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Artificial Intelligence in Clinical Medicine: A SWOT Analysis of AI Progress in Diagnostics, Therapeutics, and Safety

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Abstract

Artificial intelligence (AI) is increasingly recognized as a developing driver of innovation in clinical medicine, with reported advancements in diagnosis, treatment, and patient safety. Its capabilities may support new applications in care delivery and quality improvement, though the full extent of its impact remains under investigation. This analysis aimed to explore recent advances in AI across clinical laboratory medicine, infectious disease management, and pharmacovigilance, drawing insights from a peer-reviewed English literature published between 2019 and 2024. The study presented a descriptive literature review using the strengths, weaknesses, opportunities, and threats (SWOT) framework to examine recent AI developments in selected clinical domains, noting its emerging role and potential relevance in clinical settings. In clinical laboratories, AI has been associated with improved diagnostic accuracy and operational efficiency, while in infectious diseases, it has enabled rapid pathogen identification and precision-guided treatments. In pharmacovigilance, AI has been explored for its potential to enhance predictive analytics and real-time monitoring, which may have contributed to reducing medication-related errors and adverse drug events. Despite these reported benefits, AI adoption raised critical concerns, including data privacy, algorithmic bias, and the ongoing need for human oversight. Addressing these challenges is essential to promote ethical, transparent, and appropriate AI integration into clinical medicine. By addressing these complexities, AI may unlock new opportunities in personalized medicine, safety, and care delivery, positioning it as a supportive tool in the

evolving landscape of clinical practice.

Keywords: artificial intelligence, machine learning, clinical medicine, laboratory medicine, infectious diseases, pharmacovigilance

1. Introduction

Artificial Intelligence (AI) has already been reshaping the landscape of clinical medicine (Bajwa et al., 2021; Reali & Femminella, 2024). This AI-driven revolution is marked by the introduction of emerging approaches that are gradually influencing how patient care and outcomes are achieved and optimized (Barracca et al., 2020; Krishnan et al., 2023; Rb et al., 2024; Suh et al., 2022). The capabilities of AI to rapidly and precisely analyze extensive medical data can empower healthcare professionals to make informed, accurate, and timely decisions (Alowais et al., 2023; Bajwa et al., 2021). This progress may contribute to enhancing diagnostic accuracy, tailoring treatments to individual needs, and improving patient outcomes while increasing efficiency in healthcare delivery (Akhter et al., 2024; Alowais et al., 2023; Cesario et al., 2023; Riaño et al., 2019; Salehi, 2024; Saxena & Chandra, 2021; Stafie et al., 2023; Zavaleta-Monestel et al., 2024; Zeb et al., 2024). Additionally, the utility of AI-driven technologies extends to facilitating administrative workflows, enhancing resource allocation, and accelerating the development of groundbreaking medical interventions (Ahmadi & RabieNezhad Ganji, 2023; Alves et al., 2024; Bhagat & Kanyal, 2024; Cutler, 2023). The continued advancements of AI suggest a future where its integration into clinical medicine could support improved effectiveness and efficiency in patient care (Abbaoui et al., 2024; Dutta, 2023).

Clinical medicine involves diagnosing and treating human diseases alongside efforts in disease prevention and promoting health and well-being—key components of the United Nations' Sustainable Development Goals established in 2015 (Kruk et al., 2018). Clinical medicine integrates both primary and specialty care (Romaire, 2020). Primary care serves as the initial point of contact, addressing routine checkups and general health concerns, while specialty care involves advanced treatment provided by physicians with expertise in specific medical disciplines (Wilkinson et al., 2024).

Integrating advanced technologies such as AI into clinical medicine has become increasingly essential to meet the complex demands of modern healthcare (Bajwa et al., 2021; Barracca et al., 2020). Currently, AI is steadily transforming the practice of clinical medicine, redefining various approaches across a wide range of specialties (Bekbolatova et al., 2024; Karalis, 2024). For example, AI-driven predictive analytics have demonstrated potential in enhancing the accuracy, efficiency, and cost-effectiveness of disease diagnosis and clinical laboratory testing, offering advancements in clinical medicine (Khalifa & Albadawy, 2024; Undru et al., 2022). Moreover, AI aids population health management and the development of clinical guidelines by providing real-time, accurate insights and optimizing medication selection (Alowais et al., 2023; Krishnan et al., 2023; Maleki Varnosfaderani & Forouzanfar, 2024). On a specific note and based on the current body of evidence, in fields like clinical laboratory medicine, infectious diseases, and pharmacovigilance, AI-driven tools are being applied to enhance diagnostic accuracy, refine treatment strategies, and provide precise predictions of patient outcomes (Jafri et al., 2024; Lee et al., 2023; Salas et al., 2022; Sarantopoulos et al., 2024; Shamim et al., 2024; Undru et al., 2022; Vora et al., 2023; Xie et al., 2024; Zhao et al., 2024). These domains were selected due to their distinct roles in clinical diagnostics, population-level surveillance, and medication safety, making them important areas for evaluating AI's evolving contributions to patient care.

1.1 An Overview of AI's Development Through the Years

On a historical note, the progress of modern civilization has been profoundly influenced by technological innovation, with the adoption and integration of new tools contributing significantly to societal advancement (Ida, 2024). This development path includes the rise of artificial intelligence, a major technological development of recent decades (Bharadiya et al., 2023). Since the 1940s, AI has undergone notable advancements, evolving into a tool with applications across multiple fields, including clinical medicine (Rashid & Kausik, 2024; Roser, 2022). The evolution of artificial intelligence has been shaped by several decades of technological progress, beginning with its conceptual roots in the 1940s and 1950s. During this time, early computers were introduced, alongside foundational discussions on machine cognition and the Turing Test, proposed as a benchmark for machine intelligence (Haenlein & Kaplan, 2019), including the development of the Turing Test as a benchmark for evaluating machine intelligence (Gonçalves, 2023). A significant milestone was the 1956 Dartmouth Workshop, which formally recognized AI as a research discipline and laid the foundation for future developments (van Assen et al., 2022). Clinical relevance emerged in the 1980s with machine learning (ML) algorithms, such as decision trees and neural networks, which shifted AI from rule-based systems toward data-driven approaches capable of pattern recognition and adaptive learning (Taye, 2023). This transition enabled more adaptive, learning-based systems to identify and respond to data patterns.

A breakthrough followed in the 2010s with the development of a deep convolutional neural network model that

demonstrated substantial success in image recognition, marking a key advancement in modern deep learning (DL) and influencing computer vision (Manakitsa et al., 2024; Sarker, 2021). The 2020s witnessed the rapid evolution of generative AI (genAI), with models such as OpenAI's generative pre-trained transformer GPT-3 and GPT-4 demonstrating the ability to generate human-like text and content, with a subsequent huge influence on diverse sectors such as healthcare (Javaid et al., 2023; Sallam, 2023). By 2024, growing interest in AI integration within healthcare was observed, supported by developments in explainable AI, multimodal systems, and evolving regulatory considerations (Pahud de Mortanges et al., 2024; Sadeghi et al., 2024; Tam et al., 2024).

The major milestones in the development of AI, from its early foundations in the 1940s to its advancements in 2024, are illustrated in Figure 1.

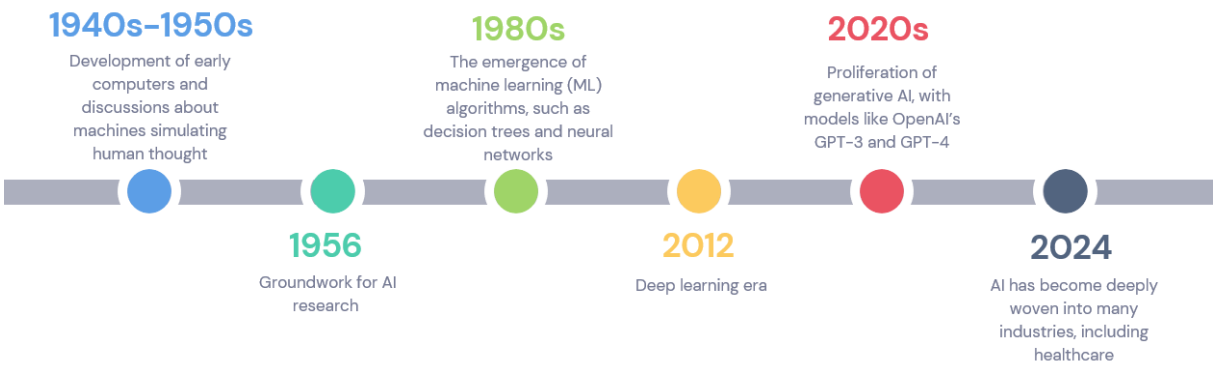

Figure 1. Key Milestones in the Evolution of AI (1940s–2024).

AI: Artificial Intelligence; ML: Machine Learning; genAI: Generative AI; GPT: Generative Pre-trained Transformer.

1.2 Definition and Scope of AI in Healthcare

AI is the branch of computer science focused on simulating aspects of human cognition through machines (Xu et al., 2021). AI encompasses several specialized domains, including ML, natural language processing (NLP), DL, robotics, and process automation, each employing distinct methodologies to address specific challenges (Ahirwal et al., 2022; Soori et al., 2023). ML and DL utilize computational techniques to identify and model complex patterns and decision rules based on data (Taye, 2023). These methods employ supervised, unsupervised, and reinforcement learning, enabling them to perform tasks such as predicting outcomes, classifying diseases, and detecting abnormalities (Asselbergs et al., 2023; Sarker, 2021). The hierarchical relationship between AI, ML, DL, and genAI is illustrated in Figure 2, showing how these fields interconnect in a nested structure.

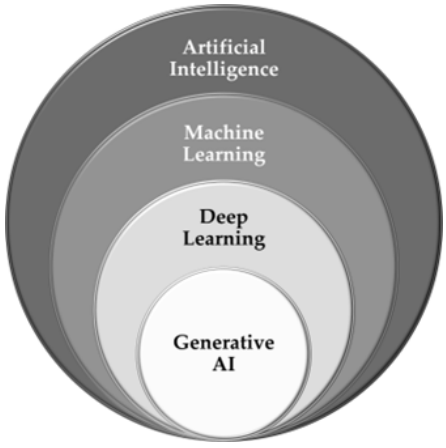

Figure 2. The Nested Structure of Artificial Intelligence, Encompassing Machine Learning, Deep Learning/Artificial Neural Networks, and Generative AI

Building on AI's diverse healthcare applications is considered a potentially valuable approach for interpreting medical texts and developing diagnostic solutions (Bajwa et al., 2021; Demner-Fushman et al., 2009; Locke et al., 2021). For example, NLP has shown practical utility in analyzing electronic health records (EHRs) (Hossain et al., 2023). By analyzing and interpreting unstructured medical texts within EHRs, NLP can extract meaningful insights, streamline diagnostic processes, and enhance clinical decision-making (Sim et al., 2024). This integration supports evidence-based medicine, improves adherence to clinical guidelines, builds knowledge repositories, and facilitates outcome-based research (Shastry & Shastry, 2023; Sheikhalishahi et al., 2019).

Investigating patient specimens is essential in clinical laboratory medicine to aid in diagnosis. Utilizing AI may significantly support the practice of laboratory medicine, enabling the interpretation of laboratory results (Theodosiou & Read, 2023; Undru et al., 2022). Additionally, the use of AI offers advantages compared to traditional clinical analysis, assisting professionals in making faster decisions and improving operational efficiency by reducing delay times and human errors (Baxi et al., 2022; Ephraim et al., 2024; Shafi & Parwani, 2023). In infectious disease management, AI has shown promise in supporting diagnosis, risk stratification, and treatment planning (Cheng et al., 2023; Santangelo et al., 2023; Sarantopoulos et al., 2024). AI's application in pharmacovigilance has been associated with improved detection and monitoring of adverse effects, aiding in detecting and preventing medication-related issues (Gamaleldin et al., 2024). Large language models (LLMs) and genAI enhance interactions, provide accurate and curated information, and improve health service delivery, highlighting the importance of responsible integration to maximize their benefits (Schueller & Morris, 2023; Xu et al., 2021).

These developments reflect AI's emerging influence across the selected domains; however, the effective application of AI continues to face limitations related to data quality, algorithmic bias, cybersecurity risks (Sallam et al., 2024a), patient data privacy and ethical concerns (Bekbolatova et al., 2024), and the limited generation of real-world evidence through clinical trials (Han et al., 2024; Salehi, 2024).

1.3 Study Aims and Objectives

This analysis aimed to explore the developing applications of AI in clinical medicine, focusing on clinical laboratory medicine, infectious disease management, and pharmacovigilance. Specifically, the study objectives included evaluating how AI could contribute to diagnostic accuracy, support decision-making, and improve operational efficiency. The paper also examined the AI's role in analyzing biological specimens, classifying diseases, stratifying risk, planning treatments, and detecting medication-related adverse events. It also highlighted the potential of large language models (LLMs) and genAI to support clinical communication, deliver curated information, and contribute to healthcare delivery (Bedi et al., 2024). Lastly, the paper addressed key challenges associated with AI integration in clinical medicine, such as data quality, bias, interpretability, privacy, and the limited availability of real-world evidence, emphasizing the need for responsible integration and consistent evaluation frameworks to ensure ethical and effective implementation.

2. Materials and Methods

This targeted descriptive literature review examined the integration of AI into three key areas of clinical medicine: clinical laboratory medicine, infectious disease management, and pharmacovigilance. To systematically evaluate current advancements, limitations, and opportunities, a narrative literature review was conducted using five databases: PubMed/MEDLINE, Web of Science, Scopus, EMBASE, and Google Scholar. The search targeted peer-reviewed English-language articles published between January 2019 and September 2024, and the search concluded on October 1, 2024.

The first and second authors collaboratively developed a multidisciplinary search strategy, leveraging AI, healthcare, infectious diseases, and pharmacovigilance expertise. The strategy employed predefined keywords including: "AI in clinical medicine," "machine learning in diagnostics," "AI in infectious diseases," and "AI in treatment optimization," which guided the search. Authors screened abstracts and full texts independently to minimize selection bias, and consensus resolved discrepancies following discussion. Studies were included if they reported on AI's impact on diagnostic accuracy, treatment optimization, patient safety, or clinical outcomes. The evaluated data covered study design, AI methodologies, clinical domains impacted, and reported outcomes. Emphasis was placed on studies detailing AI's role in enhancing diagnostic workflows, reducing medication-related harm, and supporting clinical outcomes. Articles addressing the design, performance, safety profiles, and limitations of AI tools were prioritized. Challenges, such as algorithmic bias, data privacy, security, and the need for human oversight, were systematically analyzed to ensure a balanced evaluation of AI's applications. A Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis was conducted for each domain of clinical laboratory medicine, infectious disease, and pharmacovigilance to provide a structured assessment of AI's capabilities, limitations, and areas for improvement. This analytical approach enabled a nuanced understanding of current practices and identified gaps that warrant future research and development. It provided foundational insights to guide ethical and context-appropriate implementation while addressing

potential vulnerabilities in its adoption across the studied clinical domains.

3. AI in Clinical Laboratory Medicine

Integrating artificial intelligence into clinical laboratory medicine may offer advancements in interpreting complex test results, enabling faster, more accurate, and actionable insights that could support timely clinical decision-making (Hou et al., 2024; Xie et al., 2024). The primary technique for increased diagnostic precision and efficiency has been the automation of workflows, thereby reducing turnaround times while improving quality and staff efficiency and providing advanced clinical decision support tools based on complex data patterns (Albahra et al., 2023). Automated identification of pathogens directly from biological specimens to clinical isolates, application of advanced analysis techniques, and enhanced clinical interpretation of casuistry to predict patient results are the key areas of development (Undru et al., 2022; Yang et al., 2022). Various applications of AI in laboratory medicine have been documented in the literature, including instrument automation, error detection, prediction of laboratory test values, result interpretation, optimizing laboratory test utilization, and enhancing laboratory information systems (Haymond & McCudden, 2021; Rabbani et al., 2022).

Recent studies have demonstrated the feasibility of employing text mining and artificial intelligence techniques to extract data from electronic clinical laboratory systems in microbiology. These AI classifiers, which are relatively cost-effective, have been increasingly applied to characterize infectious diseases and identify their causative agents based on laboratory test results (Chaturvedi et al., 2024; Mohseni & Ghorbani, 2024).

Major AI integration challenges in clinical laboratories are associated with a lack of robust data management systems, trained personnel, and insufficient financial and human resources (Xie et al., 2024; Yang et al., 2022). Cadamuro et al. (2023) evaluated ChatGPT's ability to interpret laboratory test results, finding that while it could detect deviations and provide general insights, it lacked the depth and accuracy needed for comprehensive clinical assessments, highlighting the importance of further validation and refinement in medical AI applications (Rabbani et al., 2022). These challenges and other ethical issues are fundamental to any AI-based clinical laboratory service on a global scale (Pennestrì & Banfi, 2022). As AI integration into clinical laboratory medicine advances, it may improve patient outcomes and elevate expectations regarding diagnostic precision and system performance (Imad-Addin, 2024).

Figure 3 presents a SWOT analysis highlighting the strengths, weaknesses, opportunities, and threats associated with implementing AI in clinical laboratory medicine.

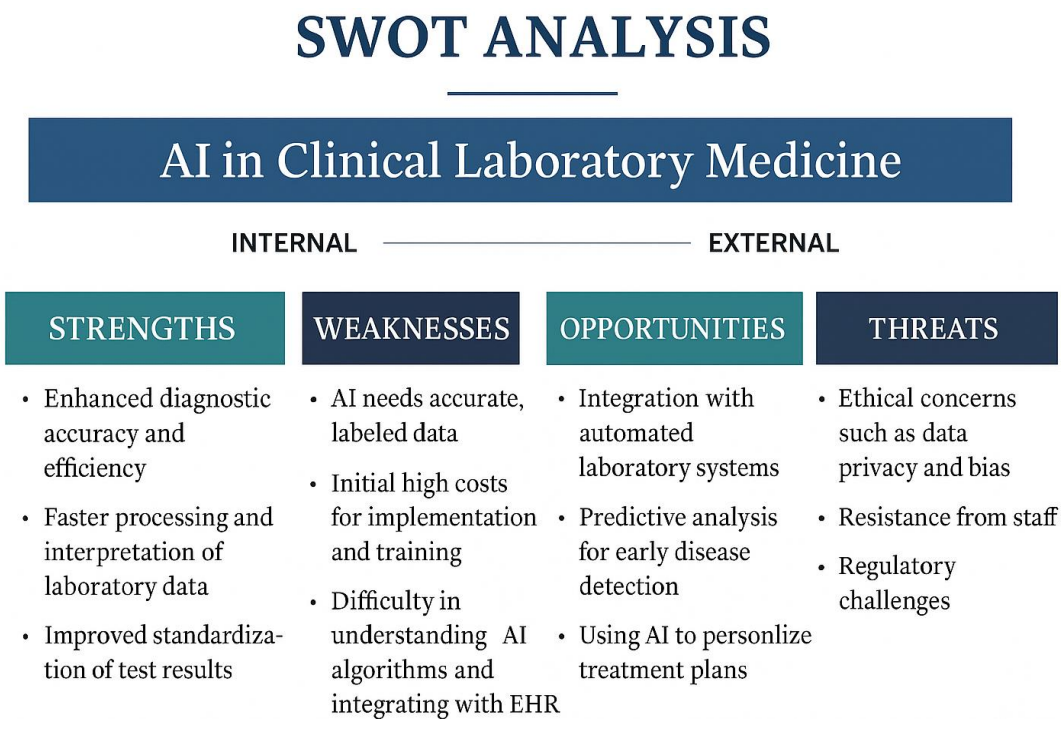

Figure 3. SWOT Analysis of AI in Clinical Laboratory Medicine

4. AI Applications in Infectious Diseases Diagnosis and Management

Despite the recent scientific and technological progress, infectious illnesses still present a notable risk to public health (Rai et al., 2024). These diseases carry the potential of spreading rapidly and causing serious health complications. Furthermore, they manifest as epidemics and impact whole populations (Piret & Boivin, 2021). The challenges of promptly and accurately diagnosing these illnesses and the rising resistance to antimicrobial treatments add complexity to their management (Timbrook et al., 2023).

