimal diagnostic and treatment tactics in the majority of patients is to ensure the bleeding cessation using conservative approaches, followed by estimation of bleeding causes and further by development of the final treatment strategy. While evaluating indications for surgical intervention, one has to consider the goal of operation, nature of the disorder, phase of hemorrhagic process and degree of compensation of homeostatic disturbances. It is advisable to distinguish urgent operations to stop the bleeding, prophylactic ones aimed at prevention of its relapses in unstable hemostasis, and planned surgery for underlying disease after restitution of basic hemostatic parameters.

A new type of two-stage radical operations in the cases of colonic cancer, complicated with hemorrhage, is proposed. If no appropriate management from both oncologic and surgical viewpoints can be applied, the operation is divided into two stages. As a rule, the first stage is to remove a tumorously affected colonic segment and to restore hemostasis, ehile the second one being regionary lymphadenectomy with removal of colonic segments, wherever necessary, and subsequent application of inter-intestinal anastomosis.

Recommendations

Application of the treatment strategy including tranexamic acid permitted to decrease amount of rebleeding and surgical interventions, performed due to failed hemostasis, from 25,6% to 12,9%. Total mortality decreased from 11,5% to 6,4%. Indications for surgical interventions in case of bleeding from the large intestine are failed medication and endoscopic hemostasis, as well as rebleeding. Rebleeding occurs more often in those who is suffered from diverticular disease of the sigmoid colon and colonic villous adenomas. In the majority of patients undergoing radical surgery for cancer, the bleeding is not a contraindication for administration of adjuvant chemotherapy. Among patients with other pathologies, complicated with colonic hemorrhages, those suffering from polyposis and non-neoplastic diseases of anal area are eligible for planned surgery. In the cases of continuing bleeding in diverticulosis and erosively-ulcerative disorders an urgent operation is indicated unless conservative therapy is ineffective. The incidences of complications and postoperative mortality are assessed. Long-term treatment results are also investigated. In the future, the application of the proposed method of diagnosis and treatment patients with colon bleeding, can be the basis of a new direction of instrumental diagnostics in surgery and oncology.

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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Synthesis of Silver Nanoparticles and Their Applications: Review

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Abstract: The synthesis of silver nanoparticles (AgNPs) has garnered significant attention due to their unique physicochemical properties and broad range of applications. This study explores various methods of synthesizing AgNPs, including chemical reduction, green synthesis using plant extracts, and physical methods such as laser ablation. The resulting nanoparticles were characterized using techniques like UV-Vis spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM) to confirm their size, shape, and crystalline structure. AgNPs exhibit remarkable antibacterial, antifungal, and antiviral properties, making them valuable in medical applications such as wound dressings, coatings for medical devices, and drug delivery systems. Additionally, their catalytic activity and optical properties enable their use in environmental remediation, water treatment, and sensor development. This study highlights the synthesis processes, characterization, and potential applications of AgNPs, emphasizing their role in advancing nanotechnology and contributing to various fields, including medicine, environmental science, and material science.

Keywords: Nanotechnology, Silver nanoparticles, Catalysis, Antibacterial activity

Introduction

Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) have emerged as a pivotal component in the field of nanotechnology due to their unique physicochemical properties and broad spectrum of applications across various domains (Bamal et al., 2021). These nanoparticles, typically ranging from 1 to 100 nanometers in size, exhibit remarkable characteristics that differ significantly from their bulk counterparts. The nanoscale dimensions of AgNPs impart them with a high surface area-to-volume ratio, which significantly enhances their reactivity and interaction with other substances, leading to exceptional antimicrobial, catalytic, and optical properties (Galatage et al., 2021).

Historically, silver has been recognized for its antimicrobial properties, with its use dating back to ancient civilizations for preserving food and treating wounds (Lansdown, 2006). The advent of nanotechnology has amplified these properties, making silver nanoparticles a subject of intense research. The synthesis of AgNPs involves a variety of techniques, including chemical reduction, biological methods using plant extracts or microorganisms, and physical approaches such as laser ablation and evaporation-condensation (Sohal et al., 2021). Each synthesis method influences the size, shape, and surface characteristics of the nanoparticles, which in turn affects their functionality and application (Sohal et al., 2021).

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

The ability to control the synthesis of AgNPs with precision has led to their incorporation into a wide range of products and applications (Zhang et al., 2016). In the medical field, AgNPs are extensively used in wound dressings, coatings for medical devices, and as carriers for drug delivery systems due to their potent antibacterial properties. Moreover, their application extends to the environmental sector, where AgNPs are utilized in water treatment processes to remove contaminants and pathogens. Additionally, the catalytic properties of AgNPs are harnessed in chemical reactions, contributing to the advancement of green chemistry.

Despite the promising applications, the widespread use of silver nanoparticles raises concerns regarding their environmental impact and potential toxicity (Marin et al., 2015). The release of AgNPs into ecosystems, either through industrial processes or consumer products, could lead to the accumulation of nanoparticles in water bodies, soil, and organisms, posing risks to environmental and human health. Therefore, understanding the synthesis, characterization, and safe use of AgNPs is crucial for advancing their application while mitigating potential risks (Mitra et al., 2023).

Finally, silver nanoparticles represent a significant advancement in nanotechnology with diverse applications in medicine, environmental science, and industry. Therefore, it is essential to focus on developing sustainable synthesis methods, characterization, applications and challenges to ensure the safe and effective use of AgNPs in various fields (Bamal et al., 2021).

Methods of Synthesis for Silver Nanoparticles (AgNPs)

The synthesis of silver nanoparticles (AgNPs) is a critical aspect of nanotechnology, as the method of production directly influences the size, shape, surface chemistry, and ultimately the functional properties of the nanoparticles (Pryshchepa et al., 2020). Several methods have been developed to synthesize AgNPs, each with its advantages and limitations. These methods can be broadly categorized into chemical, physical, and biological approaches (Table 1).

Table 1. Methods of synthesis for silver nanoparticles (AgNPs)

Sr#	Synthesis Method	Type	Processes/Examples	Toxicity
1	Top-Down	Physical Methods	High Energy Ball Milling, Melt Mixing, Physical Vapor Deposition, Laser Ablation, Sputter Deposition, Electric AC Deposition, Ion Implantation, Nano Imprinting, Electro Spinning, Thin Film Deposition, Phase Separation	Toxic
2	Bottom-Up	Chemical Methods	Chemical/Electrochemical Precipitation, Sol-Gel Process, Atomic/Molecular Condensation, Micro Emulsion, Irradiation Method, Tollens Method, UV Irradiated Photoreduction, Microwave Assisted	Toxic
3	-	Biological Methods	Bacteria, Fungi, Yeast, Algae, Plants	Non-Toxic

Chemical Reduction

The most common method for synthesizing AgNPs is chemical reduction, which involves reducing silver salts (e.g., silver nitrate, $AgNO_3$) using reducing agents (Suriati et al., 2014). This method is favored for its simplicity, efficiency, and ability to produce nanoparticles with well-defined sizes and shapes. In a typical chemical reduction process, silver ions (Ag^+) are reduced to metallic silver (Ag^0) in the presence of a reducing agent, such as sodium borohydride ($NaBH_4$), hydrazine (N_2H_4), or ascorbic acid ($C_6H_8O_6$). The reaction can be summarized as follows:

The choice of reducing agent, along with the reaction conditions (e.g., temperature, pH, concentration), plays a significant role in determining the characteristics of the synthesized nanoparticles. Additionally, stabilizing agents or capping agents, such as citrate or polyvinylpyrrolidone (PVP), are often added to prevent the aggregation of AgNPs by providing steric or electrostatic stabilization (Restrepo & Villa, 2021).

Green Synthesis

Green synthesis is an eco-friendly approach that uses biological entities like plant extracts, microorganisms, or enzymes as reducing and stabilizing agents (Priya et al., 2021). This method is gaining popularity due to its sustainability, non-toxic nature, and compatibility with biological systems. Plant extracts, for instance, contain various biomolecules (e.g., flavonoids, terpenoids, and polyphenols) that can reduce silver ions to form AgNPs, while simultaneously capping the nanoparticles to prevent aggregation. Green synthesis typically involves mixing an aqueous solution of a silver salt with a biological extract at room temperature. The reaction results in the formation of AgNPs, often characterized by their distinct color change due to surface plasmon resonance (SPR). The exact mechanism of reduction in green synthesis is still under investigation, but it is generally accepted that the phytochemicals present in the extracts act as both reducing and stabilizing agents (Singh et al., 2023).

Physical Methods

Physical methods for synthesizing AgNPs, such as laser ablation and evaporation-condensation, involve the physical manipulation of bulk silver to create nanoparticles. These methods typically do not require chemical reducing agents or stabilizers, making them ideal for producing pure AgNPs (Beyene et al., 2017).

a) *Laser Ablation*: This technique involves focusing a high-energy laser beam onto a silver target submerged in a liquid medium, resulting in the ablation of the silver material and the formation of nanoparticles (Ganash, 2022). The size and shape of the nanoparticles can be controlled by adjusting the laser parameters (e.g., pulse duration, wavelength) and the properties of the liquid medium.
b) *Evaporation-Condensation*: In this method, bulk silver is evaporated in a high-temperature furnace and then condensed into nanoparticles in a cooler region of the system. This approach can produce AgNPs with uniform size distributions, though it requires specialized equipment and high energy input (Nguyen et al., 2023).

Other Methods

Apart from the conventional chemical, green, and physical methods, other synthesis techniques have been explored, including:

a) *Electrochemical Synthesis:* Involves the reduction of silver ions at the cathode in an electrochemical cell, allowing precise control over the nanoparticle size by adjusting the current density and electrolyte composition.
b) *Photochemical Synthesis:* Utilizes light to initiate the reduction of silver ions in the presence of a photosensitizer or light-absorbing compound, resulting in the formation of AgNPs.
c) *Microwave-Assisted Synthesis:* Employs microwave radiation to accelerate the reduction process, offering a rapid and efficient route to producing AgNPs with controlled morphology.

Chemical Reduction Techniques in AgNPs Synthesis

Overview of Chemical Reduction Techniques

Chemical reduction is one of the most widely used methods for synthesizing silver nanoparticles (AgNPs) due to its simplicity, cost-effectiveness, and ability to produce nanoparticles with controlled size and shape (Kaabipour & Hemmati, 2021). This technique involves the reduction of silver ions (Ag^+) from silver salts (such as silver nitrate, $AgNO_3$) to metallic silver (Ag^0) using reducing agents in the presence of stabilizing or capping agents. The overall reaction can be represented as:

The size, shape, and distribution of the resulting nanoparticles are highly dependent on several factors, including the choice of reducing agent, reaction conditions (such as temperature, pH, and concentration), and the presence of stabilizers (Restrepo & Villa, 2021).