AI has demonstrated potential in supporting the diagnosis and management of infectious diseases through enhancements in antimicrobial drug discovery, better understanding of infection biology, and refinement of diagnostic tools (Wong et al., 2023), more accurately characterizing patients who present with acute infections, in effectively tracking outbreaks and emerging infectious diseases, and in tailoring antimicrobial therapies to patients and their specific infections (Aslan, 2024). There are many ways in which the infection community is researching methods to improve infectious disease diagnosis, management, and caregiver support (Radaelli et al., 2024). AI enhancements are likely to affect how infection occurs and develops, and it is unlikely that AI itself is directly involved in basic research (Rabaan et al., 2023). AI is used to diagnose and manage infection by analyzing and characterizing clinical and other data associated with patients and their environments—what infections look like and how they progress (Aslan, 2024). According to the literature reviewed, AI-driven data analysis may contribute to more rapid and precise generation of isolation strategies, ICU bed allocation, and antibiotic prescription potentially offering improvements over current diagnostic methods, especially in managing hospital-acquired infections and antimicrobial resistance (AMR) (Ali & Muhammad, 2023; Rabaan et al., 2022), and other outbreak or emerging pathogen scenarios by preventing ongoing disease acquisition. However, more clinical evidence and societal consensus are required to use these cutting-edge tools in infection risk prediction and management tasks (Santangelo et al., 2023). The study by Fatima et al. (2023) proposes a multidisciplinary AI-driven approach to advance precision medicine in infectious diseases, integrating genomics, proteomics, and clinical data to personalize treatment strategies while addressing data privacy and regulatory frameworks. Cheng et al. (2023) explored the rapid adoption of ChatGPT as a tool in infectious disease, emphasizing its potential for clinical practice and research while addressing its societal and ethical implications through an online survey evaluation.

Real-time population infection monitoring will be increasingly driven by data and machine learning in the coming months and years, with the urgent need to track emerging forms of the virus accelerating this transition (Elste et al., 2024). Applying AI and machine learning to environmental data could predict the scope and behavior of emerging infectious diseases months to years before such outbreaks occur (Santangelo et al., 2023). So-called “deep mining” of infectious disease data, combined with the predictive power of AI, opens pathways to preemptive infection prevention (Shausan et al., 2023). Given the resource limitations, if AI rapidly evolves into a credible nosocomial infection prevention tool, it may have the most substantial and demonstrable effect, especially in low- to middle-income settings (Huang et al., 2023). AI is increasingly being explored for its potential to support the management of routine clinical chemistry and microbiology test data, which can be used to develop, validate, and refine machine learning algorithms for diagnosing infections (Kandilci et al., 2024; Sallam, Al-Salahat, et al., 2023). Machine learning is being used to design investigational drugs and vaccines and find existing ones to reposition for tackling the pandemic (Wang et al., 2024). AI-driven systems are currently operational in infectious disease diagnosis and management. However, ethical concerns about patient privacy, data governance, informed consent, and cybersecurity remain critical when deploying AI in infectious disease settings (Isiaka et al., 2024). Healthcare professionals play a major role in fostering patient trust by addressing ethical, privacy, and data security concerns.

4.1 AI-Based Pathogen Detection

AI applications in infectious disease management have recently gained considerable attention (Wong et al., 2023). A key use of AI in infectious disease involves pathogen detection, which aims to identify and subsequently classify pathogens in a clinical sample usually directly isolated from the site of replication (Badidi, 2023; Wang et al., 2024). With increasing demands for bio-surveillance and biosecurity, there has been a steep increase in publications exploring innovative AI methods for pathogen surveillance (Huang et al., 2023; Olaboye et al., 2024; Shausan et al., 2023). Typically, in AI-integrated pathogen detection, some advanced methodologies are applied to ensure higher-dimensional information on the potential threats is collected and subsequently analyzed to ensure high accuracy and that single or multiple pathogen identifications are fast and versatile (Khan et al., 2024). Currently, machine learning models conduct most of the work presented in published research to utilize AI for pathogen surveillance (Keshavamurthy et al., 2022). While many different strategies are used to interpret and present the results to indicate a pathogen detected based on predictive analysis using machine learning, most reports present either image recognition or real-time Polymerase Chain Reaction (PCR) data modeled using a novel type of machine learning techniques (Zhao et al., 2020).

Early pathogen detection remains critical for outbreak containment worldwide (Shausan et al., 2023). Examples include the outbreak of severe acute respiratory coronavirus that was transmitted to humans from animals in China and Ebola in West Africa. AI may support timely pathogen identification and assist in delivering accurate, cost-effective diagnostic tools to enhance public health measures. Moreover, AI in infectious diseases may be utilized to predict outbreaks of emerging and zoonotic infections by facilitating the development of models that forecast future outbreaks, provide insights into new infectious threats (Guo et al., 2023; Isiaka et al., 2024), where accurately forecasting emerging infections is vital for enabling proactive, timely disease prevention strategies with global health and economic implications (Rai et al., 2024; Shausan et al., 2023). However, it may be challenging to act proactively owing to the wide variability in the features determining whether a given pathogen can survive in a new host. Consequently, the translation of animal-to-human and human-to-human pathogens can often be overlooked until the first indications become evident (Rahman et al., 2020).

In parallel to these forecasting applications, developing sensitive, rapid, and portable pathogen diagnostics can improve rather than replace pathology's front line, healthcare services, and clinical pharmacologists in diagnosis and treatment-related activities (Montastruc et al., 2023). However, technical and regulatory challenges are associated with developing and deploying AI-based pathogen diagnostics (Archana & Rohan, 2021; Zhao et al., 2020).

Figure 4 outlines the strengths, weaknesses, opportunities, and threats associated with the application of AI in infectious disease management.

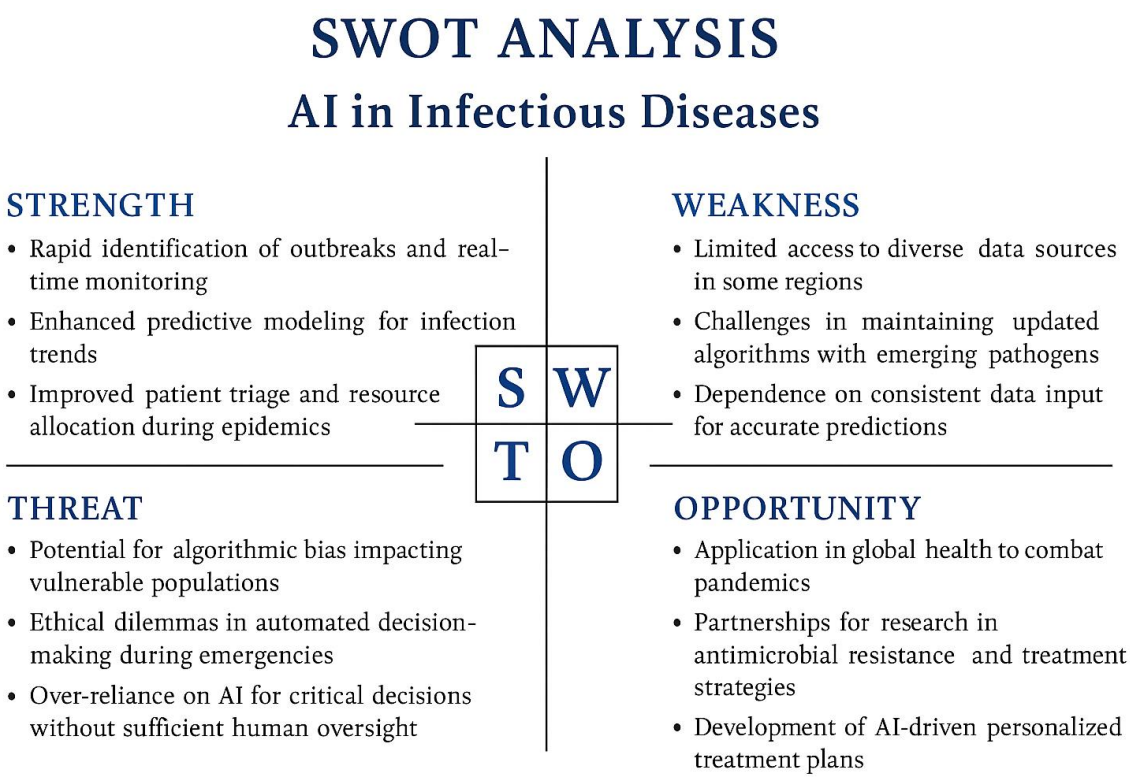

Figure 4. SWOT Analysis of AI in Infectious Diseases

5. AI in Pharmacovigilance

Pharmacovigilance represents a key component of pharmacy practice, encompassing the continuous monitoring and assessment of drug safety through systematic activities such as adverse drug reactions (ADRs) surveillance, risk assessment, post-market monitoring, and interdisciplinary collaboration with healthcare professionals to optimize patient care and prevent medication-related harm (Budha et al., 2024; Chalasani et al., 2023). Over the past sixty years, it has evolved from a voluntary, informal reporting system into a highly regulated and comprehensive framework for detecting, evaluating, and managing medication-related adverse effects (Lavertu et al., 2021). In recent years, implementing automated signal detection methods has accelerated the integration of artificial intelligence into pharmacovigilance processes (Chavhan & Uplenchwar, 2024). AI-driven systems now leverage real-world and big data from heterogeneous sources such as electronic health records and insurance claims databases to detect safety signals using diverse analytical approaches (Chen et al., 2021; Han, 2022).

These tools support associations between ADRs and medications, enable real-time surveillance of ADR trends, and contribute to the early identification of underreported drug-drug interactions (DDIs) (Hauben, 2023; Salas et al., 2022). Advanced mechanistic and data-driven techniques, including machine learning, support the detection of complex safety issues and improve pharmacovigilance workflows (Ahire et al., 2024; Alexander, 2023; Shamim et al., 2024). Several studies have demonstrated increased signal detection efficiency and accuracy with AI integration, although the broader adoption of advanced methods such as deep learning remains infrequent (Ahire et al., 2024; Kompa et al., 2022; Mockute et al., 2019).

To address current methodological limitations, integrated or federated data-sharing strategies have been proposed, enhancing AI-driven pharmacovigilance capabilities (Prasanth et al., 2024). A shared emphasis on structured and standardized quality assurance frameworks has emerged among patient safety experts, industry stakeholders, and regulators (Ahmad & Wasim, 2023; Mockute et al., 2019). Baurasien et al. (2023) further illustrated how AI may reduce medical errors and enhance patient safety by improving diagnostic accuracy, medication management, and real-time monitoring while navigating challenges related to data privacy, algorithm transparency, and system integration. While early implementations are promising, AI remains a developing field that may help address capacity constraints in pharmacovigilance systems across healthcare settings (Amponsah & Pathak, 2022). Kompa et al. (2022) concluded that despite its potential, widespread adoption of sophisticated AI tools in pharmacovigilance still requires further validation and infrastructure development.

Figure 5 provides a SWOT analysis summarizing the strengths, weaknesses, opportunities, and threats associated with implementing AI in pharmacovigilance.

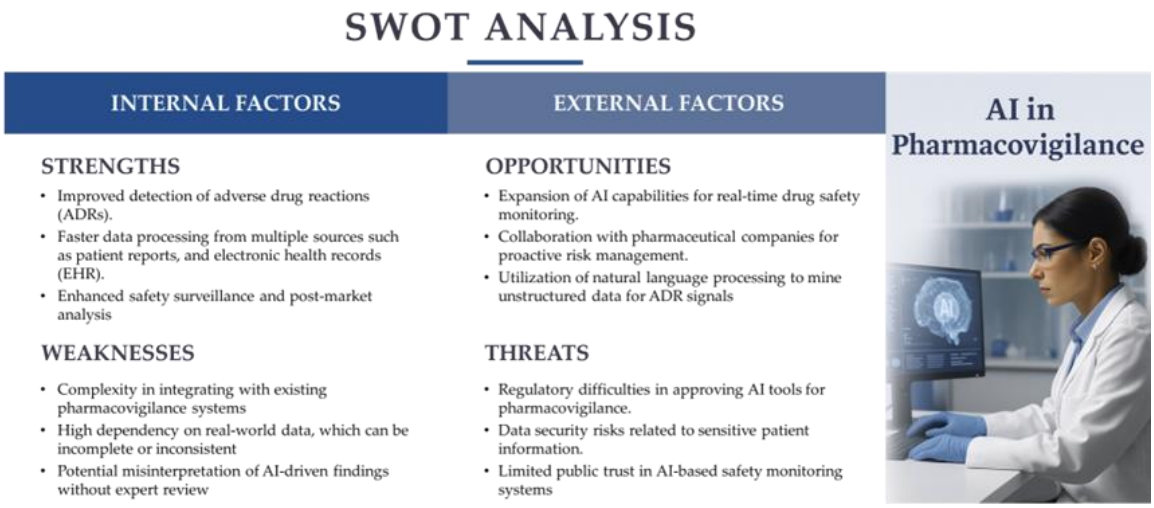

Figure 5. SWOT Analysis of AI in Pharmacovigilance

6. Discussion

6.1 Implications for Practice

The practical implications of artificial intelligence in clinical medicine are increasingly recognized as relevant (Krishnan et al., 2023). Globally, AI may support deeper insights into addressing key challenges, such as advancing disease management through improved diagnosis, treatment, and patient monitoring (Nordlinger et al., 2020). Systematic reviews and current discussions indicate that AI tools like ChatGPT in healthcare are expected to expand in use, with more targeted research appearing across various medical specialties (Bazzari & Bazzari, 2024; Egger et al., 2024). AI has the potential to assist healthcare providers in multiple ways, encompassing patient care and administrative duties (Salomon & Sibomana, 2024; Saxena et al., 2024). Socio-economic and cultural dynamics play a critical role in shaping the integration and acceptance of AI in healthcare, featuring the need for tailored adaptation strategies to ensure successful implementation and patient-centered care (Abubakr et al., 2024). Busnatu et al. (2022) reviewed the growing integration of artificial intelligence in clinical applications, highlighting advancements across various medical specialties while addressing challenges like data limitations and the need for robust methodologies in clinical practice. Aljerian et al. (2022) examined the growing role of artificial intelligence in Saudi Arabia's healthcare system, highlighting its applications in diagnostics, personalized health planning, and pandemic response, while addressing challenges such as resource limitations and the need for adequate training. Wang (2020) reviewed the application

and future potential of artificial intelligence in clinical medicine, highlighting advancements in medical diagnostics, drug development, and medical robotics while emphasizing AI's ability to improve diagnostic accuracy, enhance efficiency, and address healthcare resource shortages. Elhaddad and Hamam (2024) reviewed the integration of artificial intelligence into Clinical Decision Support Systems (CDSS), highlighting its transformative impact on healthcare decision-making, personalized treatment, and efficiency while addressing challenges like usability, bias, and ethical considerations to fully realize AI-CDSS potential.

6.2 SWOT Analysis

The comprehensive SWOT analysis of AI in clinical laboratory medicine, infectious diseases, and pharmacovigilance revealed detailed insights into its application in these areas. Across these domains, strengths included enhanced diagnostic precision, faster data processing, improved predictive modeling, real-time monitoring, and advanced safety surveillance. AI supported more efficient test standardization, rapid outbreak detection, patient triage during epidemics, and better detection of adverse drug reactions (ADRs). Despite these advantages, notable weaknesses existed, such as the need for accurate, labeled data, high initial costs for implementation and training, integration challenges with electronic health records (EHR), and dependence on consistent, high-quality data input. AI algorithms also faced difficulties adapting to emerging pathogens and maintaining up-to-date models, which complicated integration with existing systems. Opportunities included global health applications for pandemic responses, partnerships for antimicrobial resistance research (Giske et al., 2024; Shelke et al., 2023), personalized treatment plan development (El_Jerjawi et al., 2024), and expanding real-time drug safety monitoring (Desai, 2024).

Natural language processing (NLP) also showed substantial promise for analyzing unstructured data. However, threats such as algorithmic bias affecting vulnerable groups, ethical concerns in automated decision-making, reliance on AI without adequate human oversight, regulatory hurdles, data security risks, and limited public trust present significant barriers to safe and effective implementation. Continued research, refinement, and mindful implementation were essential to addressing these issues and maximizing AI's benefits in clinical practice (Egger et al., 2024).

6.3 Challenges and Future Directions

While AI in clinical medicine shows considerable promise, clinical impact, utilization, and the development of policies and regulatory frameworks to support the integration of AI in clinical practice are still in the early stages (Srivastava, 2023). The barriers to their translation into practice remain considerable. Several technical and operational issues require attention before these tools can reliably inform the care of individual patients, especially when applied to complex patients (Schwartz et al., 2023; Stevenson et al., 2023). The AI tools are only as accurate as the data used to train them (Choudhury et al., 2024). Natural language processing tools may require the expertise of a large community of researchers in computer science, linguistic science, and subject-matter experts to develop tools capable of dealing with the breadth and depth of the language used to report studies. New machine-learning tools need to be developed to generate data to help assess the variability in medical devices. Other limitations to AI tools may be difficulty integrating them across electronic health record platforms. Training and education about AI across all levels of healthcare workers are needed to create a workforce for the future for quality improvement and healthcare transformation (Sorte et al., 2024). Resistance to change and hesitancy remain notable barriers in adopting new health system technologies and must be addressed (Goldman & Patel, 2024). Incorporating clinical practice guidelines (CPGs) into large language models (LLMs) to enhance clinical decision support (CDS) through the use of collective knowledge is important to identify safety signals at an early stage (Oniani et al., 2024). Another relevant consideration is a potential algorithmic bias possibly leading to a chain reaction amplifying the effect when the algorithms are used to inform decisions, in this case, care, and a multitude of research, clinical practice, and policy questions. The development of AI that can learn continuously is an exciting scenario that challenges policy, regulators, and businesses to adapt, align interests, and engage in robust cooperation. Some studies in many clinical health domains provide a rationale for use in symptom tracking, response predictions, and treatment planning (Khalifa & Albadawy, 2024). The application of AI in clinical medicine holds great promise, but the translation into clinical practice and research poses a panoply of challenges (Sharma et al., 2022). Continuous research, ethical policy development, collaboration, sponsorship, and engagement across diverse disciplines are encouraged (Huo et al., 2023; Mashabab et al., 2024). Also, it is crucial to adopt standardized practices in the reporting, implementation, and testing of the quality of health information generated by AI-based models in clinical medicine (Sallam, Barakat, et al., 2023), reinforcing the importance of reliable methodologies and consistent reporting (Sallam et al., 2024b).

6.4 Ethical Considerations in AI Implementation

Protecting patient privacy is a primary consideration when implementing AI in clinical medicine (World Health Organization, 2021), especially in laboratory medicine, where large data sets are increasingly used for diagnostic

and prognostic purposes. Such information can provide substantial insight into community health, but often without the oversight of the clinician. In implementing AI for patient data sharing, care must be taken to secure the shared data. A second consideration is perpetuating bias, in that if an AI system predicts a healthcare outcome in a biased manner, this behavior is technically accurate but unethical. Azimi and Zaydman (2023) emphasized the importance of fairness in machine learning (ML) algorithms in laboratory medicine, addressing how biases can arise during model development and emphasizing strategies to promote equity in healthcare outcomes. Habib and Gross (2023) highlighted significant gaps in the regulatory framework for AI and ML-based clinical decision support devices, noting insufficient evidence for safety, performance bias, and effectiveness, as most FDA-authorized devices rely on older, non-AI-specific predicates. An AI system should demonstrate accuracy and transparency; however, conveying the inner workings of machine learning algorithms to human experts, particularly those trained on clinical data, remains a significant challenge. Continuing, the implementation of AI in a healthcare setting must also be a transparent system to help facilitate trust in the AI system between both the practitioner team and the patient. Additionally, with healthcare professionals increasingly treating patients from various cultural backgrounds, the rise of AI in clinical medicine highlights the importance of culturally sensitive and compassionate care (Alberto et al., 2024).

Computational technologies present a broad spectrum of opportunities in the health sector, with the potential to support more equitable and efficient resource allocation. However, to achieve these benefits, it is essential to critically evaluate the potential and limitations of technological solutions and the ethical and normative considerations currently understudied (Fatima et al., 2024; Marshall Raj et al., 2024). With harmonized and coordinated global governance, we can observe policies and regulations that focus specifically on the data, technological formulation, research techniques, and ethics for responsible application and use in a real-world setting (Marques et al., 2024). Health leaders globally are encouraged to prioritize strategies such as enhancing data quality, infrastructure, and privacy safeguards to facilitate the responsible integration of AI in healthcare (Silcox et al., 2024).

7. Conclusion

Introducing artificial intelligence into clinical medicine has the potential to improve the accuracy of diagnoses, treatment planning, and patient safety across various medical disciplines. This study examined AI's reported applications and implications in three key domains of clinical medicine, including clinical laboratory medicine, infectious disease management, and pharmacovigilance. According to the literature reviewed, the SWOT analysis suggested that AI tools in these domains may contribute to faster and more precise diagnostic workflows, support more efficient clinical processes, and enable improved utilization of resources, with possible implications for enhancing patient care. Nevertheless, concerns remain regarding data privacy, algorithmic bias, transparency, and the ongoing need for human oversight.

Realizing AI's full potential in clinical practice depends on ethical implementation, sustained research, and robust regulatory frameworks. Addressing these challenges is essential to support responsible adoption and preserve patient trust and safety. Future efforts should emphasize multidisciplinary collaboration among policymakers, clinicians, technologists, and patients to enable balanced and evidence-informed integration of AI in healthcare.

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Conflicts of Interest

The authors declare no conflicts of interest.

Institutional Review Board

Not required.

Declaration of Artificial Intelligence Use

This study utilized AI tools to enhance manuscript writing, grammar, graphics, and readability. AI-assisted in summarizing findings and technical writing, offering structures for complex descriptions.

All ideas, interpretations, and ultimate conclusions presented in this article are solely those of the authors.

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Abbreviations

AI: Artificial Intelligence

ML: Machine Learning

NLP: Natural Language Processing

LLMs: Large Language Models

ChatGPT: A Chatbot Based on Generative Pre-Trained Transformer Large Language Model

ANN: Artificial Neural Networks

DL: Deep Learning

EHR: Electronic Health Record

US FDA: United States Food and Drug Administration

ADRs: Adverse Drug Reactions

DDIs: Drug-Drug Interactions

SWOT: Strengths, Weaknesses, Opportunities, And Threats

CDS: Clinical Decision Support

CDSS: Clinical Decision Support Systems

CPGs: Clinical Practice Guidelines

AMR: Antimicrobial Resistance

PCR: Polymerase Chain Reaction

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Histopathological and Biochemical Evaluation of β-Sitosterol from Lawsonia Inermis in Aspartame-Induced Testicular Toxicity in Wistar Rats

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Abstract

Introduction: This study aims to evaluate the modulatory impact of β-sitosterol isolated from *lawsonia inermis* on Aspartame mediated testicular toxicity in Wistar rats. ***Settings and Design:*** Forty-five male Wistar rats were used for the study. The rats were divided into nine groups; each containing five rats and were treated daily for 30 days. Group 1 received 5mls of distilled water orally, group 2 received 40mg/kg of aspartame (ASP) for 20 days, while group 3 received 160mg/kg of ASP for 20 days, and group 4 was given 60mg/kg of β-sitosterol (β-sit.) for 20 days, while group 5 received 100mg/kg of β-sit. for 20 days. Group 6 received 40mg/kg of ASP for 15days followed by 60mg/kg β-sit. for the next 15 days, while group 7 had 160mg/kg ASP for 15 days, then 100mg/kg of β-sit for 15 days. Group 8 was given 60mg/kg β-sit. for 15 days, preceding 40mg/kg ASP for 15 days while group 9 received 100mg/kg β-sit. for 15 days, then 160mg/kg ASP for 15 days. ***Results:*** At the end of the experiment it was observed that there was less weight gained in group 2, 3, 6 and 8 while group 5 and 7 gained weight significantly. Histological studies using H&E showed cellular necrosis within the seminiferous tubules (ST) as well as marked edema in the intertubular spaces in groups 2 and 3 while group 4 appeared fairly normal, groups 5 and 6 showed the lumen with very few spermatozoa present. Groups 7, 8, and 9 had normal seminiferous tubules with mild edema levels in the intertubular spaces. These observations were corroborated by PAS staining technique which further revealed extensive erosion of the basement membrane and loss of connective tissue and Leydig cells in groups 2 and 3 including cellular distortion, groups 5, 6, 8, and 9 showed mild to moderate levels of edema in the intertubular spaces. An assay of GPX showed increased levels mostly in group 3 ($p<0.05$). MDA was also significantly higher in groups 2, 3 and 6 ($p<0.05$). Catalase activity decreased significantly in groups 2, 3, and 5 ($p<0.05$) with improvements in groups 6, 7, and 8. ***Conclusion:*** It was observed from the results that aspartame can induce a series of deleterious oxidative changes in the testes resulting in possible impaired testicular function, however, β-sitosterol isolated from *lawsonia inermis* may modulate the effects of aspartame when administered 15 days sequel to an initial 15 days exposure to aspartame.

Keywords: aspartame, β-sitosterol

1. Introduction

Infertility is a significant reproductive health challenge with long-term economic, social, and psychological impacts, particularly in African societies where childbirth rates are high. It is defined as the failure of a couple to achieve pregnancy after one year of regular, unprotected sexual intercourse (Leslie et al., 2020). Globally, an estimated 8–12% of couples of reproductive age experience infertility, with male factors contributing to approximately 40–50% of cases (Agarwal et al., 2021; Pandruvada et al., 2021). Historically, infertility research has focused predominantly on female reproductive health, leading to a relative lack of extensive studies on male

infertility (Petok, 2015). Societal norms and cultural beliefs have also contributed to the underestimation and underdiagnosis of male infertility.

Male infertility is often attributed to defects in spermatogenesis, with oxidative stress playing a crucial role. Approximately 30–80% of infertile men exhibit sperm damage due to reactive oxygen species (ROS) (Bui et al., 2018). Other contributing factors include environmental toxins, radiation exposure, electromagnetic waves, and various chemical substances, many of which are commonly found in processed foods, pharmaceuticals, and daily consumables (WHO, 2013).

One such chemical of concern is aspartame, an artificial sweetener widely used in food and beverages, including diet sodas, chewing gum, dairy products, breakfast cereals, and pharmaceutical formulations (EFSA, 2013). Aspartame is metabolized into phenylalanine, aspartic acid, and methanol, the latter of which is further converted into formaldehyde and subsequently into superoxide anions and hydrogen peroxide—compounds known to induce oxidative stress (EFSA, 2013). Chronic exposure to oxidative stress has been implicated in male infertility through mechanisms such as lipid peroxidation of sperm membranes, DNA fragmentation, and mitochondrial dysfunction (Anbara et al., 2020).

Several studies have linked aspartame consumption to metabolic and reproductive disorders. It has been associated with obesity, insulin resistance, and type II diabetes, conditions that indirectly impact male fertility by altering endocrine homeostasis (Almiron-Roig & Drewnowski, 2003; Sørensen et al., 2005). Furthermore, aspartame has been implicated in preterm deliveries and allergic conditions in neonates (Halldorsson et al., 2010; Maslova et al., 2013). Animal studies suggest that early exposure to aspartame may lead to metabolic dysfunction and impaired glucose tolerance in male offspring (DiPietro & Voegtline, 2017). Additionally, formaldehyde, a metabolic byproduct of aspartame, has been reported to cause DNA damage, potentially leading to genotoxicity and increased susceptibility to neurodegenerative diseases such as Parkinson's and Alzheimer's (Pontel et al., 2015; Villareal et al., 2016).

Given these concerns, there is growing interest in natural bioactive compounds with antioxidant properties that may counteract the detrimental effects of oxidative stress on male reproductive health. One such compound is β-sitosterol, a plant sterol structurally similar to cholesterol, which is abundant in rice bran, wheat germ, peanuts, corn oil, and soybeans (Rakel, 2018). β-sitosterol is known for its anti-inflammatory, anti-cancer, and analgesic properties and has been extensively studied for its role in managing benign prostatic hyperplasia (Lomenic et al., 2015). It is hypothesized that β-sitosterol may also exert protective effects on male fertility by mitigating oxidative stress.

A notable natural source of β-sitosterol is Lawsonia inermis, commonly known as henna. This subtropical and tropical plant has been widely studied for its rich content of bioactive phytochemicals with potential therapeutic applications (Yusuf, 2016). Preliminary evidence suggests that Lawsonia inermis may possess antioxidative properties that could counteract ROS-induced damage in male reproductive tissues. However, further research is necessary to elucidate its specific mechanisms of action and potential clinical applications in male infertility management.

In this study, we evaluated the effect of **β-sitosterol** on aspartame-induced toxicity in male Wistar rats.

2. Materials and Method

2.1 Chemical

Aspartame powder containing 98% aspartame, manufactured by Globexia Limited was procured from World Corsica Science Limited, Benue Crescent, Makurdi.

2.2 Lawsonia Inermis Procurement and Extraction Procedure

Fresh leaves of *Lawsonia inermis* were procured from Wadata market, Makurdi. The plant was authenticated in the Department of Botany, Benue State University by the chief taxonomist. With voucher number: BS1724.

The leaves were thoroughly washed and cut into smaller pieces, then allowed to dry at room temperature of 25^{o}C for Fourteen days. Afterward, it was ground into a fine powdered form.

The extraction of plant material was carried out using the method described by Aziz et al. (2018). Briefly: Extraction of terpene Shade-dried pulverized plant material (100 g) from plant leaves was extracted by Soxhlet apparatus with hexane (700 ml), the extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator. The hexane extract was analyzed for the presence of terpene using thin-layer chromatography (TLC) with spray reagent and confirmed by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-MS analysis. Isolation of sitosterol by preparative TLC Preparation of stationary phase Ready-made silica gel GF 254 plates with a layer thickness of 0.25 mm, dimension 20 cm×20 cm. The plates were reactivated by heating in the oven at 100°C for 15 min, left to cool, and used for application after allocation of the baseline and the solvent front. Preparation of solvent system Mobile phase for sterols:

(chloroform: acetone) was mixed in a conical flask and introduced in the jar. The jar was lined with filter paper, closed tightly, and left for saturation.

Application of sample: About 2 g of the sample was dissolved in absolute methanol and applied on the baseline of TLC plates Detection of separated spots. Detection was done by the spraying side of plate with vanillin-sulfuric acid reagent. The purity of each band was checked by analytical TLC until single spot on TLC plate is obtained for identification with a reference standard.

2.3 Experimental Animals

Fifty-four (54) adults male Wistar rats weighing between 150-180g were procured from the animal house of the College of Health Sciences, Benue State University, Makurdi. The animals were kept and maintained in the research laboratory at the Animal House of the College of Health Sciences, Benue State University, for the duration of the experiment. Before the commencement of the experiment, the animals were then housed in polypropylene cages and kept in a well-ventilated room where they were acclimatized over two weeks. During this period the animals were subjected to standard room atmospheric temperature (25±5˚C) and 12/12-hour light-dark cycle. They were also adequately fed with standard rat chow and allowed access to water *ad libitum*.

2.4 Animal Grouping and Treatment

The animals were randomly divided into nine (9) groups: I, II, III, IV, V, VI, VII, VIII, and IX, each consisting of five animals. The treatment for each group was as follows:

Group I received 5 ml/kg of normal saline for 30 days. Group II was administered 40 mg/kg of aspartame for 20 days. Group III was given 160 mg/kg of aspartame for 20 days. Group IV received 60 mg/kg of β-sitosterol for 20 days. Group V was treated with 100 mg/kg of β-sitosterol for 20 days. Group VI was administered 40 mg/kg of aspartame for 15 days, followed by 60 mg/kg of β-sitosterol for the next 15 days. Group VII received 160 mg/kg of aspartame for 15 days, followed by 100 mg/kg of β-sitosterol for the next 15 days. Group VIII was given 60 mg/kg of β-sitosterol for 15 days, followed by 40 mg/kg of aspartame for the next 15 days. Group IX received 100 mg/kg of β-sitosterol for 15 days, followed by 160 mg/kg of aspartame for the next 15 days.

2.5 Animal Sacrifice and Sample Collection

The rats were allowed to fast for 12 hours to: 1) synchronize the metabolic state of the animals, 2) Enhance the sensitivity of the biochemical assays, 3) Reduce food-related stress and 4) Ensure the accuracy of tissue sampling weighed before sacrifice then anesthetized by chloroform inhalation. Blood samples were collected from each rat immediately after sacrifice through cardiac puncture. The abdominal cavity was dissected through a midline incision to expose the reproductive organs. The testicular weights of each animal were evaluated with an electronic analytical precision balance, manufactured by ORMA Limited, Japan. The two tests of each rat were measured and the average value was obtained. One of the tests of each animal was fixed in Bouin's fluid for histological and morphometric analysis. The remaining testes of each animal were stored at – 25ºC for subsequent biochemical assays.

2.6 Histological Study

The preparation of tissue for histological study was carried out at the histopathology unit, at the University of Ibadan according to the method described by Slaoui M., & Fiette L. (2011). Testicular tissue was collected and immediately fixed in Bouin's fluid, these were allowed to fix for 48 hours. The tissues were dehydrated in ascending grades of ethanol for 1 hour each beginning with 70%, followed by 90%, and terminating finally in 2 changes of absolute ethanol each lasting for the same period. Following treatment with ethanol, the tissue was cleared in three changes of xylene lasting 15 minutes each. Impregnation with molten paraffin at 60ºC was done overnight before embedding in paraffin blocks. The blocks were trimmed and Sections (5µm) was taken using a semi-automatic Rotary Microtome manufactured by Leica, Germany, at room temperature. The sections were floated in a warm water bath at 28ºC and then collected on glass slides smeared with albumin and air-dried. The slides were stained with hematoxylin and eosin dye for Light microscopic observation.

2.7 PAS (Periodic Acid-Schiff)

The PAS staining technique was done following the protocols described by Mokobi (2022). Briefly, the tissue was deparaffinized then hydrated in water. 0.5% of periodic acid solution was added for oxidation for 5 minutes then rinsed to distilled water. The stained tissue was dehydrated by placing it in Schiff reagent for 15 minutes, then washed with lukewarm tap water for 5 minutes, the sample was counterstained with Mayer's hematoxylin for 1 minute, then washed in tap water for 5 minutes. The sample was dehydrated and mounted with DPX.

2.8 Assay of Glutathione Peroxidase (GPX) Activity

Glutathione Peroxidase activity was measured by the method described by Saalu et al. (2011). The reaction mixture contained 2.0ml of 0.4M Tris-HCL Buffer, PH 7.0, 0.01ml of 10mM sodium azide, 0.2ml of enzyme,

0.2ml of 10mM glutathione and 0.5ml of 0.2mM H_20_2. The contents were incubated at 37^0c for 10 minutes followed by termination of the reaction by addition of 0.4ml 10% (v/v) TCA, centrifuged at 5000rmp for 5 minutes. The absorbance of the product read at 430nm and expressed as nMol/mg protein.

2.9 Estimation of Lipid Peroxidation (Malondialdehyde)

Lipid peroxidation in the supernatant was estimated colorimetrically by the thiobarbituric acid reactive substances (TBARS) method described by Tsikas (2017). Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x 105 M-1 cm-1 and expressed as nmol/mg protein.

2.10 Assay of Catalase (CAT) Activity

This was measured according to the method of Weydert & Cullen (2009). The rate of nm. The activity of the enzyme was expressed as U/mg protein.

3. Results

3.1 Physical Observation: Body Weight Changes

The body weight changes observed across the experimental groups are shown on Figure 1.

Statistical analysis using one-way ANOVA reveals significant variations in initial and final body weights, as well as in body weight differences, among the groups. The control group showed a mean initial body weight of 124.00 ± 19.02 g and a final body weight of 175.00 ± 17.30 g, resulting in a weight gain of 51.00 ± 2.67 g. This represents the baseline body weight changes and serves as a reference for comparison. All treatment groups demonstrated statistically significant differences in initial body weights compared to the control group ($p < 0.05$). For the final body weights, groups 4 – 8 showed a statistically significant increase in final body weight when compared to the control group. For body weight gain (body weight changes), groups 2, 3, 6, 8, and 9 showed a significantly lower body weight gain compared to the control group ($p < 0.05$).

The result in Figure 1 implies that the treatments in Groups 2–9 variably influenced body weight changes, with some groups showing reduced weight gain compared to the control (e.g., Groups 2, 3, 6, and 8), while others exhibited comparable or enhanced growth trends (e.g., Groups 5 and 7). The variations observed may be attributed to differences in treatment effects, metabolic responses, or physiological adaptations.

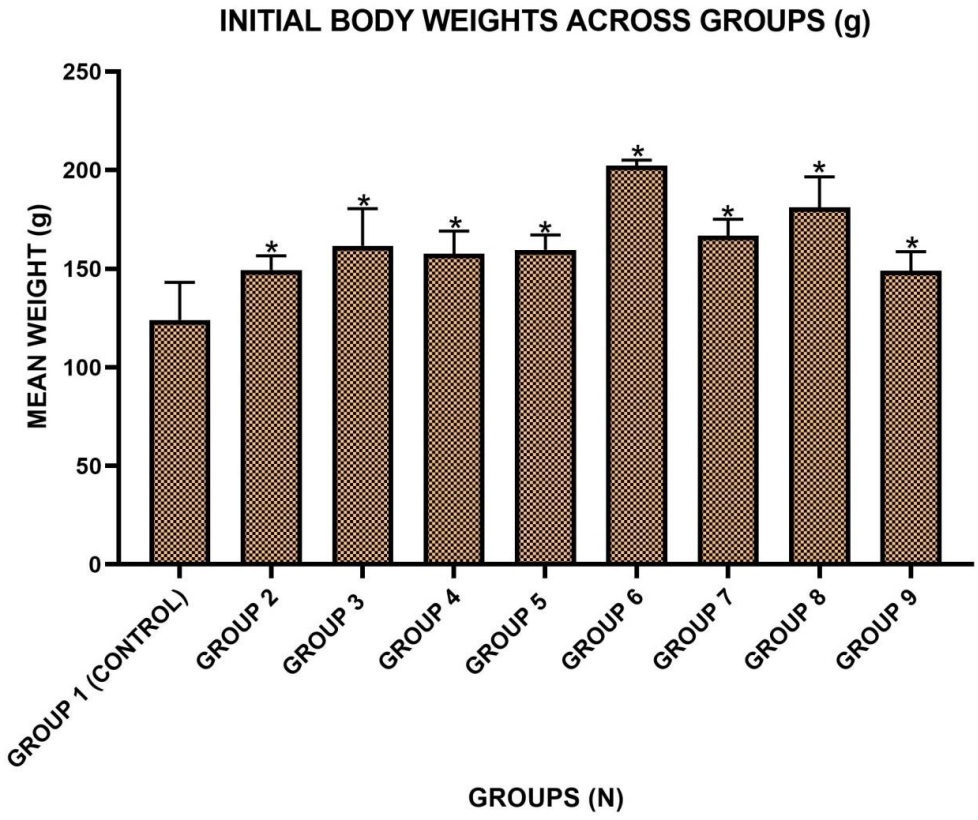


Figure 1.1 Simple Bar Chart Showing the Mean Initial Body Weights across Groups

N = 5, * = statistically significant difference in mean at $p<0.05$ compared to the control group

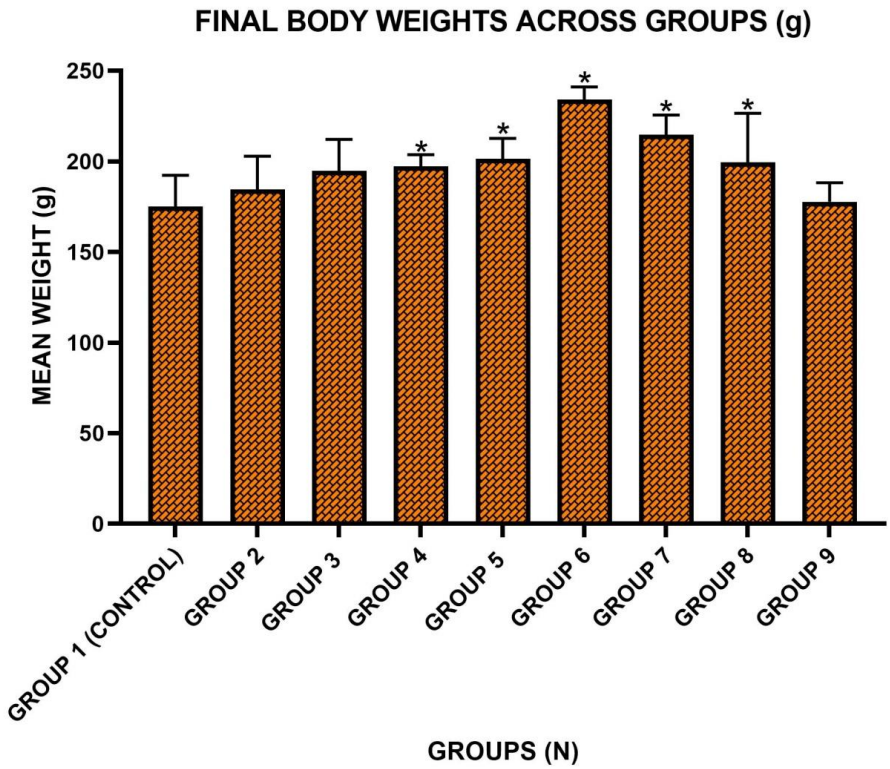


Figure 1.2. Simple Bar Chart Showing the Mean Final Body Weights across Groups

N = 5, * = statistically significant difference in mean at $p<0.05$ compared to the control group