Reducing Agents in Chemical Reduction

The choice of reducing agent is crucial in the chemical reduction process, as it determines the rate of reduction and the resulting properties of the AgNPs (Suriati et al., 2014). Common reducing agents used in the synthesis of silver nanoparticles include:

a) *Sodium Borohydride ($NaBH_4$):* $NaBH_4$ is a strong reducing agent that is often used to produce small and monodisperse AgNPs. The reduction process with $NaBH_4$ occurs rapidly, leading to the formation of nanoparticles with sizes typically in the range of 2-10 nm. The reaction can be conducted at room temperature or slightly elevated temperatures, and it is often performed in the presence of a stabilizing agent to prevent agglomeration of the nanoparticles (Cao et al., 2010). The reaction is as follows:
b) *Hydrazine (N_2H_4):* Hydrazine is another powerful reducing agent that can be used to synthesize AgNPs. It is capable of reducing silver ions even in relatively low concentrations, and the resulting nanoparticles are often larger than those produced with $NaBH_4$ (Guirguis et al., 2024). The reaction is slower than with $NaBH_4$, allowing for better control over the size distribution of the nanoparticles.
c) *Ascorbic Acid (Vitamin C):* Ascorbic acid is a milder reducing agent compared to $NaBH_4$ and hydrazine. It is often used in combination with other agents or under conditions that favor the formation of specific shapes, such as nanocubes, nanorods, or triangular nanoprisms. The slower reduction rate allows for the formation of larger and more uniform nanoparticles (Evanoff & Chumanov, 2004).
d) *Polyol Process:* In this process, polyols (such as ethylene glycol) act as both reducing and stabilizing agents. The polyol process is particularly useful for producing AgNPs with controlled size and morphology. The reaction is carried out at high temperatures (typically 120-200°C) in the presence of a silver precursor, leading to the reduction of silver ions and the simultaneous capping of the nanoparticles by the polyol (Hemmati et al., 2020).

Stabilizing Agents

Stabilizing agents, also known as capping agents, are used in the chemical reduction process to prevent the agglomeration of nanoparticles by providing steric or electrostatic stabilization (Pedroso-Santana & Fleitas-Salazar, 2023). Common stabilizers include:

a) *Citrate Ions:* Citrate is often used in conjunction with mild reducing agents like ascorbic acid. It not only acts as a weak reducing agent but also stabilizes the AgNPs by forming a negatively charged layer on the surface, preventing agglomeration due to electrostatic repulsion (Badawy et al., 2010).
b) *Polyvinylpyrrolidone (PVP):* PVP is a polymeric stabilizer commonly used in the polyol process. It adsorbs onto the surface of the nanoparticles, providing steric stabilization and preventing the particles from coming into close contact and aggregating (Gambinossi et al., 2015).
c) *Thiols:* Thiol-containing compounds, such as thioglycolic acid or mercaptoethanol, bind strongly to the surface of silver nanoparticles via the sulfur atom. This binding not only stabilizes the nanoparticles but can also introduce functional groups on the surface, allowing for further modifications or applications in sensing and bioconjugation (Sperling & Parak, 2010).

Reaction Conditions

The reaction conditions play a critical role in determining the characteristics of the synthesized AgNPs. Key factors include:

a) *Temperature:* Higher temperatures generally increase the rate of reduction, leading to faster nucleation and growth of nanoparticles. However, elevated temperatures can also result in broader size distributions if not carefully controlled (Alexander et al., 2006).
b) *pH:* The pH of the reaction medium can influence the reduction potential of the reducing agents and the stability of the silver ions. In acidic conditions, the reduction process may be slower, while in basic

conditions, the formation of hydroxide complexes can affect the morphology of the nanoparticles (Zhang et al., 2018).

c) *Concentration of Silver Ions and Reducing Agent:* The concentration of silver ions relative to the reducing agent can affect the nucleation and growth phases of nanoparticle formation. High concentrations of silver ions tend to produce larger nanoparticles, while higher concentrations of reducing agents favor the formation of smaller particles due to rapid nucleation (Restrepo & Villa, 2021).

Mechanism of Nanoparticle Formation

The formation of AgNPs through chemical reduction typically follows a two-step mechanism:

a) *Nucleation:* Upon the introduction of the reducing agent, silver ions are reduced to form small clusters or nuclei of silver atoms. The nucleation process is rapid and critical in determining the final size and distribution of the nanoparticles.
b) *Growth:* After nucleation, the silver nuclei grow by further reduction of silver ions onto the surface of the initial nuclei. The growth phase continues until the available silver ions are exhausted or the reaction conditions no longer favor further reduction. The rate of growth and the availability of stabilizers influence the final size and shape of the nanoparticles (Sugimoto, 2007).

Applications of Chemically Synthesized AgNPs

Chemically synthesized AgNPs have found extensive applications in various fields due to their controllable properties:

a) *Medical Applications:* AgNPs synthesized through chemical reduction are widely used in antimicrobial coatings, wound dressings, and drug delivery systems due to their potent antibacterial properties (Lekha et al., 2021).
b) *Catalysis:* The high surface area and active sites of AgNPs make them excellent catalysts for various chemical reactions, including the reduction of dyes and pollutants.
c) *Sensing:* AgNPs exhibit unique optical properties, such as surface plasmon resonance (SPR), which are exploited in the development of sensors for detecting chemicals, biomolecules, and environmental pollutants.

The chemical reduction method remains a cornerstone in the synthesis of silver nanoparticles, offering a versatile and effective approach to produce AgNPs with tailored properties. By carefully selecting reducing and stabilizing agents and optimizing reaction conditions, researchers can control the size, shape, and functionality of the nanoparticles, enabling their use in a wide range of applications across medicine, catalysis, and environmental science (Kim et al., 2023).

Green Synthesis of Silver Nanoparticles (AgNPs) Using Plant Extracts

Introduction to Green Synthesis

Green synthesis of silver nanoparticles (AgNPs) has emerged as an eco-friendly and sustainable alternative to traditional chemical and physical methods (Nangare & Patil, 2020). This approach leverages natural resources, such as plant extracts, to reduce silver ions (Ag^{+}) into metallic silver nanoparticles (Ag^{0}). The use of plant extracts offers several advantages, including the avoidance of toxic chemicals, the ability to scale up the process, and the potential for producing biocompatible nanoparticles. Additionally, plant extracts serve as both reducing agents and stabilizers, which simplifies the synthesis process and reduces the need for additional chemicals Kumar et al., 2021).

Components of Plant Extracts Involved in AgNP Synthesis

Plant extracts contain a diverse range of bioactive compounds that play crucial roles in the synthesis of AgNPs (Huq et al., 2022). These compounds can act as reducing agents, converting silver ions into nanoparticles, and as capping agents, stabilizing the formed nanoparticles and preventing agglomeration. Key phytochemicals involved in the green synthesis of AgNPs include:

a) *Phenolic Compounds:* Phenols and polyphenols, such as flavonoids, tannins, and catechins, are abundant in plant extracts. These compounds are potent antioxidants and can reduce silver ions through electron donation, leading to the formation of AgNPs. For example, flavonoids can reduce Ag^+ to Ag^0 while simultaneously stabilizing the nanoparticles by forming a protective layer around them (Banerjee et al., 2022).
b) *Alkaloids:* Alkaloids are nitrogen-containing compounds that can also reduce silver ions to form nanoparticles. They often act synergistically with other phytochemicals to enhance the efficiency of nanoparticle synthesis.
c) *Terpenoids:* Terpenoids, including essential oils, are another class of phytochemicals that can participate in the reduction of silver ions. These compounds often provide additional stability to the nanoparticles due to their hydrophobic nature.
d) *Proteins and Enzymes:* Some plant extracts contain proteins and enzymes that can facilitate the reduction process. For example, proteins with free amino groups can bind to silver ions and promote their reduction, while enzymes such as nitrate reductase can catalyze the reduction of Ag^+ to Ag (Rana et al., 2020)^0.

Mechanism of AgNP Formation Using Plant Extracts

The synthesis of AgNPs using plant extracts typically follows a straightforward procedure:

a) *Preparation of Plant Extract:* Fresh or dried plant material (e.g., leaves, flowers, stems, roots) is collected and washed to remove impurities. The plant material is then boiled or soaked in water or another suitable solvent to extract the bioactive compounds. The resulting extract is filtered to remove solid residues, yielding a clear solution containing the reducing and stabilizing agents (Akcil et al., 2015).
b) *Mixing with Silver Salt Solution:* The plant extract is mixed with an aqueous solution of a silver salt, commonly silver nitrate ($AgNO_3$). The mixture is stirred at room temperature or slightly elevated temperatures to facilitate the reduction process. During this process, the silver ions are reduced to silver atoms by the phytochemicals in the plant extract (Pradeep et al., 2021).
c) *Reduction and Stabilization:* As the reduction proceeds, silver atoms begin to nucleate, forming small clusters that grow into nanoparticles. The bioactive compounds in the extract not only reduce the silver ions but also stabilize the nanoparticles by capping their surfaces. This capping prevents the nanoparticles from aggregating and helps control their size and shape (Restrepo & Villa, 2021).
d) *Monitoring the Reaction:* The formation of AgNPs is often accompanied by a color change in the solution, typically from colorless to yellow, brown, or reddish-brown, depending on the concentration and size of the nanoparticles. This color change is due to the surface plasmon resonance (SPR) phenomenon, where the conduction electrons on the surface of the nanoparticles resonate with incident light (Jana et al., 2016).
e) *Characterization:* The synthesized nanoparticles are characterized using various techniques such as UV-Vis spectroscopy, which measures the SPR band, transmission electron microscopy (TEM) for size and shape analysis, and X-ray diffraction (XRD) to confirm the crystalline nature of the AgNPs.

Factors Influencing the Green Synthesis of AgNPs

Several factors influence the synthesis of AgNPs using plant extracts, affecting the size, shape, and yield of the nanoparticles:

a) *Type of Plant Extract:* Different plants contain varying amounts and types of bioactive compounds, which influence the reduction potential and stabilizing capacity. For example, extracts from plants like Azadirachta indica (neem), Ocimum sanctum (holy basil), and Cymbopogon citratus (lemongrass) have been widely studied for their effectiveness in synthesizing AgNPs (Zhang et al., 2020).
b) *Concentration of Silver Salt:* The concentration of $AgNO_3$ in the reaction mixture determines the availability of silver ions for reduction. Higher concentrations may lead to the formation of larger nanoparticles or a higher yield of AgNPs, but can also increase the likelihood of aggregation if not properly stabilized.
c) *pH of the Reaction Medium:* The pH of the solution affects the ionization of the bioactive compounds in the plant extract and the solubility of silver ions. Acidic or basic conditions can influence the reduction rate and the morphology of the nanoparticles. For example, acidic pH may lead to the formation of smaller, spherical nanoparticles, while basic pH can promote the formation of anisotropic shapes like rods or prisms.

d) *Temperature:* The temperature of the reaction influences the kinetics of nanoparticle formation. Higher temperatures generally increase the reduction rate, leading to faster nucleation and growth of nanoparticles. However, excessively high temperatures can cause uncontrolled growth and aggregation.
e) *Time of Reaction:* The duration of the reaction also plays a role in determining the final properties of the AgNPs. Prolonged reaction times may result in larger nanoparticles as the silver atoms continue to deposit on the growing nuclei (Zhang et al., 2012).