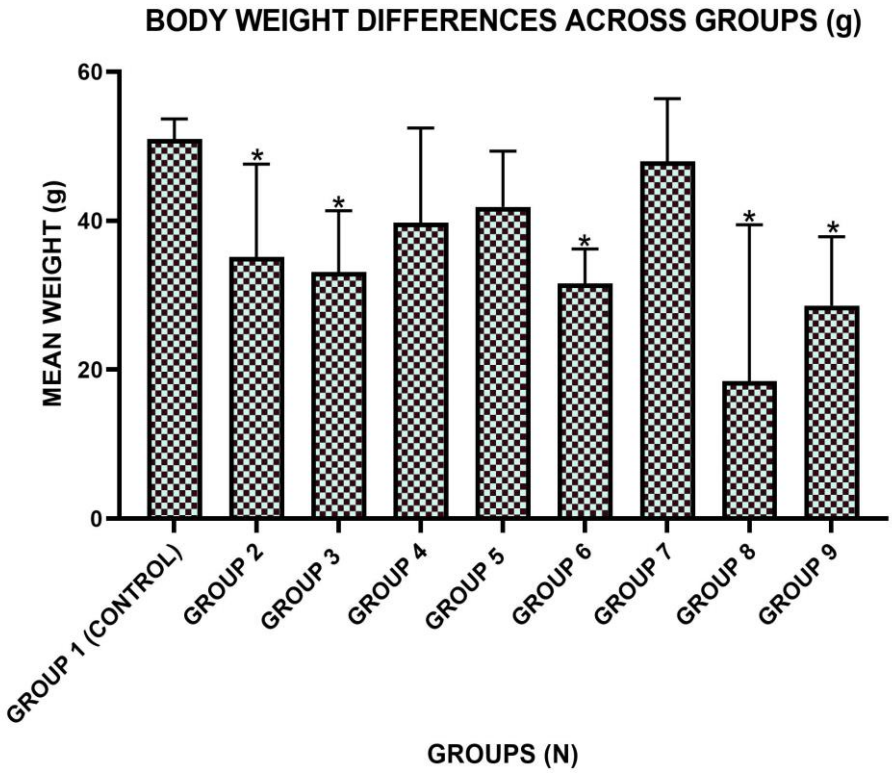


Figure 1.3. Simple Bar Chart Showing the Mean Body Weight Changes across Groups

N = 5, * = statistically significant difference in mean at $p<0.05$ compared to the control group

3.2 Histological Profile

3.2.1 H&E

The photomicrographs of the testis from group 1 (Control group) displayed the normal histological architecture of the testis which showed the seminiferous tubules, lined by spermatogenic cells surrounding a lumen, containing spermatocytes. Inter-tubular spaces containing connective tissues and interstitial cells of Leydig are also present (Plate 1.1). Slides from group 2 showed that there was cellular necrosis in the seminiferous tubules with spermatozoa present within the lumen. A marked level of edema was observed within the inter-tubular spaces and the interstitial connective tissue contained few Leydig cells (Plate 1.2). The slides from group 3 displayed; a high level of cellular necrosis in the seminiferous tubules, the presence of sperm cells within the lumen, there was also edema in the inter-tubular spaces with the interstitial connective tissue containing few Leydig cells (Plate 1.3). Group 4 slides showed normal seminiferous tubules, with a lumen containing spermatocytes and surrounding spermatogenic cells, inter-tubular spaces containing connective tissues, and Leydig cells (Plate 1.4). In groups 5 and 6, the seminiferous tubules were normal with the lumen containing spermatocytes, though there appeared to be a reduction in active spermatogenesis in some tubules. The inter-tubular spaces contained connective tissues and many Leydig cells as well (Plates 1.5 & 6.6). Groups 7, 8, and 9 had normal seminiferous tubules with lumen containing spermatocytes and surrounding spermatogenic cells (Plates 1.7, 1.8 & 1.9). The level of edema in the intertubular spaces was however mild relative to the observations in groups 2, 3, and 6.

3.2.2 P.A.S

Histopathological studies using the PAS staining technique corroborated the H&E results. Group 1 showed normal seminiferous tubules lined by spermatogenic cells enclosing a lumen containing spermatocytes. Inter-tubular spaces containing connective tissues and Leydig cells are also observed (Plate 2.1). In group 2, the seminiferous tubules were normal with spermatogenic cells surrounding the lumen containing spermatocytes; they however appeared shrunken with marked edema, there was extensive erosion of the basement membrane in some tubules with loss of connective tissues and Leydig cells in the inter-tubular spaces (Plate 2.2). The slides from group 3 showed slight tubular distortion, in addition to marked edema, erosion of the basement membrane, and loss of interstitial connective tissues with Leydig cells (Plate 2.3). The results from group 4 were normal while group 5 displayed some level of edema in the inter-tubular spaces with fewer interstitial cells and connective tissue observed (Plates 2.4 & 2.5). Groups 6, 7, 8, and 9 also showed mild to moderate levels of edema in the inter-tubular spaces (Plates 2.6, 2.7, 2.8 & 2.9).

Plate 1.1: A micrograph of the testis from group 1 showing; Normal seminiferous tubules (S) The Lumen (L) containing spermatocytes surrounded by spermatogenic cells (S). Intertubular spaces containing connective tissues and Leydig cells (I). **(H&E) X400**

Plate 1.2: A micrograph of the testis from group 2 showing; cellular necrosis in the Seminiferous Tubules (S), presence of sperm cells within the Lumen (L), Erosion of the basement membrane, Edema in the Intertubular Spaces, interstitial connective tissue contains few Leydig Cells (I). **(H&E)X400**

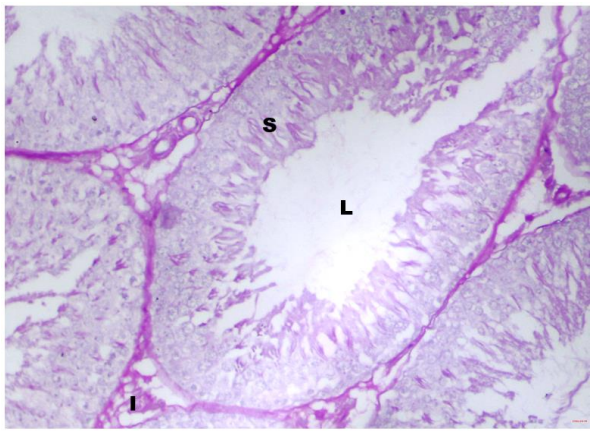
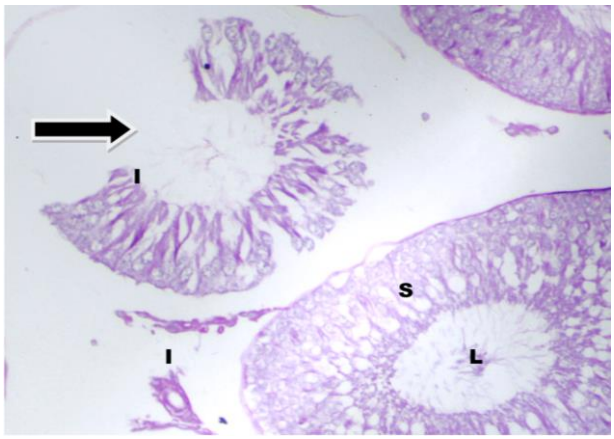


Plate 1.1



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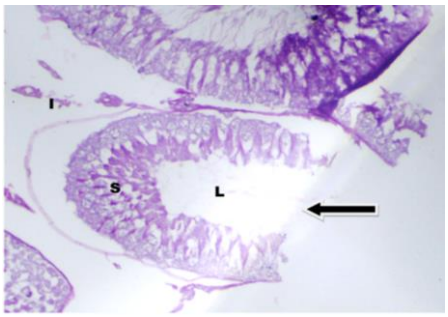

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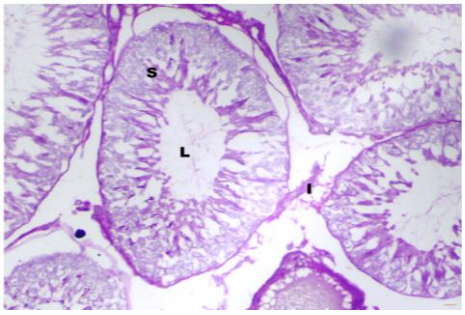

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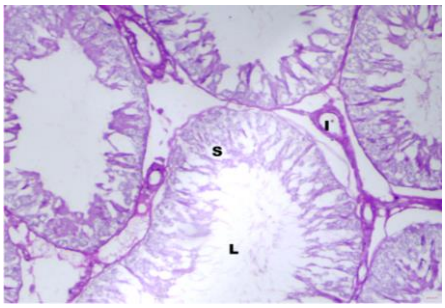

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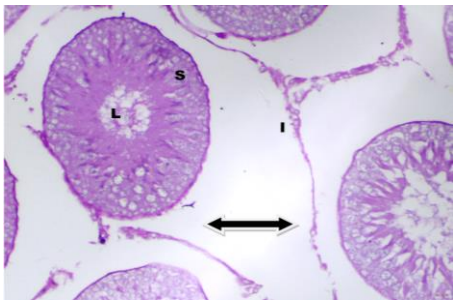

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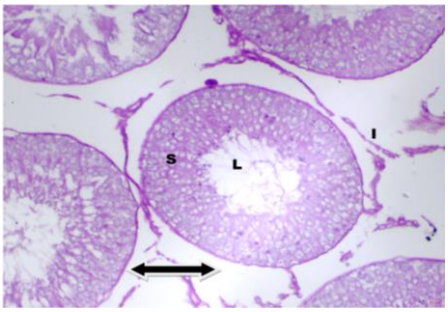

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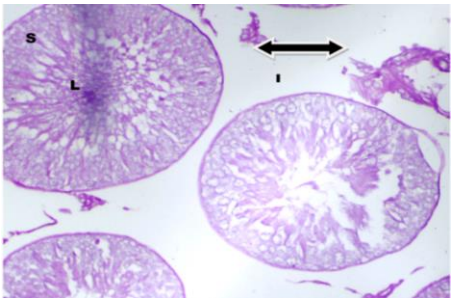

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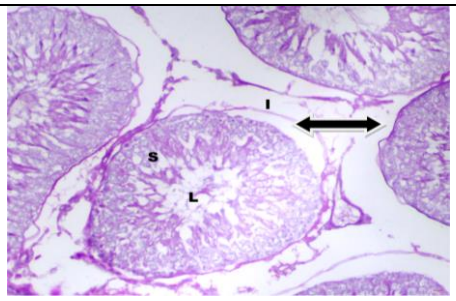
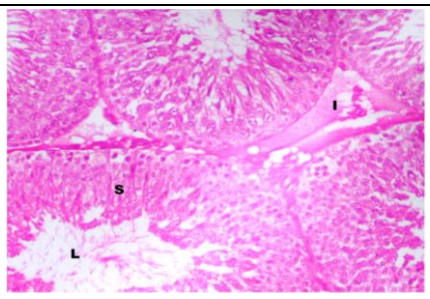


Plate 1.9

The micrographs (Plates 1.1–1.9) show normal seminiferous tubules (S) with spermatocytes and spermatogenic cells in most groups, while groups 2 and 3 exhibit cellular necrosis, basement membrane erosion, intertubular edema, and reduced Leydig cells. Group 5 shows reduced spermatogenesis, and mild to moderate edema is present in groups 6–9. (H&E) X400.



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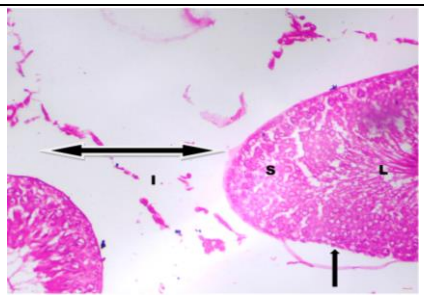

Plate 2.2

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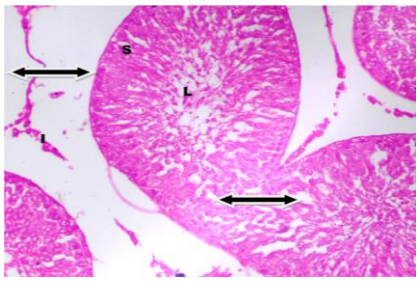
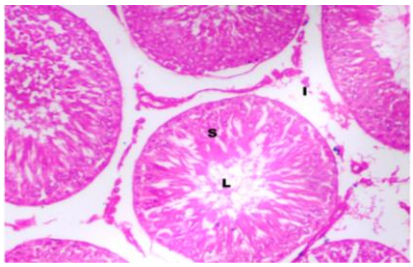
<tr><td> 
Plate 2.3</td><td> 
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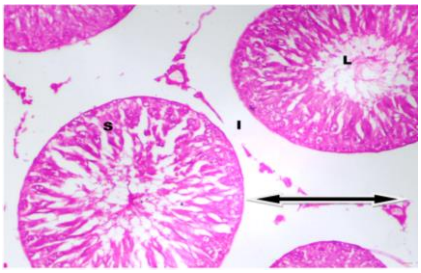
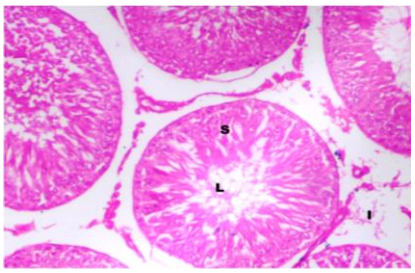
<tr><td> 
Plate 2.5</td><td> 
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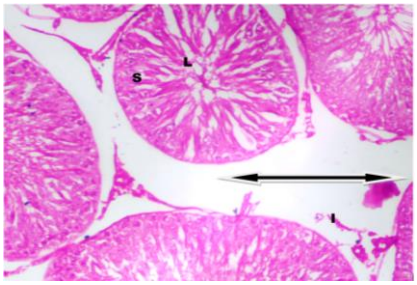
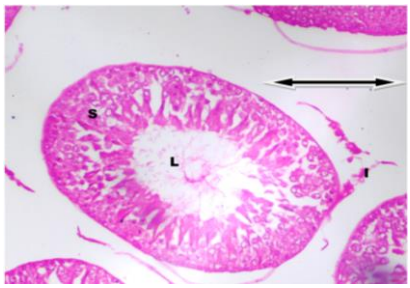
<tr><td> 
Plate 2.7</td><td> 
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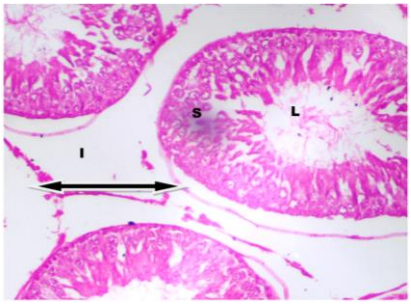
<tr><td> 
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3.3 Results from Oxidative Stress Markers

The oxidative stress markers and antioxidant enzyme activities varied across experimental groups, indicating a complex interplay between oxidative damage and defense mechanisms. Glutathione peroxidase (GPx) activity was slightly elevated in some groups, with Group 3 showing a significant increase (1.90±0.18 U/mg protein, $p<0.05$) compared to the control (1.10±0.24 U/mg protein), suggesting an adaptive antioxidant response. Malondialdehyde (MDA) levels were significantly higher in Groups 2, 3, and 6 (2.05±0.56, 2.15±0.69, and 1.95±0.56 nmol/mg protein, respectively, $p<0.05$), indicating increased lipid peroxidation and oxidative stress, while other groups maintained MDA levels closer to the control.

Catalase (CAT) activity was significantly reduced in Groups 2, 3, and 5 (15.30±1.79, 14.95±1.44, and 16.90±3.12 U/mg protein, respectively, $p<0.05$), reflecting impaired hydrogen peroxide breakdown. However, partial restoration was observed in Groups 6, 7, and 8, with Group 6 showing the most significant improvement (22.10±1.38 U/mg protein, $p<0.05$). Overall, Groups 2 and 3 exhibited pronounced oxidative stress, marked by

elevated MDA and reduced CAT activity, suggesting significant oxidative damage. Conversely, Group 7 appeared to mount a compensatory antioxidant response, with moderate CAT levels potentially mitigating oxidative stress more effectively.

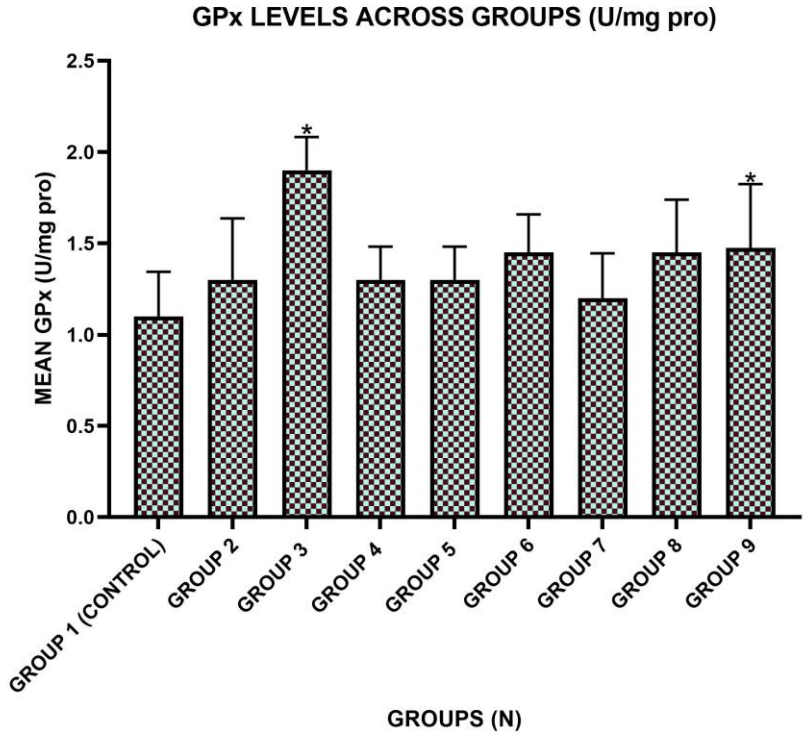

Figure 2.1. Simple Bar Chart Showing the Mean GPx Levels across Groups

N = 5; * = Statistically Significant Difference in Mean at $p<0.05$ Compared to the Control Group

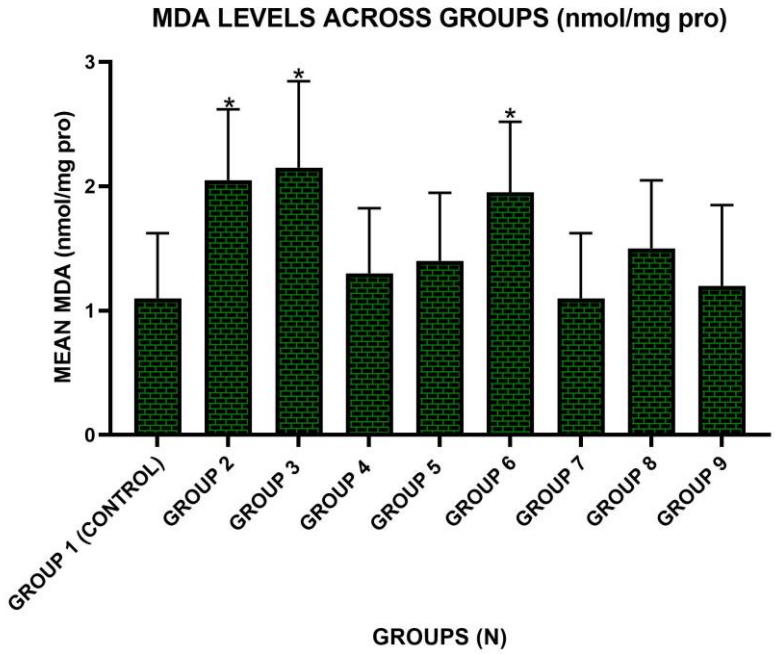

Figure 2.2. Simple Bar Chart Showing the Mean MDA Levels across Groups

N = 5; * = Statistically Significant Difference in Mean at $p<0.05$ Compared to the Control Group