Applications of Green-Synthesized AgNPs

Green-synthesized AgNPs have found applications in various fields, benefiting from their biocompatibility, reduced toxicity, and sustainable production methods:

a) *Medical Applications:* Due to their potent antimicrobial properties, AgNPs synthesized using plant extracts are used in wound dressings, coatings for medical devices, and as antibacterial agents in topical formulations. Their biocompatibility also makes them suitable for drug delivery systems and cancer therapy (Khan et al., 2022).
b) *Environmental Applications:* Green-synthesized AgNPs are employed in environmental remediation, particularly in the removal of pollutants from water and soil. Their catalytic activity can degrade organic contaminants, while their antimicrobial properties help control harmful microorganisms in water treatment processes.
c) Sensing and Biosensing: The unique optical properties of AgNPs, such as SPR, are exploited in the development of biosensors for detecting biological molecules, pathogens, and chemical substances. Plant-extract-synthesized AgNPs are particularly attractive for biosensing due to their functionalized surfaces, which can be easily modified for specific detection tasks (Selvaraj et al.).
d) *Agriculture:* AgNPs produced through green synthesis are explored for use as antimicrobial agents in agriculture, helping to control plant pathogens and promoting healthier crop growth without the environmental burden of traditional pesticides.

Advantages and Challenges of Green Synthesis

Advantages

a) *Eco-Friendly:* The use of plant extracts avoids toxic chemicals, making the process environmentally friendly and sustainable.
   Biocompatibility: Green-synthesized AgNPs are often more biocompatible, making them suitable for biomedical applications.
b) *Cost-Effective:* The raw materials (plants) are readily available and inexpensive, reducing the overall cost of production.
c) *Simplicity:* The synthesis process is straightforward, often requiring only basic laboratory equipment and conditions.

Challenges

a) *Variability:* The composition of plant extracts can vary depending on the plant species, part used, season, and geographic location, leading to variability in nanoparticle synthesis.
b) *Scale-Up:* While green synthesis is promising, scaling up the process for industrial production remains a challenge due to the need for consistent quality and yield.
c) *Characterization:* The presence of complex mixtures of phytochemicals in plant extracts can complicate the characterization and standardization of the synthesized nanoparticles (Adeyemi et al., 2022).

Physical Methods for Silver Nanoparticles (AgNPs) Synthesis

Introduction to Physical Methods

Physical methods for the synthesis of silver nanoparticles (AgNPs) involve the manipulation of bulk silver material through physical processes such as evaporation, condensation, or laser ablation (Güzel & Erdal, 2018). Unlike chemical methods, physical approaches typically do not require chemical reagents, resulting in the production of high-purity nanoparticles. These methods are advantageous for producing AgNPs with controlled size and shape, and they often offer better control over particle dispersion without the need for stabilizers or capping agents (Restrepo & Villa, 2021).

Common Physical Methods for AgNP Synthesis

a. *Laser ablation* is a widely used physical method for synthesizing AgNPs. This technique involves focusing a high-energy laser beam onto a bulk silver target that is immersed in a liquid medium. The laser pulses ablate the silver material, causing the ejection of atoms, clusters, or larger particles, which subsequently condense to form nanoparticles in the surrounding liquid (Simakin et al., 2007).

Mechanism

a) When the laser beam hits the silver target, the intense energy causes the surface atoms to absorb energy and become excited (Schou, 2006). This energy leads to the ejection of silver atoms and clusters from the surface of the target.
b) These ejected species rapidly cool and nucleate in the liquid medium, forming silver nanoparticles.
c) The surrounding liquid medium helps stabilize the nanoparticles, preventing their agglomeration.

Advantages

a) Purity: The absence of chemical reagents ensures that the resulting nanoparticles are free of contaminants, making them highly pure.
b) Control: The size and distribution of the nanoparticles can be finely controlled by adjusting the laser parameters, such as wavelength, pulse duration, and energy.
c) Versatility: This method can be applied to a wide range of liquids, including water, organic solvents, and surfactant solutions (Moradi & Yamini, 2012).

Challenges

a) Cost: Laser ablation requires specialized and expensive equipment, which can limit its use to laboratory settings or high-value applications.
b) Scalability: Producing large quantities of AgNPs via laser ablation can be challenging due to the localized nature of the laser-material interaction.

Evaporation-Condensation Method

Evaporation-condensation is a physical vapor deposition technique used to produce AgNPs by evaporating bulk silver material in a high-temperature furnace and then condensing the vapor to form nanoparticles (Bouafia et al., 2021).

Mechanism

a) The bulk silver is heated to a high temperature (usually in a vacuum or inert gas atmosphere), causing the silver atoms to evaporate.
b) The evaporated silver atoms move into a cooler region within the chamber where they condense and nucleate to form nanoparticles.
c) The nanoparticles are then collected on a substrate or suspended in a gas flow, depending on the setup (Wang & Otani, 2013).

Advantages

a) *Uniformity:* This method can produce highly uniform nanoparticles with narrow size distributions.
b) *Scalability:* The process can be scaled up by increasing the size of the furnace or by using multiple evaporation sources.

Challenges

a) ***High Energy Consumption:*** **The method requires high temperatures, leading to significant energy consumption.**
b) ***Control over Morphology:*** **While** the method can produce uniform nanoparticles, controlling their specific shapes (beyond spherical) can be challenging (Sau & Rogach, 2010).

Arc Discharge Method

The arc discharge method involves generating silver nanoparticles by creating an electric arc between two silver electrodes submerged in a dielectric liquid. The intense heat from the arc vaporizes the silver, which then condenses to form nanoparticles (Förster et al., 2012).

Mechanism

a) An electrical discharge (arc) is generated between two silver electrodes.
b) The high temperature of the arc vaporizes the silver, creating a plasma containing silver ions and atoms.
c) As the vapor cools, silver nanoparticles form and are stabilized by the liquid medium (Malekzadeh & Halali, 2011).

Advantages

a) *High Yield:* The arc discharge method can produce a large quantity of nanoparticles in a relatively short period.
b) *Purity:* Like laser ablation, this method does not require chemical reagents, resulting in pure nanoparticles.

Challenges:

a) *Equipment Complexity:* The method requires specialized equipment to generate and control the arc discharge.
b) *Agglomeration*: Without the use of stabilizers, there is a risk of nanoparticle agglomeration during synthesis (Jiang et al., 2009).

Ball Milling

Ball milling is a mechanical method that involves grinding bulk silver into nanoparticles using high-energy collisions between balls and the material in a rotating mill.

Mechanism

a) Bulk silver is placed in a cylindrical chamber with steel or ceramic balls (Velasco et al., 2016).
b) The chamber is rotated at high speeds, causing the balls to collide with the silver material.
c) The high-energy collisions reduce the size of the silver particles to the nanoscale.

Advantages

a) *Scalability*: Ball milling is easily scalable for industrial production, making it suitable for mass production of nanoparticles.
b) *Cost-Effectiveness:* The equipment used for ball milling is relatively inexpensive compared to other physical methods (Yadav et al., 2012).

Challenges

a) *Contamination:* The milling process can introduce impurities from the balls or the chamber walls into the nanoparticles.
b) *Limited Control over Size and Shape:* While effective for size reduction, controlling the exact size and shape of the nanoparticles can be difficult (An & Somorjai, 2012).

Factors Influencing the Physical Synthesis of AgNPs

Several factors influence the effectiveness and outcome of physical methods in synthesizing silver nanoparticles (Yaqoob et al., 2020):

1) *Energy Input:* The amount of energy provided (e.g., laser intensity, arc current, or rotational speed in ball milling) significantly affects the size and distribution of the nanoparticles. Higher energy inputs generally result in smaller nanoparticles due to more intense material breakage or vaporization (Kushnir & Sandén, 2008).
2) *Atmosphere and Environment:* The presence of an inert or reactive gas during processes like evaporation-condensation or arc discharge can influence the rate of nanoparticle formation and stabilization. An inert atmosphere prevents oxidation, while a reactive atmosphere might lead to the formation of silver oxide nanoparticles (Keast, 2022).
3) *Cooling Rate:* In methods like evaporation-condensation, the cooling rate determines the nucleation and growth rates of nanoparticles. Rapid cooling typically leads to smaller nanoparticles with narrower size distributions (Flagan & Lunden, 1995).
4) *Liquid Medium***:** In laser ablation and arc discharge methods, the choice of the liquid medium (e.g., water, organic solvents, or surfactants) affects nanoparticle stabilization and prevents agglomeration. Surfactants can also influence the morphology of the resulting nanoparticles (Song et al., 2021).

Applications of Physically Synthesized AgNPs

Physically synthesized silver nanoparticles have a wide range of applications due to their high purity and controlled size distribution:

1) *Catalysis*: AgNPs produced by physical methods are used as catalysts in various chemical reactions, including the reduction of organic pollutants and the oxidation of alcohols. Their high surface area and purity make them effective in these applications (Sun et al., 2018).
2) *Biomedical Applications:* The high purity and controlled size of physically synthesized AgNPs make them ideal for biomedical applications, such as in antimicrobial coatings for medical devices, wound dressings, and drug delivery systems.
3) *Optical Applications:* The unique optical properties of AgNPs, including localized surface plasmon resonance (LSPR), are exploited in the development of optical sensors and imaging systems. Physically synthesized AgNPs, with their uniform size, are particularly useful in these applications (Beyene et al., 2017).
4) *Environmental Remediation:* AgNPs are used in water treatment and environmental remediation efforts, particularly in the removal of pollutants and pathogens from water. Their high surface area and reactivity make them effective agents in these processes (Clark & Macquarrie, 1996).

Advantages and Challenges of Physical Methods

Advantages

1) *High Purity:* Physical methods often produce nanoparticles without chemical contaminants, making them highly pure and suitable for sensitive applications (Jamkhande et al., 2019).
2) *Control over Size and Distribution:* The ability to control process parameters allows for the production of nanoparticles with specific sizes and narrow size distributions.
3) *Versatility:* Physical methods can be adapted to different environments and conditions, making them versatile for various applications.

Challenges

1) *High Energy Requirements:* Many physical methods require significant energy input, which can be costly and limit their scalability.
2) *Equipment Costs:* The specialized equipment needed for methods like laser ablation and arc discharge can be expensive, making these methods less accessible.
3) *Scalability:* While some physical methods are scalable, others are limited to laboratory-scale production, making it difficult to produce large quantities of nanoparticles (Tsuzuki, 2009).

Characterization Techniques for Silver Nanoparticles (AgNPs)

Introduction to Characterization of AgNPs

Characterization of silver nanoparticles (AgNPs) is essential for understanding their physicochemical properties, which directly influence their performance in various applications (Ahmed et al., 2017). Characterization techniques provide critical information about the size, shape, surface chemistry, crystallinity, optical properties, and stability of AgNPs **(**Table 2**)**. These techniques are crucial for ensuring the consistency and quality of nanoparticle synthesis and for tailoring nanoparticles for specific uses in fields such as medicine, catalysis, and electronics (Shnoudeh et al., 2019).