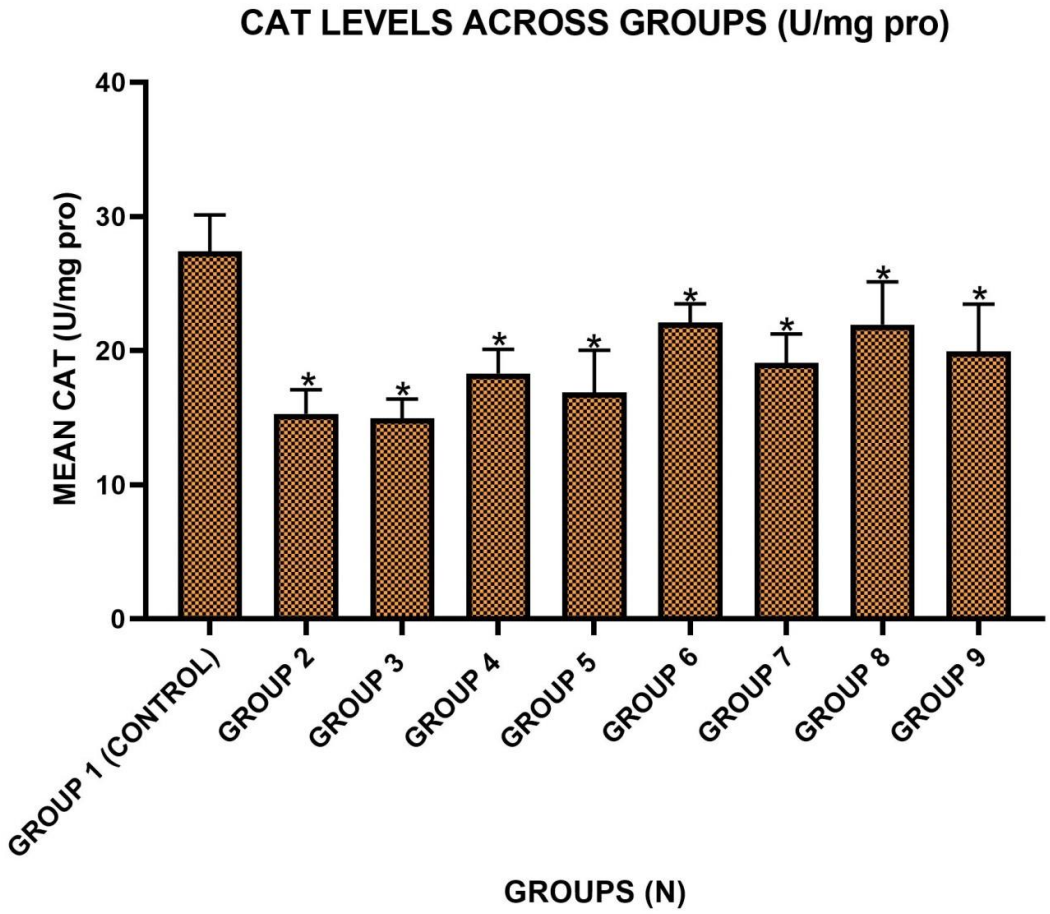

Figure 2.3. Simple Bar Chart Showing the Mean CAT Levels across Groups

N = 5; * = Statistically Significant Difference in Mean at $p<0.05$ Compared to the Control Group

4. Discussion

The results of this study demonstrate significant alterations in body weight, testicular histology, and oxidative stress markers across the experimental groups following aspartame and β-sitosterol treatments. These findings suggest that aspartame consumption may adversely affect reproductive health, while β-sitosterol exhibits potential protective and restorative effects, albeit with some inconsistencies. Body weight analysis revealed significant differences in initial and final body weights across the groups. The control group exhibited expected weight gain, while groups exposed to aspartame (Groups 2 and 3) showed significantly lower weight gain. This observation aligns with previous reports indicating that aspartame consumption can alter metabolic function and food intake regulation, possibly through its effects on hypothalamic pathways and gut microbiota (Fernandes et al., 2020; Choudhary & Lee, 2018). The lower weight gain in these groups may be attributed to metabolic disruptions induced by aspartame metabolism, leading to reduced energy efficiency or altered appetite regulation. Interestingly, groups co-treated with β-sitosterol (Groups 6 and 8) also exhibited lower weight gain compared to the control, suggesting that β-sitosterol might exert additional metabolic effects. While β-sitosterol has been recognized for its cholesterol-lowering properties (Chauhan et al., 2021), its role in weight regulation remains less understood. Notably, groups pre-treated with β-sitosterol before aspartame exposure (Groups 5 and 7) showed comparable or enhanced weight gain, suggesting a protective metabolic effect when administered before exposure to aspartame toxicity.

Histopathological analysis using H&E staining revealed that aspartame exposure (Groups 2 and 3) led to notable testicular damage, characterized by cellular necrosis, edema in inter-tubular spaces, and reduced Leydig cell populations. These findings corroborate previous reports demonstrating aspartame-induced reproductive toxicity via oxidative stress and inflammatory pathways (Ibrahim et al., 2018; Abdallah, 2016). The observed Leydig cell reduction suggests impaired testosterone synthesis, a key factor in spermatogenesis and male fertility. Conversely, groups treated with β-sitosterol (Groups 4, 5, and 7) exhibited relatively normal seminiferous tubules, with mild edema in some cases. The ability of β-sitosterol to mitigate aspartame-induced damage aligns

with its established antioxidative and anti-inflammatory properties (Baskar et al., 2020). Interestingly, groups receiving β-sitosterol post-aspartame exposure (Groups 8 and 9) still showed mild inter-tubular edema, indicating that while β-sitosterol provides some degree of protection, its efficacy is greater as a pre-treatment rather than as a post-treatment intervention.

The PAS staining technique further confirmed aspartame-induced testicular toxicity. In Groups 2 and 3, seminiferous tubules displayed extensive basement membrane erosion, marked edema, and loss of interstitial cells, indicating structural damage. These findings support prior evidence that aspartame metabolites, particularly formaldehyde, can induce oxidative stress, leading to cellular degeneration (Ashok et al., 2017). Groups treated with β-sitosterol exhibited improved basement membrane integrity, with Groups 4 and 5 showing near-normal histology. However, groups that received β-sitosterol post-aspartame exposure (Groups 8 and 9) still displayed mild to moderate edema, suggesting that while β-sitosterol offers protective effects, its ability to reverse aspartame-induced damage is limited.

Oxidative stress markers provided biochemical validation of the histopathological findings. Increased malondialdehyde (MDA) levels in Groups 2, 3, and 6 suggest enhanced lipid peroxidation and cellular damage, consistent with previous studies on aspartame-induced oxidative stress (Ibrahim et al., 2018). The concomitant reduction in catalase (CAT) activity in these groups further supports the notion that aspartame exposure impairs the testicular antioxidant defense system. Notably, glutathione peroxidase (GPx) activity was significantly increased in Group 3, suggesting a compensatory antioxidant response to oxidative stress. However, the increase in GPx was not sufficient to counteract the damaging effects of aspartame, as evident from the histological results. Groups treated with β-sitosterol (Groups 5 and 7) showed partial restoration of CAT activity, indicating its role in enhancing antioxidant defenses. This aligns with studies suggesting that β-sitosterol can upregulate antioxidant enzymes and reduce lipid peroxidation (Baskar et al., 2020).

The findings suggest that aspartame exposure induces testicular toxicity primarily through oxidative stress mechanisms, leading to histological and biochemical alterations. The protective effects of β-sitosterol may be attributed to its antioxidant, anti-inflammatory, and membrane-stabilizing properties (Chauhan et al., 2021). However, the observation that β-sitosterol was more effective as a pre-treatment than as a post-treatment suggests that its primary mode of action may be preventive rather than curative. Aspartame exposure resulted in significant testicular histopathological changes, oxidative stress, and metabolic disruptions. The administration of β-sitosterol exhibited a dose-dependent protective effect, with greater efficacy when administered before aspartame exposure. However, its ability to reverse established damage remains limited. These findings underscore the potential reproductive risks associated with prolonged aspartame consumption and highlight the need for further research into natural compounds such as β-sitosterol as potential therapeutic agents.

Future studies should extend the duration of treatment to cover a full spermatogenic cycle (approximately 62 days in rodents) to determine whether observed effects persist over longer periods. Additionally, investigations in higher animal models (e.g., primates) and clinical studies in humans are necessary to validate these findings and assess their translational relevance.

Conflict of Interest

We hereby declare that there was no conflict of interest in the course of this research.

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Mini Review: Artificial Intelligence and Systemic Lupus Erythematosus (SLE)

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Abstract

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by significant clinical heterogeneity, posing substantial challenges in diagnosis, monitoring, and treatment. Artificial Intelligence (AI), with its ability to analyze large and multidimensional datasets, offers innovative solutions to address these challenges. This review explores the current applications of AI in SLE research, highlighting its role in early diagnosis, biomarker discovery, imaging analysis, and personalized treatment strategies. We also discuss the integration of AI in disease monitoring, including the prediction of flares and remote patient management through telemedicine platforms. Despite its promise, the implementation of AI in SLE faces challenges such as data quality issues, ethical concerns, and the need for algorithm interpretability. Looking ahead, advancements in AI techniques, multi-omics integration, and interdisciplinary collaboration hold potential to overcome these barriers and transform SLE care. By synthesizing existing literature, this review underscores the transformative potential of AI in improving diagnostic accuracy, optimizing therapeutic interventions, and enhancing patient outcomes in SLE. Future research should focus on addressing current limitations and fostering equitable, clinically relevant AI applications to advance the field of lupus research and care.

Keywords: Systemic Lupus Erythematosus, artificial intelligence, machine learning, biomarkers, personalized medicine, disease monitoring

1. Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disorder characterized by widespread inflammation and tissue damage across multiple organ systems, including the skin, joints, kidneys, and central nervous system (Tsokos et al., 2016). The disease manifests with significant heterogeneity in clinical presentation, making it challenging to diagnose and manage effectively. SLE affects approximately 5 million people worldwide, predominantly women of childbearing age, and is associated with substantial morbidity and mortality (Rees et al., 2017).

Artificial Intelligence (AI) has emerged as a transformative tool in healthcare, leveraging advanced computational techniques such as machine learning (ML), deep learning (DL), and natural language processing (NLP) to analyze complex datasets and derive actionable insights (Topol, 2019). In medicine, AI applications range from diagnostic support systems to personalized treatment recommendations, offering the potential to enhance precision and efficiency in patient care.

The complexity and variability of SLE present unique challenges that traditional diagnostic and therapeutic

approaches struggle to address. AI technologies offer promising solutions by enabling the integration of multi-dimensional data, identifying novel biomarkers, and predicting disease trajectories with high accuracy (Mak et al., 2019). These capabilities align closely with the unmet needs in SLE research, where early diagnosis, accurate monitoring, and personalized interventions are critical to improving patient outcomes.

This review aims to explore the current applications of AI in SLE research, highlighting its role in diagnosis, monitoring, and management. We will examine the challenges and limitations associated with AI implementation and discuss future directions for advancing AI-driven innovations in SLE. By synthesizing existing literature, this review seeks to provide a comprehensive overview of how AI can transform the understanding and treatment of SLE.

2. Pathophysiology and Diagnostic Challenges of SLE

SLE is marked by a wide spectrum of clinical manifestations, including fatigue, joint pain, rashes, and systemic complications such as nephritis and neuropsychiatric symptoms (Lisnevskaia et al., 2014). The disease's heterogeneity complicates diagnosis, as no single symptom or test is definitive for SLE. Furthermore, disease activity fluctuates over time, with periods of flare-ups interspersed with remission. Diagnosis of SLE relies on a combination of clinical criteria, laboratory tests, and imaging studies. The American College of Rheumatology (ACR) and the Systemic Lupus International Collaborating Clinics (SLICC) have developed classification criteria, but these tools often lack sensitivity and specificity, particularly in early-stage disease (Petri et al., 2012). Additionally, serological markers such as antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA) are not always reliable indicators of disease activity.

The limitations of current diagnostic methods underscore the need for innovative approaches to improve early detection and accurate characterization of SLE. AI technologies, with their ability to process large datasets and identify subtle patterns, hold significant promise in addressing these gaps.

3. Applications of AI in SLE

3.1 AI in Early Diagnosis and Disease Prediction

Machine learning algorithms have demonstrated potential in predicting SLE onset using electronic health records (EHRs) and genetic data. For instance, a study by Zhang et al. (2020) employed ML models to analyze EHR data and achieved an accuracy of 85% in identifying patients at risk of developing SLE. Similarly, AI-driven analysis of genetic variants has identified novel susceptibility loci associated with SLE (Kariuki et al., 2018).

3.2 Machine Learning for SLE Biomarker Identification

Biomarker discovery remains a critical area of SLE research, and AI has facilitated the identification of novel biomarkers through multi-omics integration. A study by Chen et al. (2021) utilized deep learning to analyze proteomic and transcriptomic data, uncovering potential biomarkers linked to disease activity and organ involvement.

3.3 Imaging Analysis and Pattern Recognition in SLE Diagnosis

AI-based image analysis has shown promise in detecting lupus-related renal and cutaneous manifestations. Convolutional neural networks (CNNs) have been applied to renal biopsy images, achieving high accuracy in classifying lupus nephritis severity (Wang et al., 2022). Similarly, AI tools have enhanced the interpretation of dermatological lesions in SLE patients.

3.4 Natural Language Processing (NLP) for Clinical Data Extraction

NLP techniques have been used to extract valuable information from unstructured clinical notes, enabling the identification of SLE-related symptoms and comorbidities. For example, a study by Li et al. (2023) demonstrated the utility of NLP in automating the extraction of disease activity scores from EHRs.

3.5 AI for Personalized Treatment Plans and Prognostic Models

AI models have been developed to predict individualized treatment responses and long-term outcomes in SLE patients. These models incorporate demographic, clinical, and genomic data to generate tailored recommendations, enhancing the precision of therapeutic interventions (Smith et al., 2022).

4. AI in SLE Monitoring and Management

4.1 Predicting Disease Flare-Ups and Remission

Predictive models powered by AI have been designed to forecast disease flares based on longitudinal patient data. A study by Liu et al. (2021) reported a predictive accuracy of 88% in identifying impending flares, enabling timely intervention.

4.2 Monitoring Treatment Efficacy and Side Effects

AI tools have been employed to monitor the efficacy of immunosuppressive therapies and detect adverse effects. For instance, ML algorithms have been used to analyze laboratory parameters and identify early signs of drug-induced toxicity (Garcia et al., 2020).

4.3 Remote Monitoring and Telemedicine Integration

The integration of AI with wearable devices and telemedicine platforms has facilitated remote monitoring of SLE patients. These technologies enable real-time tracking of disease activity and adherence to treatment regimens (Brown et al., 2023).

5. Challenges and Limitations of AI in SLE

5.1 Data Availability and Quality Issues

The performance of AI models is heavily dependent on the availability of high-quality, annotated datasets. However, SLE research faces challenges related to data scarcity and heterogeneity, limiting the generalizability of AI applications (Johnson et al., 2021).

5.2 Ethical and Privacy Concerns in AI Applications

The use of patient data in AI research raises ethical and privacy concerns, necessitating robust data governance frameworks to ensure compliance with regulations such as GDPR and HIPAA (Miller et al., 2020).

5.3 Algorithm Interpretability and Clinician Trust

The “black-box” nature of many AI algorithms poses challenges in gaining clinician trust and acceptance. Efforts to develop interpretable models are essential to facilitate clinical adoption (Davis et al., 2022).

5.4 Generalizability Across Diverse Patient Populations

AI models trained on specific populations may not perform well in diverse settings, highlighting the need for inclusive datasets and cross-validation strategies (Taylor et al., 2021).

6. Future Directions and Perspectives

The future of AI in SLE research is marked by promising advancements and critical considerations. Emerging AI techniques, such as federated learning and transfer learning, offer innovative solutions to overcome challenges related to data scarcity and model generalizability, thereby enhancing the performance of predictive and diagnostic tools (Anderson et al., 2023). Additionally, the integration of multi-omics data — encompassing genomics, proteomics, and metabolomics — using AI-driven approaches holds immense potential to unravel the complex pathogenesis of SLE and identify novel therapeutic targets (Roberts et al., 2022). To ensure the clinical relevance and usability of AI tools, interdisciplinary collaboration between clinicians, researchers, and AI developers is essential. Such partnerships can bridge the gap between technical innovation and real-world application, fostering the creation of user-friendly and clinically meaningful solutions (Wilson et al., 2023). However, the widespread implementation of AI in SLE care also necessitates the development of robust regulatory guidelines and ethical standards to address concerns related to data privacy, algorithm transparency, and equitable access. Establishing these frameworks will be critical to ensuring the safe, responsible, and effective deployment of AI technologies in the management of SLE (Harris et al., 2022). Collectively, these efforts pave the way for transformative advancements in SLE research and patient care, leveraging the full potential of AI to improve outcomes for individuals living with this complex disease.

7. Conclusion

This review underscores the transformative potential of artificial intelligence (AI) in addressing the diagnostic, monitoring, and therapeutic challenges associated with systemic lupus erythematosus (SLE). By enabling early diagnosis, precise disease monitoring, and personalized treatment strategies, AI technologies offer innovative solutions that could significantly enhance patient outcomes. The integration of AI into SLE care holds the promise of improving diagnostic accuracy, optimizing treatment plans, and reducing healthcare costs. However, realizing these benefits requires addressing critical issues such as data quality, ethical concerns, and the interpretability of AI algorithms to ensure their safe and effective use in clinical practice. Looking ahead, future research should prioritize expanding AI applications to underrepresented populations to ensure inclusivity, validating models in real-world settings to assess their generalizability, and fostering interdisciplinary collaboration between clinicians, researchers, and AI developers to drive meaningful innovation in SLE research. These efforts will be instrumental in unlocking the full potential of AI to advance the understanding and management of SLE.

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Management Strategies of Fatal Liver Infection Due to Hepatitis C Virus (HCV)

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Abstract

Hepatitis is a liver inflammatory disease that can cause severe liver scarring. Hepatitis C is a blood-borne fatal disease of the liver that is caused by a virus called the hepatitis virus C (HCV), which lives in liver tissue and blood. It can be both an acute (short-term) illness (25-15%) and a chronic (long-term) infection (75-85%) that may gradually damage the liver. It is characterized by possible development of both liver and extra-hepatic disorders. The HCV infection is usually asymptomatic. Chronic infection with the HCV represents a major health problem worldwide that accounts for life-threatening liver disease, such as liver cirrhosis, hepatocellular carcinoma (HCC), liver failure, and ultimately needs liver transplantation, or to face unexpected premature death. Early treatment for hepatitis C is highly cost-effective and disease progression restricted, and can be avoided end-stage liver disease. The aim of this study is to identify the risk factors, transformation rout, and complication of HCV for the management of the disease to save millions of lives.

Keywords: hepatitis virus C, genotype, cirrhosis, hepatocellular carcinoma

1. Introduction

Hepatitis C virus (HCV) is responsible for the major liver infection that damages the liver, and it may take 20-40 years to damage the liver. The HCV infection can cause either acute or chronic hepatitis, the severity of the disease ranges from mild to life-threatening. After an acute infection, about 15-45% clear spontaneously (without treatment), about 55-85% progress to chronic infection, and about 80% progress to chronic HCV infection due to viral replication occurrence in liver cell. Spontaneous clearance is more likely to happen within the first 12 months from initial infection (Aisyah et al., 2018). The HCV was most prevalent in the baby boomer generation (persons born between 1945 and 1965), which comprised about 75% population (Guadagnino et al., 1997). It is a blood-borne disease, and the most common ways of infection occur through unsafe injection practices, inadequate sterilization of medical equipment, blood transfusion and blood products without examination (Kim & Chang, 2013).

From 1990 to 2019, the incidence rates of HCV infection have remained stable. During this period, it is estimated that roughly 200 million people are infected globally by the HCV (Zeng et al., 2021). In 2018, about 143,000 people in the UK were living with chronic hepatitis C infection (NICE 2013). In 2017, there were about 115 million HCV infected people worldwide, of these 11.3 million (10%) resided in Eastern Europe and Central Asia (Maistat et al., 2017). In 2020, about 71 million people have chronic HCV infections around the globe. Therefore, it is a plague of humankind (WHO, 2021). The most affected regions are Central and East Asia and North Africa, and the top three countries with the highest disease burden are China (9.48 million), Pakistan (7.39 million) and India (6.13 million) (Dugan et al., 2021). About 530,000 cases (82%) of liver cancer per year worldwide are caused by viral hepatitis infection, with 118,000 cases associated with hepatitis C (Poynard et al., 2003). It is estimated that 4.1 million (1.6%) Americans have been infected with HCV, of whom 3.2 million are

chronically infected. At present about 160 million people worldwide are suffering from HCV infection, which is the leading cause of cirrhosis, hepatocellular carcinoma (HCC), and liver transplantation (Kutala et al., 2015).