Table 2. Characterization techniques for silver nanoparticles (AgNPs)

Sr#	Characterization Technique	Abbreviation	Description
1	Atomic Force Microscopy	AFM	Surface topography measurement at nanoscale.
2	Ultraviolet-Visible Spectroscopy	UV-Vis	Measurement of absorption and transmission of UV and visible light.
3	Dynamic Light Scattering	DLS	Size distribution and particle size analysis in suspension.
4	Fourier Transform Infrared Spectroscopy	FTIR	Identification of chemical bonds and molecular structure.
5	Scanning Electron Microscopy	SEM	Surface morphology and composition analysis.
6	Transmission Electron Microscopy	TEM	Detailed imaging of internal structure at high resolution.
7	X-ray Diffraction	XRD	Determination of crystal structure and phase identification.
8	Energy Dispersive X-ray Spectroscopy	EDS	Elemental composition analysis.
9	Auger Electron Spectroscopy	AES	Surface chemical analysis and elemental identification.
10	Scanning Tunneling Microscopy	STM	Atomic-scale imaging and surface analysis.
11	Secondary Ion Mass Spectrometry	SIMS	Surface analysis and depth profiling by detecting sputtered ions.
12	Low-Energy Ion Scattering	LEIS	Surface composition and structure analysis.
13	X-ray Photoelectron Spectroscopy	XPS	Surface chemistry analysis through electron binding energies.
14	Zeta Potential	-	Measurement of surface charge and stability of colloidal dispersions.
15	Scanning Probe Microscopy	SPM	High-resolution surface imaging and analysis of electrical, mechanical properties.

Morphological and Structural Characterization

a. Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is one of the most widely used techniques for characterizing the morphology and size of AgNPs.

Principle

a) TEM operates by transmitting a beam of electrons through an ultra-thin sample. As electrons pass through the sample, they interact with the atoms and are scattered, forming an image on a detector.
b) The resulting images provide information about the size, shape, and internal structure of the nanoparticles.

Advantages

a) *High Resolution:* TEM offers atomic-scale resolution, allowing for the detailed visualization of nanoparticle morphology.
b) *Size Distribution Analysis:* TEM images can be analyzed to determine the size distribution of the nanoparticles, providing statistical data on their uniformity.

Challenges

a) *Sample Preparation:* TEM requires complex and time-consuming sample preparation, including the creation of ultra-thin sections or dispersions on a grid (Schrand et al., 2010).
b) *Electron Beam Damage:* Prolonged exposure to the electron beam can cause damage or alteration to the nanoparticles.

b. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is another key technique for examining the surface morphology and size of AgNPs.

Principle

a) SEM uses a focused beam of electrons to scan the surface of a sample. The interaction between the electrons and the sample surface generates secondary electrons, backscattered electrons, and characteristic X-rays, which are detected to create an image.
b) SEM images provide topographical information and can be used to assess the surface texture and shape of nanoparticles.

Advantages

a) *3D Imaging:* SEM provides three-dimensional surface images, which can give insights into the nanoparticle morphology.
b) *Elemental Analysis:* Coupled with Energy Dispersive X-ray Spectroscopy (EDX or EDS), SEM can also provide elemental composition information.

Challenges

a) *Resolution:* While SEM has high resolution, it is generally lower than that of TEM.
b) *Sample Preparation:* Conductive coatings may be required for non-conductive samples, which can introduce artifacts (Dang & Frisk, 1998).

c. X-ray Diffraction (XRD)

X-ray Diffraction (XRD) is a powerful technique for determining the crystalline structure and phase purity of AgNPs.

Principle

a) XRD involves directing X-rays at a sample and measuring the intensity of the diffracted rays (Whittig & Allardice, 1986). The diffraction pattern is analyzed to determine the crystal structure, lattice parameters, and phase composition of the nanoparticles.
b) The characteristic peaks in an XRD pattern correspond to the specific crystallographic planes within the silver lattice.

Advantages

a) Crystallinity Analysis: XRD can determine the degree of crystallinity of AgNPs, which is crucial for applications where crystallinity impacts performance, such as in catalysis.
b) Phase Identification: XRD can identify different crystalline phases, including any impurities or secondary phases that may be present.

Challenges

a) Size Limitation: XRD is less effective for characterizing very small nanoparticles (below 5 nm) due to peak broadening.
b) Complex Data Interpretation: (Holder & Schaak, 2019).

d. Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) is a technique used to analyze the surface topology and roughness of AgNPs at the nanoscale.

Principle

a) AFM involves scanning a sharp tip over the surface of a sample, which is mounted on a cantilever (Moreno-Herrero & Gomez-Herrero, 2012). The interaction between the tip and the sample surface (van der Waals forces, electrostatic forces, etc.) causes deflections in the cantilever, which are measured to create a topographical map of the surface.
b) AFM provides information about the height, width, and overall shape of nanoparticles on a surface.

Advantages

a) High Spatial Resolution: AFM can resolve surface features at the atomic scale.
b) Non-Destructive: AFM does not require conductive coatings, preserving the original state of the sample.

Challenges

a) Slow Scanning Speed: AFM is typically slower than SEM or TEM, making it less suitable for characterizing large sample areas.
b) Surface Effects: The technique is sensitive to surface contamination, which can affect accuracy (Kohli, 2012).

Optical and Spectroscopic Characterization

a. UV-Visible Spectroscopy (UV-Vis)

UV-Visible Spectroscopy (UV-Vis) is a commonly used technique to study the optical properties of AgNPs, particularly their surface plasmon resonance (SPR) (Alzahrani, 2020).

Principle

a) UV-Vis spectroscopy measures the absorption of light in the ultraviolet and visible regions of the electromagnetic spectrum. When light interacts with AgNPs, it excites the collective oscillation of conduction electrons on the nanoparticle surface, known as surface plasmon resonance (SPR).
b) The SPR phenomenon results in a characteristic absorption peak in the UV-Vis spectrum, which can provide information about the size, shape, and concentration of the nanoparticles (Amendola & Meneghetti, 2009).

Advantages

a) *Quick and Non-Destructive:* UV-Vis spectroscopy is a fast and non-invasive technique.
b) *Size and Shape Estimation:* The position and shape of the SPR peak can give insights into the average size and shape of the nanoparticles.

Challenges

a) *Limited Information:* UV-Vis provides limited structural information and is often used in conjunction with other techniques.
b) *Aggregation Effects:* Nanoparticle aggregation can shift and broaden the SPR peak, complicating analysis (Ghosh & Pal, 2007).

b.Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy (FTIR) is used to identify the functional groups on the surface of AgNPs, providing insights into the surface chemistry and capping agents (Sousa et al., 2017).

Principle

a) FTIR measures the absorption of infrared radiation by the sample, which causes molecular vibrations that are characteristic of specific functional groups. By analyzing the resulting spectrum, it is possible to identify the chemical bonds and functional groups present on the nanoparticle surface.
b) This technique is particularly useful for identifying organic molecules that may be adsorbed on or attached to the nanoparticles.

Advantages

a) *Surface Chemistry Analysis:* FTIR is highly effective at identifying organic molecules and functional groups on the nanoparticle surface.
b) *Insight into Capping Agents:* FTIR can confirm the presence and nature of capping agents used to stabilize nanoparticles.

Challenges

a) *Sensitivity:* FTIR is less sensitive to small quantities of surface-bound species.
b) *Overlapping Peaks:* The complexity of some spectra, especially in the presence of multiple functional groups, can make interpretation challenging (Coates, 2000).

c. Raman Spectroscopy

Raman Spectroscopy is another technique for probing the surface chemistry of AgNPs, particularly useful for studying molecular interactions on the nanoparticle surface.

Principle

a) Raman spectroscopy relies on inelastic scattering of monochromatic light (usually from a laser) by molecules, resulting in a shift in the energy of the scattered photons (Rostron et al., 2016). This shift provides information about the vibrational modes of the molecules on the surface.
b) The enhancement of the Raman signal, known as Surface-Enhanced Raman Scattering (SERS), can occur when molecules are adsorbed onto the surface of AgNPs, making it highly sensitive to surface-bound species.

Advantages

a) High Sensitivity: SERS can detect even low concentrations of molecules on the nanoparticle surface.
*b) Non-Destructive**:*** Raman spectroscopy is non-destructive and requires minimal sample preparation.

Challenges

a) Complex Data: The interpretation of Raman spectra can be complex due to overlapping bands and background noise.
b) Instrumentation Cost: Raman spectroscopy, especially with SERS capability, requires sophisticated and expensive equipment (Zhang et al., 2017).

Surface Area and Porosity Characterization

a. Brunauer-Emmett-Teller (BET) Surface Area Analysis

Brunauer-Emmett-Teller (BET) surface area analysis is used to determine the specific surface area of AgNPs, which is crucial for applications in catalysis and adsorption (Shah et al., 2021).

Principle

a) BET analysis involves the adsorption of gas molecules onto the surface of the nanoparticles. By measuring the amount of gas adsorbed at different pressures, the surface area of the nanoparticles can be calculated.
b) This technique is particularly useful for determining the surface area of porous materials or nanoparticles with high surface-to-volume ratios (White et al., 2009).

Advantages

a) Quantitative Analysis: BET provides a quantitative measurement of the specific surface area, which is important for evaluating the reactivity of nanoparticles.
b) Applicable to Porous Materials: BET is effective for characterizing the surface area of porous and non-porous materials.

Challenges

a) Sample Preparation: The sample must be degassed to remove adsorbed contaminants, which can be time-consuming.
b) Complexity in Interpretation: For materials with complex pore structures, interpreting BET data can be challenging (Cychosz et al., 2017).

Stability and Zeta Potential Measurement

a. Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) is a technique used to measure the hydrodynamic size and size distribution of AgNPs in suspension, as well as their stability over time (MacCuspie et al., 2011).

Principle

a) DLS measures the fluctuations in the intensity of scattered light caused by the Brownian motion of nanoparticles in suspension. The rate of these fluctuations is related to the size of the particles, providing information on their hydrodynamic diameter.
b) DLS is also used to assess the stability of nanoparticles by measuring changes in size over time, indicating aggregation or degradation (Gontijo et al., 2020).

Advantages

a) *Quick and Easy:* DLS is a fast, non-invasive method that requires minimal sample preparation.
b) *Size Distribution Analysis:* It provides information on the size distribution of nanoparticles in a colloidal suspension.

Challenges

a) Polydispersity Sensitivity: DLS is sensitive to polydispersity, and the presence of aggregates can skew the size distribution data.
b) Interpretation: The hydrodynamic diameter measured by DLS includes the nanoparticle core and any attached or adsorbed layers (such as capping agents), which may differ from the actual core size (Leong et al., 2018).

b. Zeta Potential Measurement

Zeta Potential is a measure of the surface charge of nanoparticles and is an important parameter for understanding the stability of colloidal suspensions.

Principle

a) Zeta potential measures the electrostatic potential at the slipping plane of a particle in a colloidal suspension (Lowry et al., 2016). This potential reflects the charge on the nanoparticle surface and the surrounding ion cloud, which affects the stability of the suspension.
b) A high zeta potential (positive or negative) typically indicates good colloidal stability, as the electrostatic repulsion between particles prevents aggregation.