At present about 325 million people worldwide have hepatitis B or C, and majority of them do not have access to life saving medications. There is no vaccine against HCV. Therefore, it is a major challenge worldwide for controlling the disease (Liang, 2013). In the absence of vaccination most exposed neonates and young children will be infected and become lifelong carriers (Gow & Mutimer, 2001). In 2020, HBV and HCV related disease led to 1.1 million deaths worldwide. Some patients progress quickly to liver cirrhosis and may develop HCC (Zeng et al., 2021). The burden of HCV is a global health concern as liver-related morbidity and mortality continue to rise due to people living with human immunodeficiency virus (HIV), hepatitis B, or both, and people who inject drugs. Antiviral medicines, such as pegylated interferon and ribavirin can be used to limit liver damage (Liang et al., 2000).

2. Literature Review

In any research, the literature review is a section where research works of previous researchers are introduced briefly to make familiar with the new researchers in the research arena (Polit & Hungler, 2013). It is a hard-working search, scholarly inquiry, and investigation that aim for the discovery of new facts and findings (Adams et al., 2007). It helps the researchers to understand the subject, and it serves as an indicator of the subject that has been carried out previously (Creswell, 2007). Yasir Waheed and his coworkers have found that the prevalence, genotypes, and factors associated with HCV infection in the Pakistani population. They have observed that the most prevalent genotype of HCV is 3a and the prevalence is moderate in the general population but very high in injecting drug users and multi-transfused populations (Waheed et al., 2009).

Amal Ahmed Mohamed and his coauthors have found that about 130-175 million HCV patients are chronically infected, and over 350,000 die each. They have realized that chronic HCV is the primary cause of cirrhosis, HCC, and end-stage liver disease. They have stressed on the management and prevention of chronic HCV through the reduction of the risk of HCC and treating extra hepatic complications (Mohamed et al., 2015). Blaise K. Kutala and his coauthors have realized the beneficial effect of achieving a sustained virological response (SVR) after antiviral treatment against HCV. But they are in confusion whether unsuccessful treatment (non-SVR) also improves patient survival, especially in patients with advanced liver fibrosis. They have evaluated the incidence of death or liver transplantation in the 427 naive patients with a Child-Pugh score of A and advanced fibrosis (Kutala et al., 2015).

Francesco Negro has provided a thorough review on the extent of the HCV epidemic across Europe, with a discussion of the most important subgroups affected, and of the risk factors of infection, both traditional and new (Negro, 2014). Ellen Dugan and her coworkers have aimed to estimate the global prevalence of viremic HCV in 2019 among women of childbearing age through the literature search, modeling work, and extrapolation. They have applied test-and-treat strategies to reduce vertical transmission and total disease burden (Dugan et al., 2021). Roberta D'Ambrosio and Alessio Aghemo have studied antiviral treatment of chronic HCV for the persistent eradication of the virus through the sustained virological response (SVR) (D'Ambrosio & Aghemo, 2012).

T. Jake Liang has shown that despite major advances in the understanding and treatment of hepatitis C, a preventive vaccine has not been discovered yet. Therefore, the ultimate path to a successful preventive vaccine requires comprehensive evaluations of all aspects of protective immunity, innovative application of state-of-the-art vaccine technology and properly designed vaccine trials that can affirm definitive endpoints of efficacy (Liang, 2013). Mario U. Mondelli and his coauthors have shown that prevention with an effective vaccine is the best option to eradicate dangerous human pathogens. Since at present there is no vaccine of HCV, prevention of chronic liver disease is regarded as a more realistic and equally important goal to fight the deleterious effects of chronic HCV infection (Mondelli et al., 2005).

3. Research Methodology of the Study

To lead in the academic world an academician takes the research as an essential and influential work of his/her way of life (Pandey & Pandey, 2015). Methodology is a proper guideline of any valuable research that is considered as an organized procedure and follows scientific methods appropriately (Kothari, 2008). It provides the research design and analysis procedures to perform good research (Hallberg, 2006). Therefore, research methodology is a strategy for planning, arranging, designing, and conducting a meaningful and valuable research that tries to develop logic to generate theory within which the research is conducted (Remenyi et al., 1998).

This study is an exploratory and descriptive procedure that deals with a qualitative research approach (Mohajan, 2017, 2018, 2020). I have studied research papers, books and handbooks of renowned authors, and have collected materials from internet, websites, etc. to enrich this paper (Mohajan, 2024a-g).

4. Objective of the Study

Main objective of this article is to discuss the basic concept of acute and chronic HCV infection. Hepatitis C is an infection of the liver that may gradually damage the liver. The most people who are infected cannot able to clear the virus and develop a lifelong chronic infection. At present it is the leading cause for liver transplants in the USA (Franciscus, 2017). Other minor objectives of the study are as follows:

1) to focus on symptoms and transmission of HCV,

2) to highlight on virology and risk factors of HCV, and

3) to discuss diagnosis and treatment of HCV.

5. Virology of HCV

HCV is a small and single-stranded RNA Flaviviridae family and the genus Hepacivirus, encoding for a capsid protein, two envelope proteins, and some non-structural proteins. There are no direct morphological markers of the virus. The virus replicates in the hepatocyte and in other cells, such as lymphocytes, and macrophages that cause acute or chronic hepatitis, cirrhosis, and HCC (Waheed et al., 2009). The HCV contains dual layered wrapped nucleocapsid with lipid bilayer (Hakim et al., 2008).

The existence of HCV was first fully recognized in 1975 when the infection was not associated with hepatitis A virus (HAV) or hepatitis B virus (HBV) infections, and then defined the disease a non-A, non-B hepatitis (Feinstone et al., 1975). It cannot be clinically distinguished from other viral hepatitis with any reliability. Its only reservoir is human beings. Hepatitis C virus (HCV) is discovered in 1989 by three scientists Harvey J. Alter, Michael Houghton and Charles M. Rice as the major causative agent of “non-A, non-B hepatitis” (Choo et al., 1989). The HCV genome is found to consist of about 9,600 nucleotides in length, and it has a single open reading frame about that encodes a 3010 amino acid polyprotein that undergoes proteolytic processing to form structural and nonstructural viral protein. It is flanked at the termini by 5′ and 3′ untranslated regions critical for viral replication and translation (Gottwein & Bukh, 2008).

The HCV has 7 genotypes (GTs) and over 90 subtypes. The GTs 1, 2, and 3 are more common in the northern hemisphere (Smith et al., 2014). GT1 is the most common and is estimated to account for 83.4 million (46.2%), with wide geographical distribution in Northern and Western Europe, Asia, North and South America, and Australia (Messina et al., 2015). GT2 is mostly present in West and Central Africa that accounts for 16.5 million (9.1%). GT3 is the most common after GT1 that accounts for 54.3 million (30.1%) cases globally, about 75% of this number occur in south Asia (Lavanchy, 2011). GT4 is the most common in Egypt, GT5 is present only in South Africa, and GT6 is endemic in Hong Kong and Southern China. To date, only one GT7 infection has been reported that is isolated in Canada from a Central African immigrant (Murphy et al., 2007).

5.1 Symptoms of HCV

Most people (70-80%) have no symptoms when they are first infected with hepatitis C. Over time people with chronic HCV may develop various symptoms related to liver damage that is associated with a wide variety of related conditions (Franciscus, 2017). Sometimes the symptoms may develop within one to three months of infection. Chronic HCV infection is normally a slow progressive disease that may produce for many years after infection (Chuang et al., 2009). Some symptoms of it are jaundice, anorexia, vague abdominal discomfort, loss of appetite, nausea and vomiting, weight loss, fatigue, tiredness, abdominal pain, joint pain, fever, itching, abdominal swelling due to fluid, clay-colored or pale stools, and dark grayish urine (Purcell, 1997). Usually alanine aminotransferase (ALT) remains about seven times upper than the normal limit. Most HCV infected people carry the virus for the rest of their lives, and about 70% of them develop chronic liver disease (Maheshwari et al., 2008).

5.2 Risk Factors of HCV

People at the highest risk of infection are a person sexual relationship with a HCV infected person, and have a sexual partner with hepatitis C infection and are HIV positive (Mesquita et al., 1997). Some sensitive of HCV infection people are injecting or inhaling for drug users, have been in prison for a long time, persons born between 1945 and 1965, take medicine sharing needles, chronic hemodialysis patients, and was born in a country with a high prevalence of hepatitis C (NICE, 2013). Also, the people are in the risk of HCV infection; the recipients of clotting factor concentrate before 1987, recipients of blood transfusions before 1992, and have a practice of tattooing, acupuncture and skin piercing. Children born from HCV-positive mothers are also in the risk of HCV transformation. Homeless populations are at extremely high risk for infection with HCV (Manzini et al., 1995).

5.3 Transmission of HCV

The main route of HCV transmission is parenteral, and can be spread in human body through the contact with infected blood and blood products (horizontal transmission) without testing especially through the hemophiliacs, dialysis patients, and intravenous drug users (Tremolada et al., 1992). Transmission of HCV may also occur with

activities involving percutaneous exposure and the use of contaminated equipment (NICE, 2013). Other common modes of transmission are sexual, perinatal, and idiopathic. The HCV transmission (vertical transmission) from mother to infant during pregnancy or childbirth has been documented but the case is rare (less than 5%). If the mother is infected with both HCV and HIV, the probability of vertical transmission increases 4 to 5 folds (Rehermann & Nascimbeni, 2005).

Sexual contact is less likely to transmit HCV but it depends on the type of sexual activity. Epidemiological studies show low rates of HCV infection in high promiscuity groups, such as prostitutes, homosexuals, and patients with sexually transmitted diseases (Tedder et al., 1991). The risk is higher with increased number of sexual partners, engaging in high-risk sexual practices, co-infection with sexually transmitted infections (STIs) and HIV, and engaging in sexual activities with those who are involved in high-risk behaviors. The HCV transmission is lowest with oral sex, higher with vaginal sex, and highest with anal sex (Mesquita et al., 1997).

The HCV can be transmitted through the ear and nose piercing, tattooing, acupuncture, reuse of syringes and needles in healthcare settings, use of injection drugs, blood transfusion, unsterilized dental equipment, surgical instruments, shaving and cosmetology instrument sharing, etc. (Raza, 2007). Other sources of HCV transmission are sharing drug injection; snorting or smoking equipment, such as needles, syringes, tourniquets, straws, pipes, cookers, wash and filters, etc.; household exposure through sharing of personal hygiene equipment, such as toothbrushes, razors, nail clippers, sex toys, etc.; practices using unsterilized objects, such as scarification, circumcision, etc. (Liou et al., 1992).

The HCV is not transmitted by casual contact like hugging or shaking hands, kissing on the cheek, coughing or sneezing, or sharing a bathroom. It also cannot spread through food, water, or in cross-contact, or sharing food and drinks, or sharing eating utensils and drinking glasses with the infected patients. The HCV has not been found in breast milk (Ogasawara et al., 1993).

5.4 Diagnosis of HCV

Diagnosis for HCV is recommended if a person is in one or more of the symptoms of HCV infection, such as fatigue, jaundice, nausea and vomiting, etc. The HCV is often remains undiagnosed for many years and usually diagnosed accidentally when patients present to physician with increased liver enzymes, or cryptogenic chronic liver disease. Infection with HCV can be diagnosed by specific antibody test using enzyme immunoassay (EIA), chemiluminescence immunoassays, and recombinant immunoblot assays (Ghany et al., 2009). Generally, HCV antibodies develop on 7-8 weeks after infection. Real-time polymerase chain reaction (RT-PCR) can be helpful in the detection of HCV in early onset of infection. It detects HCV RNA (Allain et al., 1991). The HCV viral load test estimates the number of viral particles per milliliter of blood that is useful in assessing prognosis and planning and monitoring treatment. It is often used to guide initial treatment decisions and to follow the progress of individuals undergoing treatment (Yuki et al., 1992).

5.5 Treatment of HCV Patient

There is no vaccine for hepatitis C, but several vaccines are currently under development (Strickland et al., 2008). The HCV vaccines will be difficult to develop due to the virus' different genotypes and its ability to change or mutate during infection (Franciscus, 2017). Treatment of the HCV is palliative and supportive. The effectiveness of the treatment varies according to many factors, such as the genotype, treatment regimen, and characteristics of the infected person. Response to treatment varies depending on the genotype (GT) with which the person is infected. Early detection and treatment can prevent serious liver damage and lead a long-term healthy life (Strickland et al., 2008). Identification of HCV genotype is useful in assessing prognosis and planning treatment. The treatment improves liver health by stopping liver damage caused by the HCV and even reversing some of the damages that have already occurred (Arcaini et al., 2016).

About 15-45% HCV infected patients cure automatically within six months without any treatment, and the rest may develop life-threatening chronic hepatitis that is the major cause of liver fibrosis, liver cirrhosis, HCC, and liver failure (Thomas & Zoulim, 2012). In 1996, only 9% has a chance of being cured of hepatitis C, but now treatments usually for 12-24 weeks, HCV has progressed to the point that more than 90% of the people who take the treatments can be cured (Franciscus, 2017).

The HCV is successfully treated with pegylated interferon-alfa injections along with oral drugs, such as ribavirin has been effective (Jacobson, 2007). In 2013, the chronic HCV infection with the approval of second-generation direct-acting antivirals led the way for IFNfree combination regimens. About 30% of the infected patients are cured within six months and about 70% can develop chronic infection (D'Ambrosio & Aghemo, 2012). The combination of PegIFN-alpha and ribavirin for 24 or 48 weeks is the standard of care for treatment of HCV infection. First-generation NS3 protease inhibitors introduced in the market of HCV therapy since 2011 are telaprevir and boceprevir that are approved as a new standard line of therapy for GT1 (Aghemo et al., 2013). Recently, the combinations and newer treatments, such as polymerase inhibitors, protease inhibitors, and NS5A

inhibitors are recommended for HCV (Berg et al., 2006). GT1 patients are treated with a combination of HCV inhibitors and ribavirin for 8-24 weeks. GT2,3,4,5, and 6 people are treated with a combination of an HCV direct-acting antiviral (DAA) and ribavirin for 12-24 weeks and the cure rates are 80-100% (Franciscus, 2017).

Bed rest and fluids may be prescribed especially during the acute phase. Medicines can heal more than 90% of people with HCV that can be treated through the combination therapy with interferon and ribavirin. The ribavirin is not effective when used alone (Nahon et al., 2018). The recombinant immunoblot assay (RIBA) is a more specific test for anti-HCV antibody. HCV screening test of pregnant women is necessary to save the infants from hepatitis C (Naderi, 2014). Direct-acting antivirals (DAAs) treatment for chronic HCV infection may improve liver function and decrease the portal pressure of HCV patients with compensated cirrhosis (Berzigotti et al., 2015).

The HCV RNA appears in the blood within 1-2 weeks of infection in a majority of patients. About 30% of patients develop symptoms of acute HCV infection within 3-12 weeks after infection, with an average of 7 weeks (Carrat et al., 2019). It has a cytopathic effect and causes immune disorders. The infection can be very serious. The incubation period of this virus is 15 to 150 days. This virus may stay in the liver for years and it is not discovered until much damage is done in the liver (Hosry et al., 2016).

6. Nutrition Diet and Healthy Habits

A healthy, well-balanced diet is essential for a HCV infected patient. Such a diet is low in fat and sodium, high in complex carbohydrates, and has adequate protein. Consumption of processed foods, such as canned, frozen, and other preserved foods must be reduced, as these foods often contain chemical additives (Denniston et al., 2012). Non-dairy nutritious foods, such as soy, almond, cashew, coconut, hemp or rice milk must take properly. The patients should avoid high fat, salt, or sugar foods. Over-consumption of coffee, tea, and soda is harmful for HCV patients (Franciscus, 2017).

Heavy consumption of alcohol can severely accelerate HCV disease progression. Therefore, the people with HCV should avoid alcohol. They should avoid other recreational drugs and tobacco as usual (North et al., 2014). Moderate exercise, such as walking and swimming are generally recommended for all individuals who are not in an acute or end-stage phase of HCV. Exercise can reduce stress and is important for maintaining good health (Denniston et al., 2014).

7. Conclusions

From this study, I have observed that HCV can cause the liver disease hepatitis C that is a major health concern worldwide. At present more than 170 million people are infected with HCV worldwide. About 71 million people are chronically infected, and about 3-4 million people are newly infected every year, and from these about 700,000 people die each year worldwide from chronic HCV infection. The highest prevalence rates of HCV are seen in developing poor countries of Africa and Asia, with low prevalence rates are in developed countries of Europe and North America. Most patients with HCV infection are unaware about their infection for many years due to no symptoms. As a result, liver cirrhosis and HCC are developed before the identification of HCV infection. The high-cost HCV medications, regular outpatient visits, diagnostic, and routinely monitoring blood tests, etc. are common problems for patients and over burden to the family members. Successful treatment will stop the progression of liver disease, and maximum cases the patient will recover completely, and consequently, the numbers of HCV infected individuals will be reduced. To reduce the risk of further liver damage of a HCV patient needs to avoid alcohol if addicted, and should eat a healthy balanced diet, and undertake a regular physical exercise. At present the medical providers are much more knowledgeable about diagnosis, management and treatment of hepatitis C. More researches into several important areas of HCV infection must be increased through the development of more effective and better tolerated antiviral therapies and medications. Consumption of balanced diet and medications the patients must adopt lifestyle changes, such as good nutrition, exercise, and stress management to alleviate some side-effects to slow disease progression.

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The Risk Assessment for Malignant Conversion of Cervical Pre-Neoplastic and Other Lesions by High Risk HPV and Relevant Markers

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Abstract

Objective is to identify high risk cases of cervical pre -neoplastic lesions through the study of p16 (HR-HPV), Ki-67, DNA ploidy & Apoptosis. Western Blot analysis was performed to identify the high-risk HPV association. Immunohistochemistry was done to study Ki-67; DNA content study was performed with the help of FACS & Apoptotic index was studied by Tunnel Assay. The association of p16 with the cervical pre-neoplastic pathology was not only strong but also very significant. In this study, 72% cases were positive, and 28% cases were negative for p16 study by Western Blot analysis. Only 2 cases of other associated conditions (Koilocytosis) showed positivity for p16. The study of Ki-67 was also highly significant & showed positivity in 73% cases and has similarity with other studies. The test of proportion showed that patients with positive TUNEL assay (63%) were significantly higher than patients with negative TUNEL assay (37%) ($p<0.00024$). To conclude, in India, Cervical cancer is the second most common cancer among women. It is preceded by cervical pre neoplastic lesions (CINS). Apart from HR HPV expressed in CIN's, condyloma, cervicitis, reactive cellular and koilocytotic changes are also caused by HR HPV. Therefore, study of HR HPV association along with identification of DNA status, Ki67 expression and Apoptosis play a very important and crucial part for risk assessment and should be done at the earliest for cervical cancer prevention.

Keywords: HR-HPV, pre-neoplastic lesion, cervical cancer, Western Blot, immunohistochemistry, DNA ploidy, Tunnel assay

1. Introduction

Worldwide, among all cancer cases, cervical cancer has been placed in the fourth position with approximately 600400 new cases and 34200 deaths[1].

It is classified into two major histopathologic types, Squamous cell carcinoma (72.6%) and adenocarcinoma (21.8%) (S. Nagase, T. Ohta, F. Takahashi et al., 2022). Almost all the Squamous cell carcinomas (95%) and more than 80% of cervical adenocarcinoma are HPV associated. But 10–15% of them, such as gastric-type adenocarcinoma and clear cell carcinomas, are HPV independent (The Cancer Genome Atlas Research Network,

[1] WHO 2020.

2017; S. Stolnicu, L. Hoang, R.A. Soslow et al, 2019).

Majority of cervical cancer preceded by pre-neoplastic lesions designated as cervical intra- epithelial neoplasia and classified as mild (CIN I), moderate (CIN II) and severe (CIN–III) lesions. Annually, approximately 1.5 per 1000 women in developed countries is diagnosed with CIN II/CIN III (K. Tainio, A. Athanasiou, K.A.O. Tikkinen et al., 2018). Most of the cervical cancer occurred in the squamocolumnar junction (F. Bray, J. Ferlay, L. Laversanne et al., 2015).

Human papillomavirus (HPV) infection and integration of cervical epithelium has been considered as the key factor for the development of cervical cancer (K. Munger, A. Baldwin, K.M. Edwards, et al., 2004). All-over the world, in general, HPV infection among women is about 9 to 13% and in Indian women it varies between 7.5% to 16.9% (A. Sridevi, R. Javed, A. Dinesh et al., 2015).

Papillomaviruses are small, non-enveloped, icosahedral DNA viruses with a diameter of 52–55 nm. The viral particles consist of a double-stranded DNA molecule of about 8000 base-pairs that is bound to cellular histones and contained in a protein capsid composed of 72 pentameric capsomers. Apart from general population it is also widely distributed in the animal kingdom.