Advantages

a) *Stability Indicator:* Zeta potential is a reliable indicator of the stability of nanoparticle suspensions.
b) *Simple Measurement*: The measurement process is relatively straightforward and can be performed on most nanoparticle suspensions (Wells et al., 2017).

Challenges

a) *Sensitivity to Ionic Strength:* Zeta potential measurements can be influenced by the ionic strength of the suspension medium, which may affect the accuracy.

b) *Interpretation:* While zeta potential provides insights into stability, it does not provide direct information about particle size or shape.

Characterization techniques are essential tools for understanding the properties of silver nanoparticles, which in turn affect their performance in various applications. Techniques such as TEM, SEM, and XRD provide detailed information about nanoparticle morphology and structure, while UV-Vis spectroscopy, FTIR, and Raman spectroscopy offer insights into optical properties and surface chemistry. BET analysis and zeta potential measurements are crucial for evaluating surface area and colloidal stability. By employing a combination of these techniques, researchers can obtain a comprehensive understanding of AgNPs, enabling the development of tailored nanoparticles for specific uses in medicine, catalysis, environmental remediation, and other fields (Ahmed et al., 2022).

Applications of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) have garnered significant attention due to their unique physicochemical properties, which make them suitable for a wide range of applications across various fields (Beyene et al., 2017). These include medicine, environmental science, electronics, and catalysis, among others. The following sections provide a detailed exploration of these applications.

Medical Applications

Antibacterial and Antifungal Agents

Mechanism of Action

a) AgNPs exhibit strong antibacterial and antifungal properties, making them effective in combating a broad spectrum of pathogens, including antibiotic-resistant strains (More et al., 2023). The antimicrobial activity of AgNPs is primarily attributed to the release of silver ions ($Ag+$) from the nanoparticles, which interact with microbial cell membranes, causing structural damage and disrupting cellular functions.
b) AgNPs also generate reactive oxygen species (ROS), which can further damage microbial cells, leading to cell death.

Applications

a) *Wound Dressings:* AgNPs are incorporated into wound dressings to prevent infections and promote healing. Their ability to inhibit microbial growth reduces the risk of wound-related complications, such as sepsis.
b) *Topical Creams and Gels:* Silver nanoparticle-based creams and gels are used to treat skin infections, burns, and ulcers due to their antimicrobial properties.
c) *Medical Device Coatings:* Coating medical devices, such as catheters, implants, and surgical instruments, with AgNPs reduces the risk of biofilm formation and device-associated infections (Cao & Liu, 2010).
d) *Antiviral Agents*

Mechanism of Action

AgNPs have been shown to possess antiviral properties by interacting with viral particles and inhibiting their ability to infect host cells. This is achieved through several mechanisms, including binding to viral surface proteins, disrupting viral envelopes, and preventing viral replication (Klasse et al., 1998).

Applications

a) *Preventive Measures:* AgNPs are explored as potential coatings for personal protective equipment (PPE), such as masks and gloves, to provide an additional layer of defense against viral transmission.

b) *Therapeutic Use:* Research is ongoing into the development of AgNP-based antiviral therapies, which could be used to treat viral infections, including those caused by influenza, HIV, and SARS-CoV-2 (Gurunathan et al., 2020).
c) *Drug Delivery Systems*

Mechanism of Action

AgNPs can be functionalized with therapeutic agents, enabling targeted drug delivery (Ivanova et al., 2018). The surface of AgNPs can be modified with specific ligands that recognize and bind to target cells, such as cancer cells. Once delivered, the drug can be released in a controlled manner, enhancing its therapeutic efficacy while minimizing side effects.

Applications

a) *Cancer Treatment:* AgNPs are being investigated for use in cancer therapy due to their ability to deliver chemotherapeutic agents directly to tumor cells, reducing the impact on healthy tissues.
b) *Gene Therapy:* AgNPs are explored as carriers for delivering genetic material, such as siRNA or DNA, into cells for the treatment of genetic disorders (Pędziwiatr-Werbicka et al., 2020).
c) *Imaging and Diagnostics*

Mechanism of Action

The optical properties of AgNPs, particularly their strong surface plasmon resonance (SPR), make them excellent candidates for use in imaging and diagnostic applications. AgNPs can be conjugated with antibodies or other targeting molecules to visualize specific cells or biomolecules (Prasher et al., 2020).

Applications

a) *Biosensors:* AgNPs are used in biosensors to detect biomolecules, pathogens, or environmental toxins. Their strong SPR enhances the sensitivity and accuracy of detection.
b) *Medical Imaging:* AgNPs can be used as contrast agents in imaging techniques, such as optical coherence tomography (OCT) and Raman imaging, providing high-resolution images of biological tissues (Das et al., 2019).

Environmental Applications

a. Water Treatment

Mechanism of Action

o AgNPs exhibit strong antimicrobial activity, making them effective in disinfecting water by killing bacteria, viruses, and other pathogens. Their catalytic properties also enable the degradation of organic pollutants in water (Lu & Astruc, 2020).

- **Applications**

o ***Water Filtration Systems:*** AgNPs are incorporated into water filtration membranes and filters to enhance their antimicrobial efficiency, providing safer drinking water.
o ***Pollutant Degradation*:** AgNPs can catalyze the breakdown of organic pollutants, such as dyes and pesticides, in wastewater, reducing environmental contamination (Rani et al., 2020).

b. Environmental Remediation

- **Mechanism of Action**

o The high reactivity and large surface area of AgNPs make them suitable for environmental remediation, including the removal of heavy metals and other contaminants from soil and water (Bhatt et al., 2022).

- **Applications**

o *Soil Remediation:* AgNPs are used to stabilize and remove heavy metals, such as lead and mercury, from contaminated soils, preventing their uptake by plants and entry into the food chain.
o *Air Purification:* AgNPs are integrated into air filtration systems to remove airborne pathogens and pollutants, improving indoor air quality (A. Sharma et al., 2021).

Catalysis

Chemical Catalysis

- **Mechanism of Action**

o AgNPs act as catalysts by providing active sites for chemical reactions, such as oxidation and reduction processes. Their large surface area and high surface energy enhance their catalytic efficiency (Zhou et al., 2011).

- **Applications**

o *Reduction of Pollutants:* AgNPs catalyze the reduction of organic pollutants, such as nitro compounds and dyes, in industrial wastewater.
o *Synthesis of Fine Chemicals:* AgNPs are used as catalysts in the synthesis of fine chemicals, including pharmaceuticals and agrochemicals, by facilitating reactions such as hydrogenation and oxidation (Sharma et al., 2021).

b. Photocatalysis

- **Mechanism of Action**

o AgNPs can enhance the photocatalytic activity of materials like titanium dioxide (TiO_2) by promoting electron transfer and reducing recombination of electron-hole pairs. This enhances the degradation of organic pollutants under light irradiation (Skiba et al., 2022).

- **Applications**

o *Solar Energy Conversion:* AgNPs are used in solar cells to improve the efficiency of light absorption and energy conversion.
o *Degradation of Organic Pollutants:* AgNPs-enhanced photocatalysts are employed to degrade organic pollutants, such as dyes and pesticides, under sunlight, contributing to environmental clean-up (Azeez et al., 2021).

c. **Electronics and Sensors**

1) **Conductive Inks**

2) **Mechanism of Action**

a) AgNPs are highly conductive, making them ideal for use in conductive inks, which are used to print electronic circuits on flexible substrates (Mo et al., 2019).

3) **Applications**

a) *Flexible Electronics:* AgNP-based inks are used in the production of flexible and wearable electronics, such as RFID tags, sensors, and flexible displays.
b) *Printed Circuit Boards (PCBs):* AgNPs enable the production of highly conductive printed circuit boards, which are essential components in various electronic devices (Mo et al., 2019).

Sensors

- **Mechanism of Action**

o The optical and electrical properties of AgNPs make them suitable for use in various types of sensors, including chemical and biological sensors. AgNPs can enhance the sensitivity and selectivity of these sensors (Montes-García et al., 2021).

- **Applications**

o *Chemical Sensors:* AgNPs are used in sensors for detecting gases, such as hydrogen and methane, as well as chemicals like glucose and alcohol.
o *Biosensors:* AgNP-based biosensors are employed in medical diagnostics to detect biomarkers, pathogens, and other biological molecules with high sensitivity and specificity (Nishat et al., 2019).

Cosmetics and Personal Care

Antimicrobial Additives

1) **Mechanism of Action**

a) AgNPs are incorporated into cosmetic and personal care products for their antimicrobial properties, which help to prevent microbial contamination and extend the shelf life of these products (Stewart et al., 2016).

2) **Applications**

a) *Deodorants and Antiperspirants*: AgNPs are added to deodorants and antiperspirants to inhibit the growth of odor-causing bacteria.
b) *Skin Care Products:* AgNPs are used in creams, lotions, and sunscreens to provide antimicrobial protection and enhance product stability (Pillay et al., 2024).

Anti-Aging and Skin Health

3) **Mechanism of Action**

a) AgNPs have been explored for their potential benefits in anti-aging skin care products due to their antioxidant properties and ability to promote wound healing (Bold et al., 2022).

4) **Applications:**

a) *Anti-Aging Creams:* AgNPs are included in formulations to reduce wrinkles and fine lines by promoting collagen production and protecting against oxidative stress.
b) *Acne Treatment:* AgNPs are used in acne treatment products to reduce inflammation and prevent bacterial infections (Bold et al., 2022).

Food Packaging

Antimicrobial Food Packaging

Mechanism of Action

a) AgNPs are incorporated into food packaging materials to provide antimicrobial protection, extending the shelf life of perishable goods by inhibiting the growth of foodborne pathogens (Kumar et al., 2021).

Applications

b) *Active Packaging:* AgNPs are used in active packaging films that release antimicrobial agents over time, providing continuous protection against microbial contamination.
c) *Edible Coatings:* AgNPs are incorporated into edible coatings applied to fruits, vegetables, and other perishable items to preserve freshness and prevent spoilage (Jafarzadeh et al., 2021).

Textile Industry

a. Antimicrobial Textiles

- **Mechanism of Action**

o AgNPs are incorporated into textiles to impart antimicrobial properties, preventing the growth of bacteria and fungi on fabrics (Rodrigues et al., 2019).

- **Applications**

o **Medical Textiles:** AgNP-treated fabrics.