So far, more than 200 genotypes of HPVs have been identified (I. Lesnikova, M. Lidang, S. Hamilton-Dutoit, et al., 2009). HPVs are classified as High Risk HPVs (HR HPV) and Low Risk HPVs (LR HPV) (D. Uyar, J. Radar., 2014). Most of the HPVs are low risk HPV and produce benign warts (Münger, K., Werness, B. A., Dyson, N., Phelps, W. C., Harlow, E., & Howley, P. M., 1989). The International Agency for Research on Cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans Biological Agents, 2012). IARC working group classified HPV as Groups 1–4 based on the risk of carcinogenesis. Group 1 or Group 2A with a high risk of carcinogenesis includes 13 types of HPV; HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Particularly, HPV types 16 and 18 are responsible for 70% of the HPV genotypes detectable in cervical cancer (Y. Azuma, R. Kusumoto-Matsuo, F. Takeuchi, et al., 2014).

However, immune protection clears 90% of all HPV infection spontaneously but some cases have persistent viral infection and leads to the development of cervical cancer (Hu, Z., et al., 2015).

The key event of carcinogenesis is the integration of HPV genome with the host genome(chromosome). It often occurs at the common fragile sites of host genome (Thorland, E. C., et al., 2003). HPV integration follows more specific pattern with relation to the HPV genome and as well as clonal proliferation of epithelial cells.

Expression of viral E6 & E7 genes leads to constant presence of E6, E7 proteins where as other portions of the viral DNA are deleted (V.V. Baker., 2008). Viral E6 protein binds to P53 tumour suppressor protein causing degradation of P53 protein and E7 protein inactivates retinoblastoma (Rb) protein resulting inhibition of apoptosis and leading to abnormal cell cycle and proliferation. In addition to this fact, E7 also triggers upregulation of CDKN2A tumour suppressor gene resulting marked accumulation & overexpression of p16 genes as p16 protein (Aupperle, K. R., et al., 1998; M. Narisawa-Saito & T. Kiyono, 2007).

Though, histopathology is considered as ‘gold Standard,’ it fails to assess cancer conversion risk of cervical pre-neoplastic lesions. This fact accentuates the importance for biomarker study in pre neoplastic lesions.

Currently, high risk HPV marker, CDKN2A (M. Narisawa-Saito & T. Kiyono, 2007), proliferative marker ki 67 (R. SalesGi & P. Vagnarelli, 2018), DNA ploidy analysis (Melsheimer, P., et al., 2004), and apoptosis are getting importance for the risk assessment of cervical pre-neoplastic lesions.

2. Materials and Methods

Study Design:

The study took Place under the Dept. of Pathology and Cancer Screening, and the samples were collected at Gynaecology OPD and CDC OPD of Chittaranjan National Cancer Institute, Kolkata, India.

Both the cervical smear samples and punch Biopsy specimens were taken from 100 pre- diagnosed cases. The samples were processed according to the standard protocol.

P16 Estimation–Western Blot

(i) Sample collection: Cervical punch biopsy specimen was collected, in I X PBS in Eppendorf tube separately for100 cases and the whole protein was extracted from each of the sample within 24 hours.

(ii) Protein extraction: Tissue samples were washed with 1 x PBS thoroughly and transferred to a 2mL Eppendorf tube. Next, 250 uL of lysis butter added to the tube and kept on ice for 15 to 20 minutes and homogenised thoroughly. Homogenized tissue was sonicated for 1.5 minutes and kept on a rocker at 4° C for 2 hours and centrifuged at 13500 rpm for 15 minutes at 4°C and the supernatant was placed in a clear and sterile Eppendorf tube for further experiment.

(iii) Protein estimation: 1 ml of supernatant was added to 200 uL of Bradford reagent mixed with 49 ul of distilled water. The soln. was placed in a ELISA plate & mixed well. The reagent turned blue. The protein estimation was done with the help of ELISA Reader at an absorbance of 595 nm & the reading was taken. Rest of the supernatant (extracted protein, was stored at –70°c for further use).

(iv) Western Blot Analysis: For identification of P16 protein — the experiment was performed using SDS — PAGE following standard protocol.

Immunohistochemistry (IHC) for Ki- 67

Ki-67 IHC was performed in 100 cases of deparaffinised tissue sections and positive control according to the protocol of IHC World (IHC World, 2008) with slight modification. Commercially available kit (IHC Select-HRP/DAB Kit Millipore) was used for detection.

Briefly, antigen retrieval was carried out by boiling the slides in 0.01 M citrate buffer, pH 6.0 in a microwave oven at 900 watts for 10 minutes allowed to cool down at room temperature, washed with rinse buffer and blotted. Endogenous peroxidase was blocked by 3% hydrogen peroxide in water for 10minutes.Sections were washed with rinse buffer and blotted. Non -specific binding was blocked by blocking reagent (provided in the detection kit, Millipore) for 5 minutes in a humid chamber and excess reagent was blotted. Next primary monoclonal antibody (Sigma, USA, clone PP 67, dilution 1:50) were added to the sections and incubated overnight in 4 degree centigrade. Slides were washed thoroughly with rinse buffer to remove excess primary antibody and blotted. Secondary antibody (Millipore) was added to the sections and incubated in a humid chamber for 10 minutes, washed and blotted. Next, Streptavidin HRP was applied on the tissue sections and incubated for 10 minutes. After washing and blotting freshly prepared DAB solution was added to the tissue sections and incubated for 10 minutes then sections were washed for 5 minutes and blotted. Counter staining was done by Meyer's haematoxylin for 1 minute. The slides were then passed through a series of graded alcohol and xylene and mounted with DPX mountant. Primary anti-body was omitted from the negative control slides.

Scoring method:

Ki 67 expression were analysed semi quantitively by scoring 1000 cells at 40x under light microscope. Ki 67 immunoreactivity was considered in cases having 10% positive nucleus (Qin, L. X., et al., 2002).

DNA ploidy Analysis:

The DNA content study was done following the protocol of University of Virginia[1].

Method: From a total of 100 previously diagnosed pre-neoplastic cases of cervix along with controls, cells were collected using cytobrush in 12x75 mm tube containing 4.5 ml of 70% ethanol kept on ice. The collected samples were immediately dipped in 5 ml of PBS pH-7.6.

Samples and controls were put to test tubes containing PBS PH 7.6 and centrifuged for 5 minutes in 300 g. The pellet was re-suspended in PBS PH 7.6. A total of 10^6 to 10^7 cells were taken from it in 5 ml PBS PH 7.6 and suspension was made. The suspension again centrifuged at 300g for 5 minutes and the supernatant was discarded, and cells were re-suspended as single cell suspension in 0.5 ml PBS. The cells were then transferred into a test tube containing chilled 70% ethanol and kept for 2 hours. The ethanol fixed cells were again centrifuged for 5 minutes at 300g and the supernatant was discarded thoroughly. The cell pellet was suspended in 5 ml PBS for 1 minute and again centrifuged for 5 minutes in 300 g. The supernatant was discarded, and the cells were suspended in 1 ml PI solution and kept under dark for30 minutes at room temperature.

Flow cytometry study was carried out with the help of FACS calibre Flow cytometer of Becton Dickinson make using an ergon laser, wavelength 485 nm run at 15 mV. Calibration was carried out using latex beads. Maximum permitted error was 3%. Histogram data was generated, and DNA index was calculated according to the following formula.

DNA Index=Mean Go/G1peak of test sample/Mean Go/G1 peak of control. Histogram interpretation was done according to the method of Bergers, E., van Diest, P. J., & Baak, J. P., (1997).

Apoptosis:

From 100 study cases and controls, tissue sections were deparaffinised following standard protocol. Sections were rehydrated with PBS PH 7.6, blotted and were treated with proteinase K at 37degree centigrade for 12 minutes and rinsed with 2 changes in PBS for 10 minutes. The sections were incubated with 3% H2O2 in PBS for 10 minutes for blocking endogenous peroxidase. The tissue sections were then rinsed with PBS for 3 times, 2 minutes each. The sections were then treated with reaction buffer for 10 minutes followed by incubation in TdT reaction mixture for1.5 hours at 37-40 degree centigrade in humidified chamber. The reaction was stopped by

[1] http://hsc.virginia.edu/intrnet/cytometry/protocols/dnacontent.efm

adding stop wash buffer and incubated for 10 minutes. The sections were thoroughly washed with PBS thrice for 2 minutes each. Streptavidin HRP antibody (ultrasensitive S2438, Sigma, USA) was added to the sections and incubated for10 minutes then rinsed 3 times with PBS. The sections were treated with DAB solution for 2 minutes and rinsed in running tap water for 5 minutes. Slides were then dehydrated through graded alcohol and dried and cleaned in 2 changes of xylene for 5 minutes each and mounted with DPX and visualised under light microscope.

Statistical Analysis

Statistical Analysis was performed with the help of Epi Info (TM) 7.2.2.2. EPI INFO is a trademark of the Centres for Disease Control and Prevention (CDC). Descriptive statistical analyses were performed to calculate the means with corresponding standard deviations (s.d.). Test of proportion was used to find the Standard Normal Deviate (Z) to compare the difference proportions and Chi-square ($\chi 2$) test was performed to find the associations. In the cases where one of the cell frequencies were less than 5 corrected Chi-square ($\chi 2$) was used to find the association between variables. t-test was used to compare the means. $p<0.05$ was taken to be statistically significant.

3. Results

Status of p16 expression by Western Blot of the patients

The positivity for p16 expression by western blot is 72.0%, which was significantly higher than that of negativity (28.0%) ($p<0.00001$).

Table 1. Status of p16 expression by Western Blot

Status of p16 by Western Blot	Number	%	p-value
Positive	72	72.00%	
Negative	28	28.00%	p<0.00001*
Total	100	100.00%	

* Significant level ($p<0.05$), >0.050 -not significant.

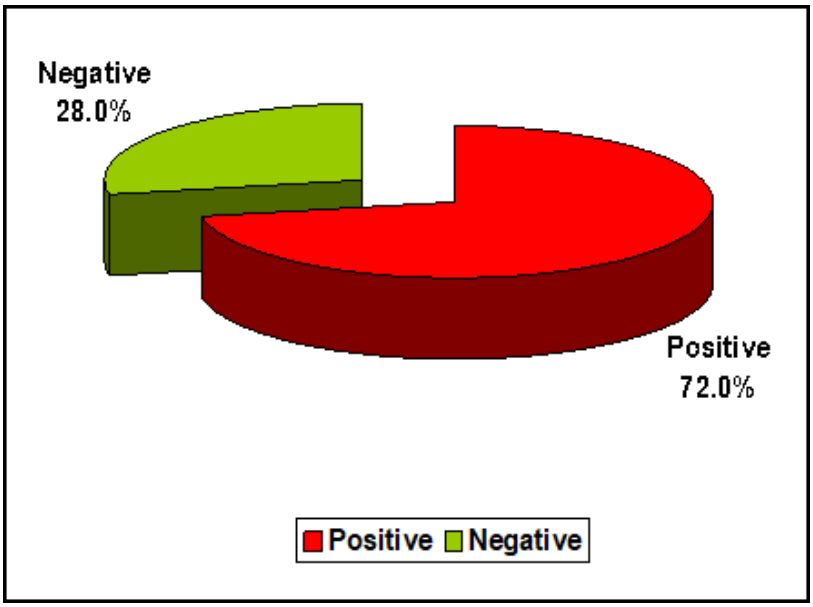


Figure 1. Pie chart showing Status of p16 expression by Western Blot

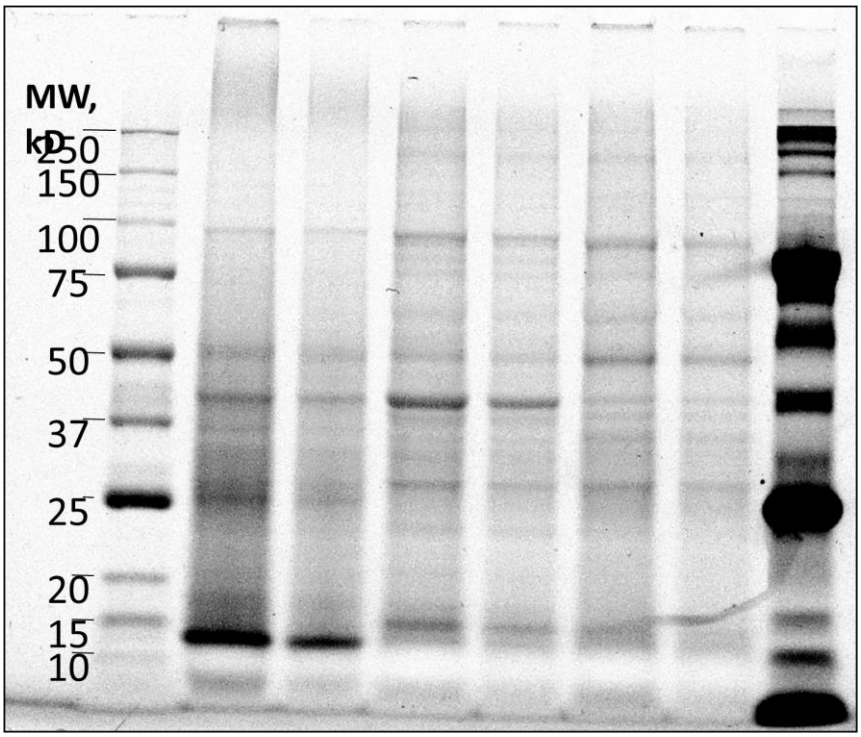


Figure 2. Stain-Free Gel image of Tissue protein samples

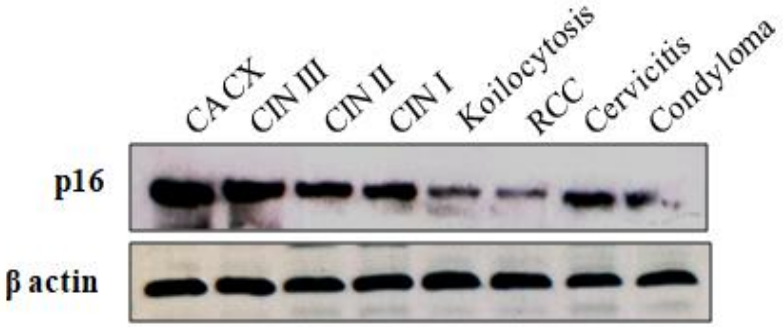


Figure 3. Western Blot of p16 protein in different cervical lesions

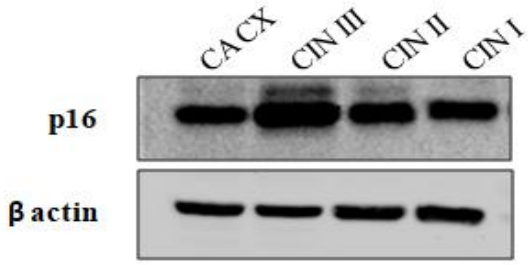


Figure 4. Western Blot of p16 protein in different grades of cervical intraepithelial neoplasia

Status of Ki-67 expression

Out of 100 cases, Ki67 immunopositivity (73%) was significantly higher than negative cases (27%) ($p<0.001$). CIN I (72.2%), CIN-II (77.8%) and CIN-III (67.6%) all were significantly prevalent among patients with Ki67 immunopositivity ($p<0.00001$). The expression for Ki67 increased from CIN I to CIN II (CIN I – 72.2%, CIN II

– 77.8%) and slightly decreased in CIN III (67.6%). 8 cases (88.9%) of other associated lesions showed Ki67 positivity (condyloma 1, cervicitis 2, RCC 1, Koilocytosis 4). Ki67 positivity with strong intensity (58.9%) was significantly higher than moderate intensity and mild intensity staining (26.0% and 15.1%, respectively) ($p<0.001$). Ki67 expression was significantly seen in the whole of the epithelium (87.67%) ($p<0.001$).

Table 2. Status of Ki-67 expression

Biomarkers	Result of IHC	Number	%	p-value
Status of Ki67	Positive	73	73.0%	p<0.001*
	Negative	27	27.0%	
	Total	100	100.0%	

* Significant level ($p<0.05$), >0.050 -not significant.

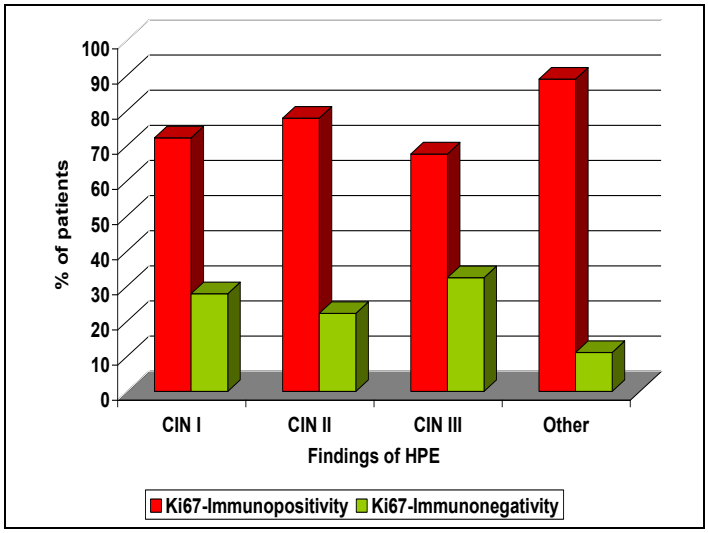
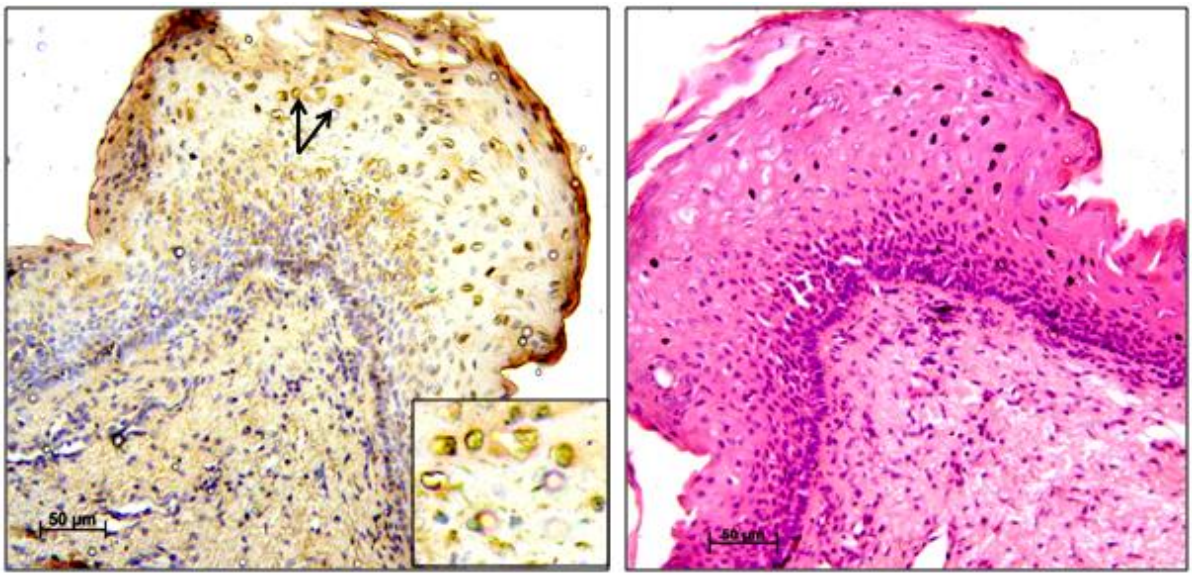


Figure 5. Bar diagram showing the association between Ki67 Immunohistochemical result and findings of HPE of the patients