Future Perspectives and Challenges in Silver Nanoparticles (AgNPs)

a) Silver nanoparticles (AgNPs) offer significant potential across various fields due to their unique properties, but their development and application face several challenges.
b) Future advancements in AgNPs are expected to focus on refining synthesis techniques to achieve more controlled and uniform particles.
c) Innovations in green synthesis methods using biological materials could lead to more sustainable production processes.
d) Enhanced functionalization strategies will likely improve targeted drug delivery systems, enabling more precise and personalized treatments. AgNPs may also be integrated into smart materials with responsive properties, benefiting applications in environmental monitoring, healthcare, and wearable technology.
e) Further exploration in environmental and biomedical integration could enhance the effectiveness of AgNPs in water purification, soil remediation, and advanced imaging technologies.
f) Sustainable practices, including recycling and lifecycle assessment, will be crucial to mitigate environmental and health risks associated with AgNPs. Developing standardized protocols and regulatory frameworks will ensure consistent quality and safety across various applications.
g) However, several challenges need to be addressed. New research is needed to assess their impact on human health and the environment, as well as strategies for minimizing these risks.
h) The cost of synthesis and scalability of production methods pose significant obstacles, requiring more cost-effective and scalable solutions.
i) Stability issues, such as aggregation and degradation, must be resolved to maintain AgNPs' effectiveness and safety.
j) Public perception and acceptance of nanomaterials, including AgNPs, will influence their adoption. Transparent communication and addressing ethical concerns will be important for gaining public trust.
k) Lastly, interdisciplinary collaboration among chemists, biologists, engineers, and other experts will enhance innovation and address commercialization and regulatory challenges.
l) AgNPs present exciting opportunities for advancements in medicine, environmental science, and technology, addressing the associated challenges through continued research, responsible development, and collaboration will be key to realizing their full potential.

Conclusion

In conclusion, silver nanoparticles (AgNPs) represent a versatile and transformative class of materials with a wide range of applications due to their unique physicochemical properties. Their potential extends across various fields including medicine, environmental science, electronics, and catalysis. AgNPs offer remarkable antibacterial, antifungal, and antiviral properties, making them valuable in medical applications such as wound dressings, drug delivery systems, and coatings for medical devices. They also hold promise in environmental remediation, water treatment, and as catalysts in chemical processes. The synthesis of AgNPs has evolved through several methods, including chemical reduction, green synthesis using plant extracts, and physical techniques like laser ablation. Each method offers distinct advantages and challenges, influencing the properties and applications of the nanoparticles. Characterization techniques such as TEM, SEM, XRD, UV-Vis spectroscopy, and DLS are essential for understanding the size, shape, and stability of AgNPs, ensuring their effective use in various applications. Future advancements in AgNPs are expected to focus on enhancing synthesis techniques, improving functionalization for targeted applications, and integrating them into innovative materials and technologies. Sustainable practices and regulatory frameworks will play a crucial role in addressing environmental and health concerns associated with AgNPs. However, challenges remain, including understanding their toxicity, managing production costs, ensuring stability, and addressing public perception. Overall, AgNPs offer significant benefits and hold substantial promise for future technological and scientific advancements, their development must be approached with careful consideration of their potential risks and challenges. But continuous research, interdisciplinary collaboration, and responsible development practices will be essential with the link of silver nanoparticles while mitigating any associated risks.

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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Damage and Morphometric Effects of Prohibited Substance Use as Doping on Tissues and Organs

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Abstract: Uncontrolled acute or chronic use of very high doses by athletes to enhance athletic performance, improve muscle strength and physical appearance (Pope et al, 2017; Gök et al 2016) Erythropoietin, methenolone Enanthate (rhEPO), Testosterone propionate, Testosterone phenylpropionate, Testosterone isocaproate, Testosterone decanoate (Özdemir & Yalçın, 2011; Bozkurt et al., 2011a-b; Özdemir, 2020), which are among the most common AAS used for doping purposes, can cause irreparable serious organ damage (Al-Otaibi, 2024). Prohibited substances are also widely used as pharmacological drug therapy in patients with chronic diseases to improve the quality of life by achieving the appropriate effects on the body (Handelsman, 2006). Despite their use in clinical treatments, banned substances have also been found to have some negative effects on patients and the health of external users (Mutalip et al 2013). Testosterone is used in drug therapy for chronic respiratory or heart failure, anemia due to bone marrow failure, increasing or decreasing erythropoietin in renal failure, and for muscle and bone healing in autoimmune diseases. The abuse of these substances is often used illegally in large doses for non-medical purposes, especially in strength sports and bodybuilding. In parallel with effective findings that reduce the abuse of banned substances in elite sports, there is a need to focus more attention on non-sporting cosmetic, recreational, exertional and occupational abuse (Handelsman, 2006). Athletes use banned substances to improve performance regardless of health risks (El-Gendy, et al., 2021). In studies conducted in various countries, the lifetime prevalence of banned substances used for exertion, physical appearance and performance enhancement in young men is reported at rates ranging from 3-12% (Gök et al., 2016). The use of banned substances has continued to increase in recent years and there is a need for more research on this subject. In this study, it is aimed to inform the society and especially the sports community about the damage caused by banned substances used as doping on tissues and organs.

Keywords: Doping, Organs, Tissues

Introduction

Anabolic-androgenic steroids (AAS) are synthetic compounds derived from testosterone and its related precursors. AAS are commonly used illegally, particularly among adolescents and athletes, to enhance muscle growth and achieve a lean body mass for aesthetic purposes. This illegal use can lead to serious health problems and various dysfunctions. Sudden cardiac death (SCD) is noted as the most common medical cause of death among athletes (Torrisi et al., 2020).

The misuse of anabolic-androgenic steroids (AAS) has become quite prevalent among both professional and recreational athletes. These steroids are used to quickly increase muscle mass and achieve an aesthetic

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appearance. However, many of the adverse effects of AAS on testicular morphology, tissue redox state, and androgen receptor levels during training are not yet fully understood (Sretenovic et al., 2021).

Testosterone is a hormone produced through the interaction between the pituitary gland, hypothalamus, and testes. Pulsatile gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus stimulates the release of luteinizing hormone (LH) from the anterior pituitary, which then interacts with Leydig cells in the testes to produce testosterone. More than 95% of circulating testosterone is produced in the testes (Şensoy, 2024).

It is evident that AAS used in clinical-therapeutic trials by medical doctors are not free of side effects. These side effects vary depending on the type and dosage of the steroids and include increases in liver enzyme levels, cholestatic jaundice, and peliosis hepatis (Maravelias et al., 2005). Additionally, effects such as stunted growth and thickening of the bone cortex have been observed (Özdemir and Yalçın, 2011; Bozkurt et al., 2011a-b).

Common side effects of AAS include increased water retention, liver dysfunction, edema, jaundice, increased cardiac workload, increased risk of benign and malignant liver tumors, increased blood pressure, kidney dysfunction, increased cholesterol levels, tumor growth stimulation, increased risk of cardiovascular disease, elevated blood sugar levels, pustulation, nosebleeds, muscle cramps and spasms, thyroid dysfunction, tendon damage or rupture, psychological disorders, euphoria, and aggression (Gök et al., 2016).

In women, the side effects of anabolic-androgenic steroids (AAS) include hirsutism, nymphomania, hair loss, menstrual irregularities or early menopause, reduced breast size, voice deepening, and clitoral enlargement. In prepubescent boys, side effects include premature epiphyseal closure and stunted growth. Additionally, AAS use can lead to hair loss, infertility, reduced production of male sex hormones, gynecomastia, decreased libido, testicular shrinkage, impotence, and reduced sperm production. Male sex hormones can cause the prostate gland to enlarge, lead to prostate cancer, reduce sperm production, and cause Wilms tumors and abnormal sperm (Livanelioğlu, 2010).

Furthermore, prolonged androgen use can reduce height growth in young individuals and lead to early epiphyseal closure in children and adolescents (Al-Ismail, 2002; Özdemir and Yalçın, 2011; Bozkurt et al., 2011a-b). Anabolic steroids can suppress the production of estrogen and testosterone (Kaşıkçıoğlu, 2009). Testosterone administration has been shown to reduce albumin and total serum protein levels ($p<0.05$), while increasing cholesterol, aminotransferase, and aspartate levels ($p<0.05$). Therefore, long-term testosterone administration during adolescence may lead to organ defects (Lök and Yalçın, 2010; El-Gendy et al., 2021).

Erythropoietin (EPO) is a glycoprotein hormone primarily synthesized in the kidneys and has a gene locus on the seventh chromosome (Glöckner et al., 1998). EPO's primary function is to stimulate the proliferation and maturation of erythroid cells in the bone marrow. Besides its use in treating anemia, EPO also has anti-inflammatory, antioxidant, anti-apoptotic, and angiogenic effects. However, this hormone is sometimes used by athletes for doping purposes.

Mitchell et al. (2005) and Kaşıkcıoğlu (2009) reported that bone loss occurs a few months after steroid use, with this loss being more prevalent in trabecular bone of the vertebrae and femoral head compared to cortical bone. A morphometric study on rats examining the effects of AAS on the humerus and femur found that AAS reduced cortical bone density in males (Özdemir and Yalçın, 2011; Bozkurt et al., 2011a, 2011b). Mitchell et al. (2005) and Kaşıkcıoğlu (2009) noted that weight-bearing exercises could reduce AAS-induced bone loss.
EPO is a hematopoietic growth factor that stimulates the formation of red blood cells and is known as a doping agent in high-performance sports, particularly cycling. It is used in clinical settings to treat anemia caused by insufficient endogenous EPO production, especially in chronic kidney failure. In recent years, the non-hematopoietic functions of EPO have also been extensively researched. These non-hematopoietic capabilities, including osteogenic and angiogenic potentials, are of particular interest to orthopedic and musculoskeletal engineering (Rölfing, 2014).

In clinical settings, EPO administered to anemic patients can increase the number of red blood cells by 35%, change the shape of cells, and enhance their oxygen-carrying capacity. Erythropoietin is an important hematopoietic growth factor necessary for erythropoiesis. This hormone is a glycoprotein composed of 165 amino acids and four carbohydrate chains, with a molecular weight of 30,400 kD (Koury et al., 1998; Wang et al., 1985).

This study comprehensively examined the biological and psychological effects of anabolic-androgenic steroids (AAS). The findings indicate that AAS use leads to serious health problems in both men and women. In men, increased testosterone levels support the development of secondary sexual characteristics, while excessive use leads to testicular atrophy, reduced sperm production, and loss of libido. In women, AAS use causes side effects such as hirsutism, voice deepening, and menstrual irregularities. These findings clearly show that AAS negatively impacts sexual health and the reproductive system.

Effects on the Heart

One of the most affected systems by the side effects of anabolic-androgenic steroids (AAS) is the cardiovascular system. AAS use increases vascular resistance and blood pressure, raises levels of pro-inflammatory biomarkers, and leads directly to myocardial toxicity. To assess the relationship between AAS use and sudden cardiac death, it is crucial to focus on the organs where these side effects are most frequently observed (Torrisi et al., 2020).

A recent case study highlighted the cardiovascular risks associated with androgen use (Scarth and Bjørnebekk, 2021). Long-term AAS consumption has been shown to cause liver, heart, and vascular injuries in adolescent rats, along with increased serum CK-MB and AST activity (Lok, Tasgin, Demir, and Özdemir, 2010; Tasgin, Lok, and Demir, 2011). The abuse of erythropoietin and AAS can result in severe and irreversible organ damage (Al-Otaibi, 2024).

Even therapeutic use of AAS has been associated with adverse effects. Anabolic effects often manifest with numerous physical and physiological side effects. Reported side effects include acne, testicular atrophy, gynecomastia, hypertension, arrhythmia, myocardial infarction, depression, increased red blood cell count, impaired diastolic function, reduced sperm count, and increased mortality (Mutalip et al., 2013; Nascimento et al., 2016). Left ventricular hypertrophy has been observed in individuals engaging in resistance exercise and using high doses of AAS. Özdemir et al. (2013) found that the use of methenolone enanthate, an AAS, increased muscle hypertrophy and left ventricular heart rate in pubertal female rats.

The toxic effects of anabolic steroids on the heart and skeletal muscles of albino rats have been exacerbated by treatment with fenugreek seed extract and silymarin (Hassan et al., 2023). Abdollahi et al. (2016) reported that the combination of chronic nandrolone administration and long-term high-intensity swimming exercise increased the incidence of ventricular fibrillation in male rats. Sretenovic et al. (2016) reported that nandrolone administration increased left ventricular wall thickness in Wistar albino rats, with the thickening ranging from 6% (nandrolone only) to 30% (nandrolone with exercise) compared to the control group. Exercise alone also led to greater wall thickening (16%) compared to the control group. AAS use has been observed to have adverse and lasting effects on left ventricular function (Johnson et al., 2024). Chronic use of testosterone enanthate at doping doses has been found to cause hypertrophy and fibrosis in the heart muscle and liver of rats, leading to loss of organ function at both histological and biochemical levels (El-Gendy et al., 2021).

Effects on the Liver

The use of anabolic-androgenic steroids (AAS) can lead to various adverse effects, depending on the dosage. AAS, often used illegally in high doses by athletes, pose significant health risks. Supraphysiological and long-term use of AAS can result in cardiovascular, neurological, endocrine, gastrointestinal, renal, and hematological disorders (Petrovic et al., 2022). Hepatotoxicity, in particular, is one of the major concerns associated with AAS therapy and abuse. It has been shown that testosterone and its derivatives can induce specific cholestasis, peliosis hepatis, and the formation of benign and malignant hepatic tumors (Cunningham et al., 2013; Zelleroth et al., 2021). Prominent side effects of AAS therapy include elevated liver enzyme levels, cholestatic jaundice, peliosis hepatis, and various neoplastic lesions, all indicating hepatotoxicity (Nasrollah & Shahidi, 2001).

In recent years, the use of anabolic steroids among athletes and young individuals has significantly increased. This rise has particularly heightened the toxic effects of orally administered steroids on the liver (Boada et al., 1999). Even three months after discontinuing steroid use, destructive effects on liver cells persist. However, some of the negative impacts on liver enzymes have been observed to decrease significantly over time (Barbalho and Barreiros, 2015).

Studies by Parkinson and Evans (2006) found that bodybuilders who used steroid medications during exercise exhibited significantly increased liver enzymes and blood factors. Elevated ALT and AST levels indicate that liver and muscle enzymes have entered the bloodstream. The liver detoxifies various drugs or sends them to the bile, converting steroid hormones not found in tissues into androsterone and dihydroepiandrosterone, which are then excreted through the bile or urine after sulfation. The damage to liver tissue from these drugs can vary depending on the type of drug, duration of use, and dosage (Robergs and Roberts, 2000).

The most common forms of tissue damage in the liver include vascular hyperemia, degeneration, inflammation, and an increase in cytoplasmic fat vacuoles. This condition leads to liver deformation and hardening, destroying liver tissue and replacing it with connective tissue. In some cases, this damage can lead to liver failure, encephalopathy, and ultimately death (Susan et al., 2007).

The increased use of anabolic steroids among athletes and young people has raised concerns, particularly about the hepatotoxicity of orally administered steroids. Various in vivo effects of AAS have been reported, including liver microsome damage in male rats. A five-week study in mice reported that AAS use increased markers of hepatic necrosis, centrilobular vessels, and collagen accumulation in liver parenchyma (Boada et al., 1999).

Anabolic-androgenic steroids (AAS) are used in medical settings for two primary purposes. Firstly, they are used in androgen replacement therapy for patients with androgen deficiency due to genetic disorders of the hypothalamus, pituitary gland, or testes. Secondly, AAS are applied as pharmacological androgen therapy (PAT) to improve the quality of life in patients with chronic diseases without androgen deficiency, aiming to achieve optimal testosterone effects. However, chronic use of testosterone enanthate at supraphysiological (doping) doses can lead to significant toxic effects. Research indicates that long-term administration of such doses can cause histological and biochemical changes in cardiac and hepatic tissues, resulting in hypertrophy, fibrosis, and potential loss of function. Understanding these effects is crucial for healthcare providers and patients considering AAS therapy (El-Gendy et al., 2021).

Effects on the Nervous System

The hippocampus is a region notable for its structural plasticity and its significant role in learning and memory. This has led to considerable interest in studies examining the effects of androgens on the brain. Animal studies have demonstrated that supraphysiological doses of androgens can lead to neurodegeneration, reduced levels of brain-derived neurotrophic factor (BDNF), increased inflammation, and decreased neuronal density. Data from human studies suggest that prolonged androgen use may result in similar behavioral and cognitive deficits (Scarth and Bjørnebekk, 2021).

Damio et al. (2021) investigated the effects of two different steroid treatments, testosterone cypionate and stanozolol, on neuronal density in the limbic, motor, and sensory cortical areas, as well as in the hippocampal CA1, CA2, and CA3 regions of both male and female mice. They found that both testosterone cypionate and stanozolol significantly reduced neuronal density in the limbic region in male mice, with stanozolol alone significantly reducing neuronal density in the CA1 region.

A study involving 99 weightlifters and 130 androgen users suggested that long-term androgen use may accelerate brain aging. The brain ages of androgen users were found to be higher than those of controls, and the longer the exposure to these substances, the more rapid the brain aging (Scarth and Bjørnebekk, 2021). Abuse of AAS may lead to neurotoxicity, activating cell necrosis (Bertozzi et al., 2018). This was observed as a significant reduction in neuronal density in animals supplemented with AAS compared to control and exercise groups (Corsini et al., 2022), indicating that similar neurotoxic processes may affect the nervous system in these animals.

Corsini et al. (2022) conducted a study examining the impact of physical exercise on neuronal body damage in the brains of male rats. They found that steroids influenced cell death mechanisms, leading to cell death that affected the nervous system, particularly impacting the locus coeruleus and causing significant neuron loss in this region. Behavioral disorders associated with AAS may reflect notable psychopathological comorbidities between drug addiction and neural circuit adaptations, including neurotrophic changes. There is a high expression of steroid receptors and enzymes involved in steroid synthesis and metabolism in brain regions implicated in the onset of anxiety and aggression associated with AAS abuse. Chronic administration of high-dose AAS has been shown to promote anxiety-like behaviors (Bertozzi et al., 2018). Long-term use of AAS negatively affects ERα or ERβ receptors in brain regions responsible for aggression control (Melloni and Ricci, 2010).

In conclusion, research on the effects of androgens on the brain examines how banned substances used for doping impact healthy brain function and behavior. These effects arise from various changes occurring in specific brain regions, such as the cerebral cortex, hippocampus, amygdala, and hypothalamus. High-dose androgen therapy has been associated with increased oxidative stress and impaired neuroplasticity. However, most of these findings are derived from animal studies, and their direct applicability to humans may not yield accurate results.

Effects on Body and Organ Weights

The primary aim of steroid use is to achieve rapid muscle mass gain and an aesthetic appearance. However, the adverse effects of combining anabolic-androgenic steroid (AAS) abuse with training on testicular morphology, tissue redox status, and androgen receptor levels are still not fully understood (Sretenovic et al., 2021). The androgenic and anabolic effects of testosterone can have significant impacts on body weight and various organs (Özdemir and Yalçın, 2011).

Experimental studies have shown that testosterone administration does not significantly alter body weight in rats but does lead to a significant reduction in testicular weight (Özdemir and Yalçın, 2011; Sretenovic et al., 2021). This reduction is attributed to decreased testosterone levels, leading to apoptosis in spermatogenic cells and affecting spermatogenesis, sperm count, morphology index, viability, progressive motility, and testicular injury (Al-Otaibi, 2024).

In a study by El-Gendy et al. (2021), a significant reduction in body weight was observed in the group treated with a doping dose of testosterone enanthate for 8 and 12 weeks compared to the control group. It is also known that mild hyperthermia conditions can reduce testicular weight, trigger apoptosis in germ cells, and cause damage to the seminiferous epithelium (Lue et al., 2000). In a four-week study by Chuffa et al. (2011), no significant weight loss was observed in the exercise group, while Nandrolone Decanoate showed a statistically insignificant decrease in body weight despite its anabolic effects in experimental animals.

The body weights of rats given anabolic steroids, protein supplements, and exercise were found to be the lowest among all groups. Testicular weight significantly decreased in the anabolic groups, and the groups that exercised had the lowest hematocrit values. All serological values remained within normal ranges, and no pathological changes were observed in tissues taken from specific "target organs" (Bauman et al., 1988).

In a study by Özdemir and Yalçın (2011), testosterone administration in male rats did not affect the weight of the heart, right kidney, or left kidney, but led to a slight decrease in spleen weight compared to the control group. Additionally, a significant reduction in liver weight and the weights of the right and left testes was observed in hormone-treated male rats. Blystone et al. (2007) reported that administering testosterone to adolescent male rats significantly reduced the weight of some organs. These findings align with Carson et al.'s (2002) report that nandrolone administration significantly decreased testicular weight in male rats. Balkaya et al. (2002) also observed a significant reduction in the weight of certain organs in male rats administered testosterone. In this study, the female rats in the experimental group showed only a quantitative decrease in heart, liver, and spleen weights compared to the control group, while the weight of the right kidney was statistically increased.

Effects of AAS on Sexual Behavior and the Reproductive System

Anabolic-androgenic steroids (AAS) are synthetic compounds derived from the testosterone molecule, naturally produced in the interstitial Leydig cells of the testes. AAS are typically used in two main medical scenarios: first, in androgen replacement therapy for patients with androgen deficiency due to genetic disorders of the hypothalamus, pituitary gland, or testes; and second, as pharmacological androgen therapy (PAT) to improve the quality of life in individuals with chronic diseases without androgen deficiency (Mutalip et al., 2013).

The effects of AAS on the reproductive system in men include decreased libido, sexual impotence, impaired spermatogenesis, and prostate hypertrophy. In women, observed effects include hirsutism, voice deepening, and menstrual irregularities (Mutalip et al., 2013). Experimental studies in rats have examined the effects of AAS on testicular morphology and histology, revealing significant reductions in the number and size of Leydig cells in the interstitial space (Naraghi et al., 2010).

In a study conducted by Grokett and colleagues (1992), rats treated with AAS showed weights of the testes, prostate gland, and seminal vesicles that were 69%, 50%, and 29% lower, respectively, compared to control groups. During a three-hour incubation period, testicular testosterone production in treated animals dropped to as low as 1.3% of control values. Additionally, serum levels of FSH (11.7% of control) and LH (undetectable) were significantly lower in treated animals compared to controls. Histological findings showed a cessation of late spermatid development and a marked decrease in Leydig cell populations in the interstitial area (Grokett et al., 1992; Naraghi et al., 2010). These findings suggest that treatment with oxandrolone in immature male rats can have profound and multi-level effects on the adult male reproductive system (Grokett et al., 1992).

The pathophysiological effects of AAS are not limited to the reproductive system; they are also associated with increased oxidative stress in the heart, liver, and kidneys (Frankenfeld et al., 2014). Previous studies have indicated that excessive production of reactive oxygen species (ROS) can lead to male infertility by disrupting steroidogenic activity in the testes and affecting the integrity of cell membrane macromolecules (Manna et al., 2003; Sretenovic et al., 2021).

It has been found that estrogens derived from androgens during early developmental stages significantly influence male sexual preferences. An increase in sexual preference for female stimuli among males exposed to supraphysiological testosterone levels around postnatal day 21 suggests that males exposed to high testosterone levels might experience disadvantages in reproductive success under natural conditions where competition among males is high. Conversely, males not exposed to testosterone may have a higher likelihood of approaching females, potentially enhancing their reproductive success (Domínguez et al., 2002; Henley et al., 2010).

In earlier studies, Beach and colleagues (1949) administered varying doses of testosterone propionate injections to castrated male rats daily. Doses below 50 micrograms did not produce significant changes in mating behavior, and treated animals showed a significantly lower likelihood of exhibiting sexual response to females compared to normal animals or castrated animals receiving higher testosterone doses. These results suggest that high early androgen levels play a role in bisexual social preferences rather than homosexual preferences (Hanley et al., 2010).

Research by Clemens et al. (1978) demonstrated that females developing between two males in utero exhibited more male-like sexual behavior in adulthood compared to females developing between two females. Prenatal treatment with anti-androgens prevented this effect. Neumann and colleagues' (1966) study showed that male offspring of mothers treated with anti-androgens during pregnancy exhibited feminized behaviors in adulthood. In another study by Pollak and Sachs (1975), females treated with testosterone during both prenatal and postnatal periods displayed more pronounced male-like sexual behavior in adulthood compared to those treated only postnatally.

Effects on Bone Tissue

The use of anabolic-androgenic steroids (AAS) can have various adverse effects on bone development, particularly during the growth period. One of the most significant side effects of AAS is the premature closure of the growth plates (epiphyseal plates), which can lead to stunted growth in animals, indicating that prolonged use of these substances can cause similar effects in humans (Özdemir & Yalçın, 2011). Additionally, some studies have reported that AAS use can negatively impact the healing of injuries, thus suggesting cautious use in clinical treatment (Arslan & Besoluk, 2023).

Methenolone Enanthate (ME) is used, particularly for the treatment of anemia due to bone marrow failure, wasting syndromes, osteoporosis, and sarcopenia (Tauchen et al., 2021). The administration of high doses of ME in rats can lead to early epiphyseal closure in the femur and humerus, halting bone growth and potentially adversely affecting bone development, especially in young athletes and sedentary individuals using AAS (Özdemir & Yalçın, 2011).

In a study by Bozkurt et al. (2011a-b), it was reported that intraperitoneal (IP) administration of Methenolone Enanthate at a dose of 5 mg/kg for four weeks, five days a week, in 40-day-old Sprague-Dawley male and female rats resulted in a decrease in femur length in males and an increase in females. A reduction in corpus thickness was observed in males, while an increase was noted in females. No difference in cavum medullare diameter was found in either gender, and no difference in cortex thickness was observed in males, while an increase occurred in females.

Another study by Lok and Yalçın (2010) found that the intraperitoneal administration of Nandrolone at a dose of 10 mg/kg for four weeks, five days a week, in 30-day-old male and female rats led to a reduction in femur length, with no differences found in femur corpus thickness, cavum medullare diameter, or cortex thickness. Additionally, subcutaneous administration of Testosterone at a dose of 5 mg/kg for ten weeks, five days a week, in 50-day-old Sprague-Dawley rats resulted in a reduction in femur length in males and an increase in females; a decrease in corpus thickness in males, with no effect observed in females; no difference in cavum medullare diameter in males, with narrowing observed in females; and no effect on cortex thickness in either gender (Özdemir & Yalçın, 2011).

In studies by Özdemir et al. (2020), the effects of Methenolone Enanthate on scapula height, width, and surface area in rats were found to be similar to other AAS, suppressing bone development in male pubescents while promoting bone development in females. It is emphasized that commonly used doping substances have negative effects on bones in males in the medium and long term.

Among the side effects of AAS observed in prepubescent males are the early ossification of cartilage and stunted growth (Özdemir & Yalçın, 2011). Testosterone and synthetic androgen analogs have been widely used in pharmacological androgen therapy (PAT) to produce androgenic effects on bone marrow, muscle, or bone. While PAT has increasingly been replaced by newer, more expensive drugs, androgens continue to play a significant role in many traditional applications due to their cost-effectiveness (Handelsman, 2006).

The effects of anabolic-androgenic steroids (AAS) on bone tissue have been extensively studied in both clinical and experimental settings. While AAS have been reported to have positive effects on bone density, it is also highlighted that long-term and high-dose use can lead to adverse outcomes on bone health. AAS have the potential to reduce bone fragility by increasing bone mineral density; however, their unbalanced use can lead to abnormal growth and deformities in bone structure (Bhasin et al., 2001).

In one study, the effects of nandrolone decanoate on bone mineral density (BMD) were investigated, and it was found that this steroid increased BMD but also caused an increase in bone marrow fat content. This could negatively affect the hematopoietic potential of the bone marrow, leading to impairments in bone marrow function (Van Brussel et al., 1999).

AAS use can also affect bone remodeling processes. Specifically, testosterone and other androgens have bone-forming effects; however, these effects can vary depending on the dose and duration of use. High-dose and long-term AAS use can increase bone resorption, leading to bone loss rather than net bone gain (Seeman, 2001). Moreover, the effects of AAS on bone tissue can differ based on age, gender, and physiological condition. For instance, while bone density-enhancing effects may be observed in young adults, increased bone fragility may occur in older individuals. Therefore, it is crucial to consider individual factors when assessing the effects of AAS on bone tissue (Clark et al., 1999).

Testosterone and synthetic androgen analogs have also been used in pharmacological androgen therapy (PAT) to produce androgenic effects on marrow, muscle, or bone. While PAT has been increasingly replaced by newer, more expensive drugs, androgens remain cost-effective in many traditional applications (Handelsman, 2006). In conclusion, the effects of anabolic-androgenic steroids (AAS) on bone tissue should be carefully evaluated, considering both positive and negative aspects. More comprehensive research is needed on the potential risks and benefits of AAS on bone health. Healthcare professionals should carefully manage the use of these substances, considering their effects on bone tissue. These assessments are critical for minimizing the long-term health impacts of AAS use and enhancing safety in treatment processes.

Psychological Effects

Research on the effects of anabolic-androgenic steroids (AAS) on brain development and behavioral outcomes has revealed extensive impacts on neurological and psychological health, including during adolescence. The influence of AAS on brain organization has been observed in both humans and animal models, showing significant alterations in neurotransmitter functions and neural structures (Cunningham et al., 2013). Adolescence is a critical period for brain development, and exposure to AAS during this time can disrupt the normal pattern of brain development, leading to long-term behavioral changes.

The effects of AAS on aggression have been extensively studied. Aggression is a behavioral effect frequently associated with AAS exposure, which can result from a combination of various factors. The chemical composition of AAS, hormonal context, environmental conditions, physical provocation, and social interactions all play significant roles in the expression of aggression (Kanayama et al., 2010; Lumia et al., 2010). These factors can influence AAS users' tendencies to respond to social encounters with increased vigilance and enhanced motivation.

High doses of AAS used by athletes can have psychological and neurological side effects. It has been shown that high-dose AAS use increases the risk of emotional and psychotic syndromes, as well as psychological dependence (Bahrke et al., 1996; Zelleroth et al., 2021). Additionally, the phenomenon known as "roid rage" suggests that behavioral changes in AAS users are side effects of these substances (Lumia et al., 2010).

Endogenous androgens and estrogens affect the nervous system in both males and females, altering behavioral responses. However, the notion that AAS directly causes aggression has been re-evaluated by recent studies. It has been highlighted that men with AAS dependence are not typically more violent, but they are more likely to have a history of behavioral disorders and psychopathology (Kanayama et al., 2010).

In conclusion, studies on the effects of AAS on brain development and behavior indicate that the use of these substances can lead to a wide range of neurological and psychological effects. Exposure to AAS during adolescence can alter the normal course of brain development and increase vulnerability to psychopathological conditions and maladaptive behaviors. Therefore, further research on the neurological and psychological effects of AAS is essential, and healthcare professionals should carefully manage the use of these substances.

Evaluating the potential risks and benefits of AAS use requires comprehensive and ongoing research to understand and manage their health impacts. This research will be crucial in determining the long-term effects of AAS and in developing more informed approaches to their clinical use.

Conclusion

This study comprehensively examines the biological and psychological effects of anabolic-androgenic steroids (AAS). The findings demonstrate that AAS use can lead to significant health issues in both men and women. In men, increased testosterone levels support the development of secondary sexual characteristics; however, excessive use has been associated with testicular atrophy, reduced sperm production, and loss of libido. In women, AAS use has been linked to side effects such as hirsutism, voice deepening, and menstrual irregularities. These findings clearly indicate that AAS have detrimental effects on sexual health and the reproductive system.

Beyond biological effects, the psychological impacts of AAS use are also profound. Research indicates that AAS users show increased tendencies towards aggression and violent behavior, which are often associated with pre-existing psychopathology, behavioral disorders, and a history of drug addiction. The phenomenon known as "roid rage" suggests that AAS users attribute their behavioral changes to the side effects of AAS, such as psychosis or paranoia, which has even been used as a legal defense. This highlights that AAS use has implications not only for individual health but also for public safety and the legal system.

Long-term and high-dose AAS use leads to serious pathophysiological changes in vital organs such as the heart, liver, and kidneys. Effects such as myocardial hypertrophy, increased liver enzyme levels, and impaired kidney function indicate that AAS use causes systemic toxicity. Additionally, changes in muscle tissue and overall body composition negatively impact the general health status of AAS users. Psychologically, AAS use alters behavioral responses to sensory and social stimuli. Users often become more aggressive and active, negatively affecting social relationships and daily life. These findings suggest that AAS have broad impacts not only on physical health but also on psychosocial well-being.

In conclusion, the biological and psychological effects of AAS use result in significant health and behavioral impairments in users. Therefore, it is crucial to regulate AAS use and develop more effective policies to prevent the abuse of these substances. Awareness campaigns should be conducted to inform the public, especially young athletes, about the adverse effects of these substances. There should be collaboration among healthcare professionals, educators, and policymakers to develop comprehensive strategies to prevent AAS abuse. In this context, early intervention and treatment programs should be implemented to prevent AAS dependence.

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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