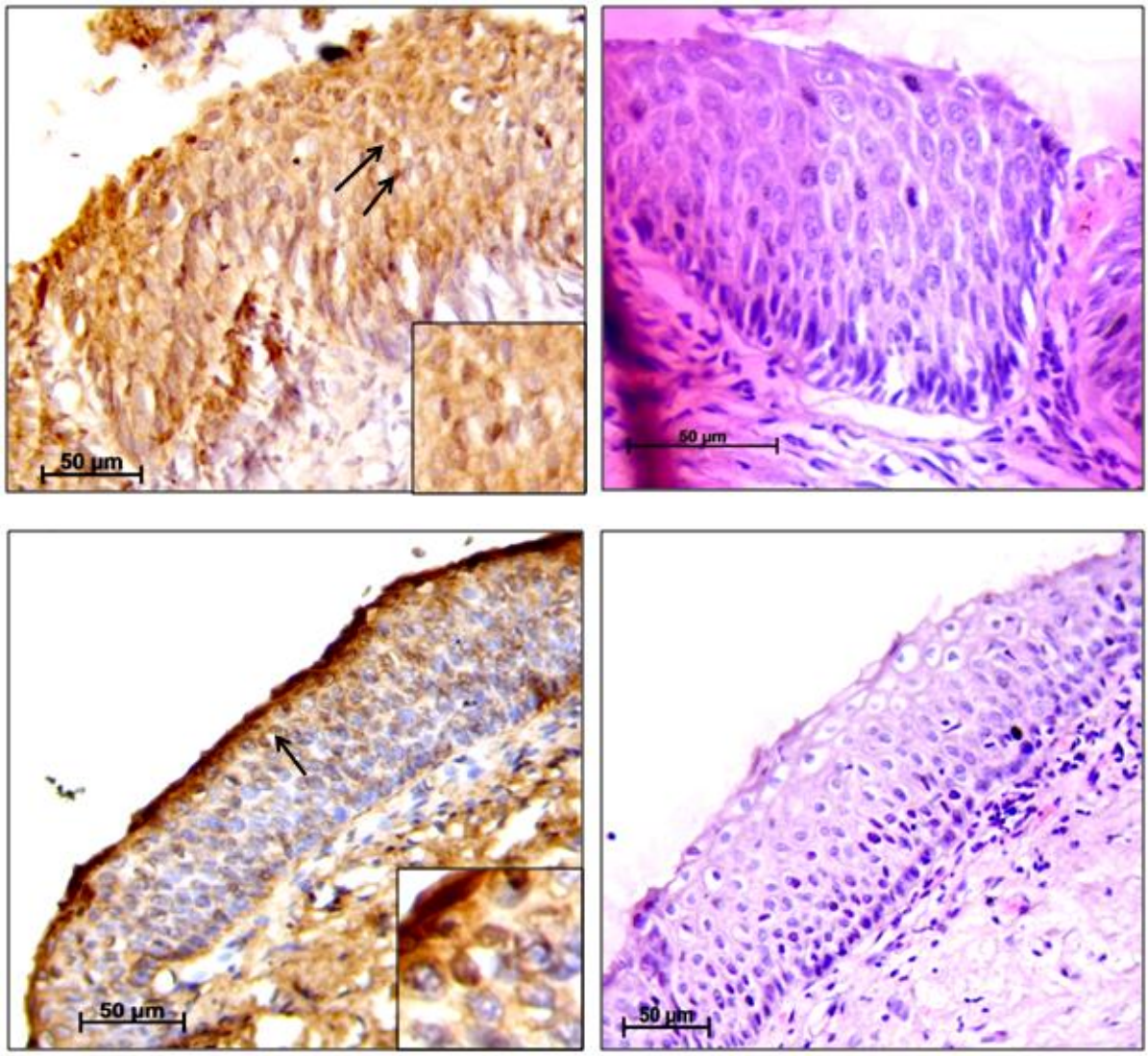


Figure 6. A(a) Ki67 immunopositivity in Cervical Intraepithelial Neoplasia I (CIN I), A(b) Corresponding Histopathology (HP) image (H/E stain); B(a) Ki67 immunopositivity in CIN II, B(b) Corresponding HP image (H/E stain); C(a) Ki67 immunopositivity in CIN III, C(b) Corresponding HP image (H/E stain). The magnification of the main and inset image is 20X and 40X, respectively. Black arrows denote Ki67 immunopositive cells.

Status of TUNEL assay in patients

The test of proportion showed that patients with positive TUNEL assay (63%) were significantly higher than patients with negative TUNEL assay (37%) ($p<0.00024$).

Table 3. Status of TUNEL assay of the patients

Status of TUNEL assay	Number	%	p-value
Positive	63	63.00%	
Negative	37	37.00%	p<0.00024*
Total	100	100.00%	

* Significant level ($p<0.05$), >0.050 -not significant.

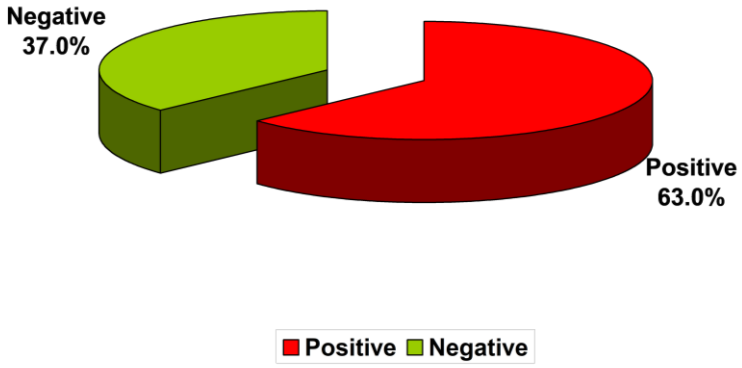


Figure 7. Pie chart showing the status of TUNEL assay of the patients

Association between the status of TUNEL assay and Histopathological findings

CIN I (58.3%), CIN II (61.1%), and CIN III (83.8%) were significantly prevalent among patients with positive for TUNEL assay (p=0.023; p=0.002; p<0.00001).

Apoptotic Index significantly increased from CIN I (58.3%) to CIN II (61.1%) to CIN III (83.8%). 1 case (11.1%) with other pathological conditions (condyloma) showed positive for TUNEL assay.

Univariate analysis showed that CIN III had a significant risk for apoptosis. However, no risk of apoptosis was found in CIN I and CIN II cases.

Table 4. Association between Result of TUNEL Assay and findings of HPE of the patients

Result of TUNEL Assay	**Histopathological Grades**				**TOTAL**
	CIN I	CIN II	CIN III	OTHER	
Positive	21 (58.3%) p=0.023*	11 (61.1%) p=0.002*	31 (83.8%) p<0.00001*	1 (11.1%)	63 (63.0%)
Negative	15 (41.7%)	7 (38.9%)	6 (16.2%)	8 (88.9%)	37 (37.0%)
TOTAL	36 (100.0%)	18 (100.0%)	37 (100.0%)	9 (100.0%)	100 (100.0%)

*Significant level (p<0.05), >0.050 -not significant.

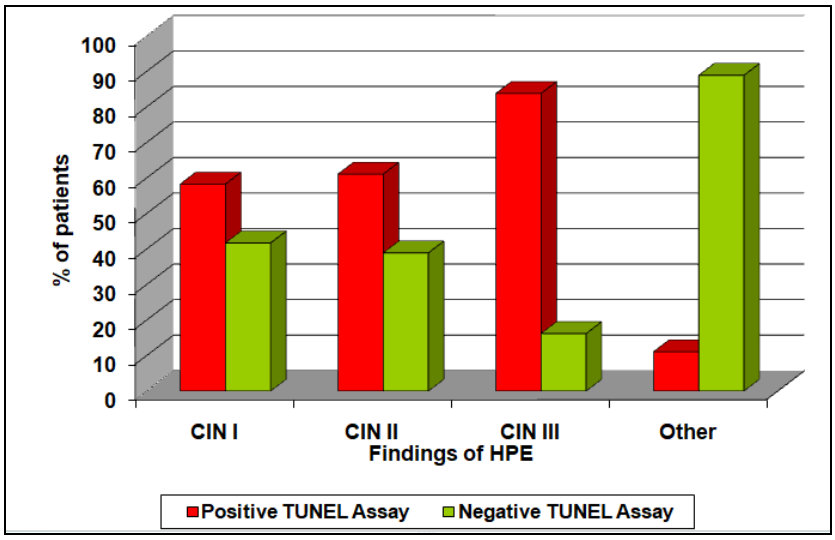
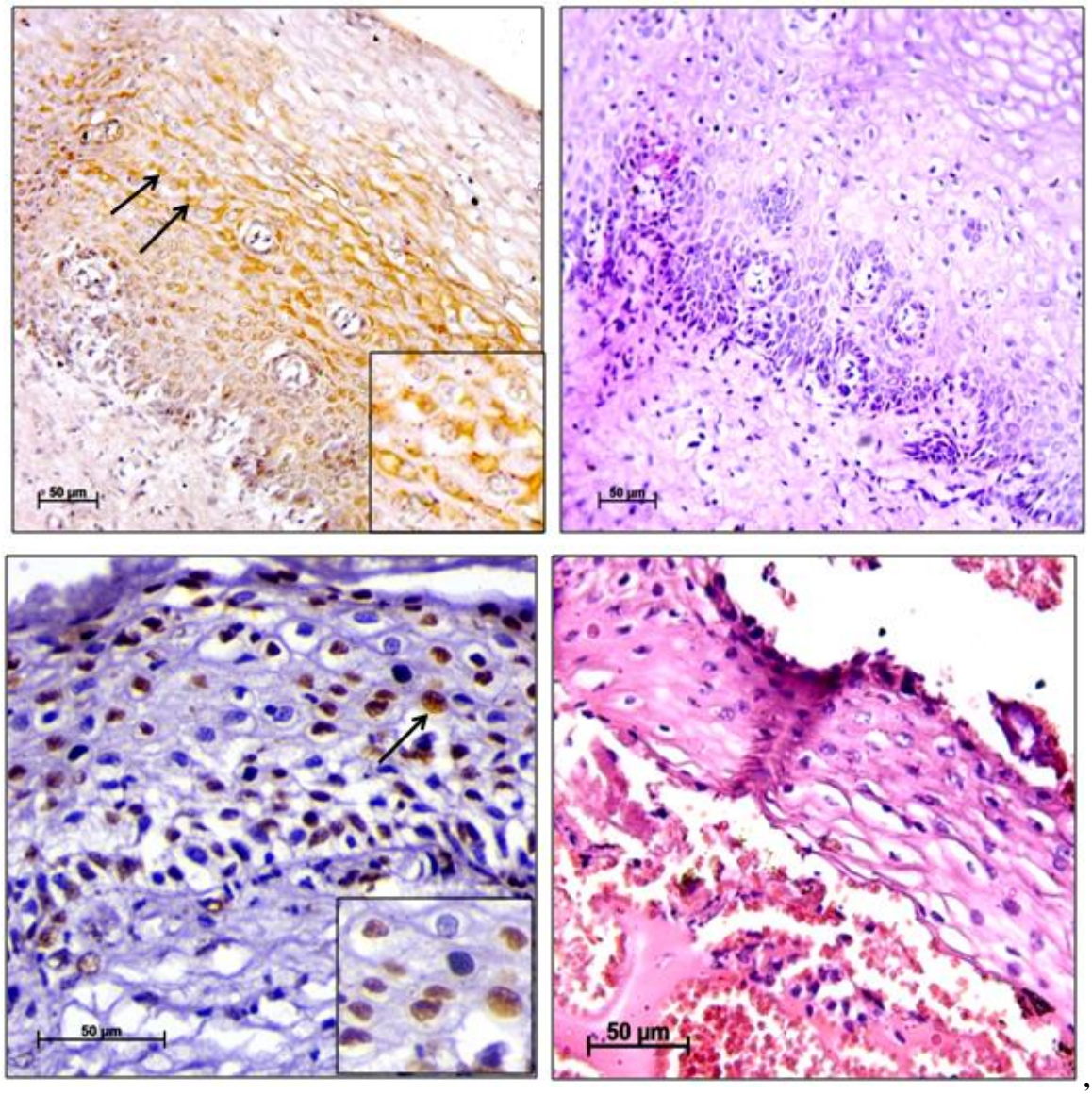


Figure 8. Bar diagram showing association between Result of TUNEL Assay and findings of HPE of the patients

Table 5. Risk factors associated with Apoptotic Index content and Histopathological grades

Biomarkers	**Histopathological grades**					
	CIN-I		CIN-II		CIN-III	
	OR with 95% Confidence Interval	p-value	OR with 95% Confidence Interval	p-value	OR with 95% Confidence Interval	p-value
Positive TUNEL assay	0.733(0.316,1.698)	0.468	0.859(0.300,2.475)	0.777	5.005(1.833,13.661)	<0.0001*

* Significant level (p<0.05), >0.050 -not significant.



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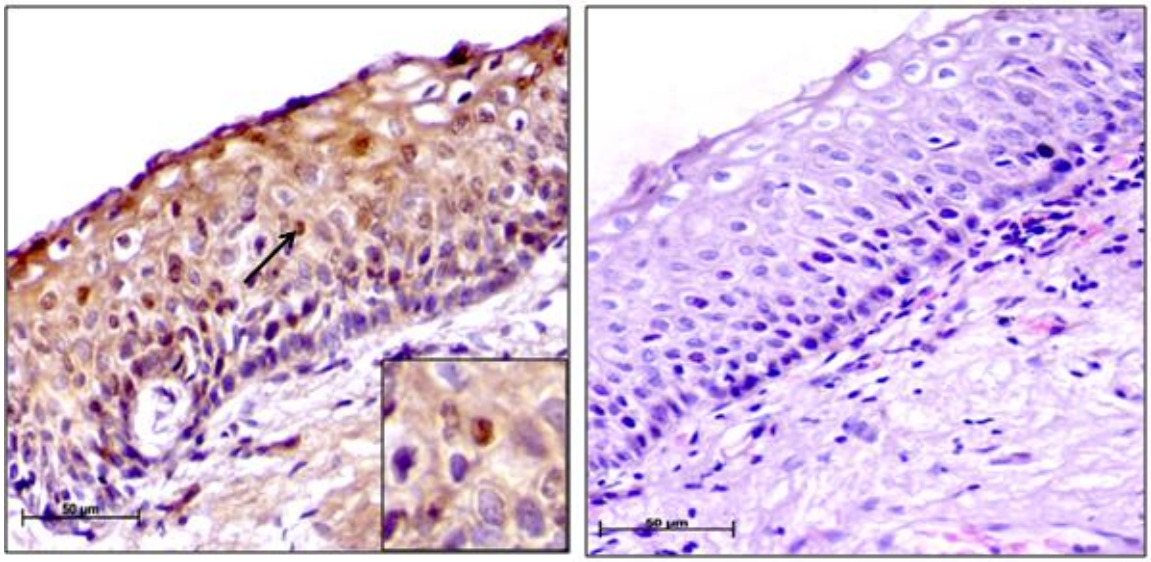

Figure 9. A(a) Cervical Intraepithelial Neoplasia I (CIN I) with TUNEL positive cells, A(b) Corresponding Histopathology (HP) image (H/E stain); B(a) CIN II with TUNEL positive cells, B(b) Corresponding HP image (H/E stain); C(a) CIN III with TUNEL positive cells, C(b) Corresponding HP image (H/E stain). The magnification of the main and inset image is 20X and 40X, respectively. Black arrows denote TUNEL positive cells.

Status of DNA content in patients

All 100 cases were included in this study, and DNA content was detected by flow cytometry. 57% of the cases showed aneuploid DNA content, and the remaining 43% had diploid DNA content. Thus, Patients with aneuploid DNA content (57.0%) were significantly higher than patients with diploid DNA content (43.0%%) ($p<0.047$).

Aneuploid DNA content was significantly prevalent among patients with CIN II (66.7%) and CIN III (70.3%) ($p<0.00001$) and significantly increased from CIN I (50.0%) to CIN II (66.7%) to CIN III (70.3%). 1 case (11.10%) with other pathological conditions (condyloma) showed aneuploid DNA content.

Univariate analysis showed that CIN III had a significant risk for aneuploidy. The risk for aneuploidy was also found among CIN II patients, but it was not significant. However, no risk of aneuploidy was found in CIN I cases.

Table 6. Status of DNA content (FACS) of the patients

Status of DNA content	Number	%	p-value
Aneuploid	57	57.00%	
Diploid	43	43.00%	p=0.047*
Total	100	100.00%	

* Significant level ($p<0.05$), >0.050 -not significant.

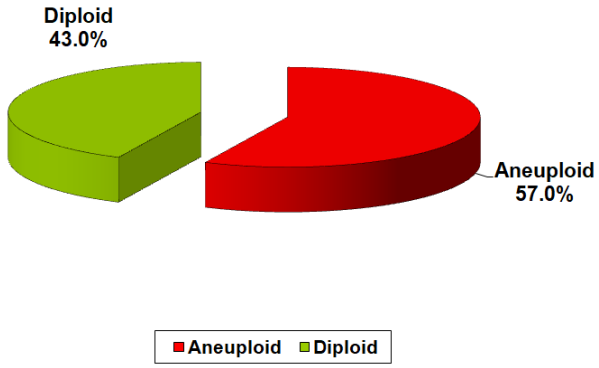

Figure 10. Pie chart showing the status of DNA content (FACS) of the patients

Table 7. Association between DNA Content Status and findings of HPE of the patients

DNA Content Status	Histopathological Grades				TOTAL
	CIN I	CIN II	CIN III	OTHER	
Aneuploid	18 (50.0%)	12 (66.7%) p<0.00001*	26 (70.3%) p<0.00001*	1 (11.1%)	57 (57.0%)
Diploid	18 (50.0%)	6 (33.3%)	11 (29.7%)	8 (88.9%)	43 (43.0%)
TOTAL	36 (100.0%)	18 (100.0%)	37 (100.0%)	9 (100.0%)	100 (100.0%)

* Significant level (p<0.05), >0.050 -not significant.

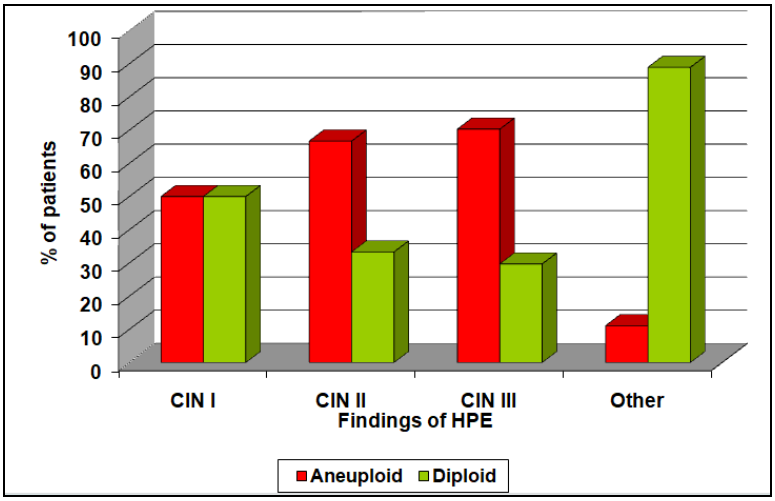

Figure 11. Bar diagram showing the association between DNA Content Status and findings of HPE of the patients

Table 8. Risk factors associated with Aneuploid DNA content and Histopathological grades

Biomarkers	Histopathological grades					
	CIN-I		CIN-II		CIN-III	
	OR with 95% Confidence Interval	p-value	OR with 95% Confidence Interval	p-value	OR with 95% Confidence Interval	p-value
Aneuploid DNA content	0.641[0.281,1.461]	0.288	1.644[0.562,4.804]	0.360	2.439[1.031,5.770]	0.039*

* Significant level (p<0.05), >0.050 -not significant.

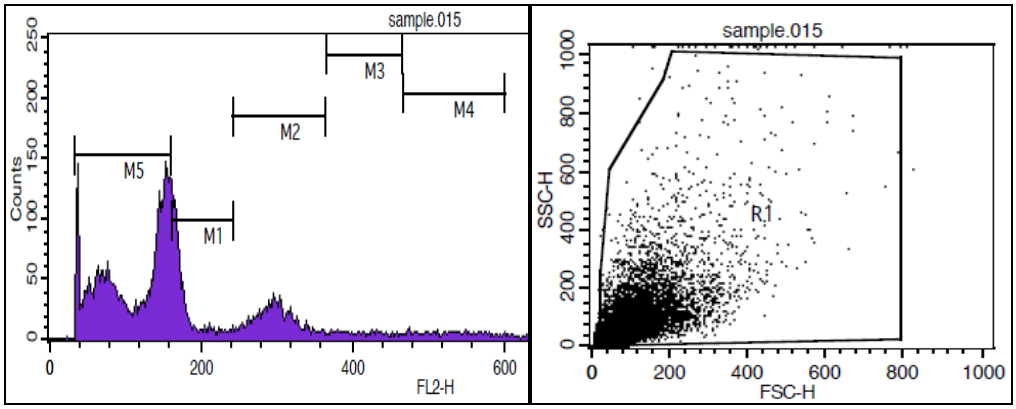

Figure 12. A. Histogram and Dot plot of CIN I showing diploid DNA content [DNA Index (DI) - 0.88]; B. Histogram and Dot plot of CIN II showing diploid DNA content (DI- 0.84); C. Histogram and Dot plot of CIN III showing diploid DNA content (DI- 0.97)

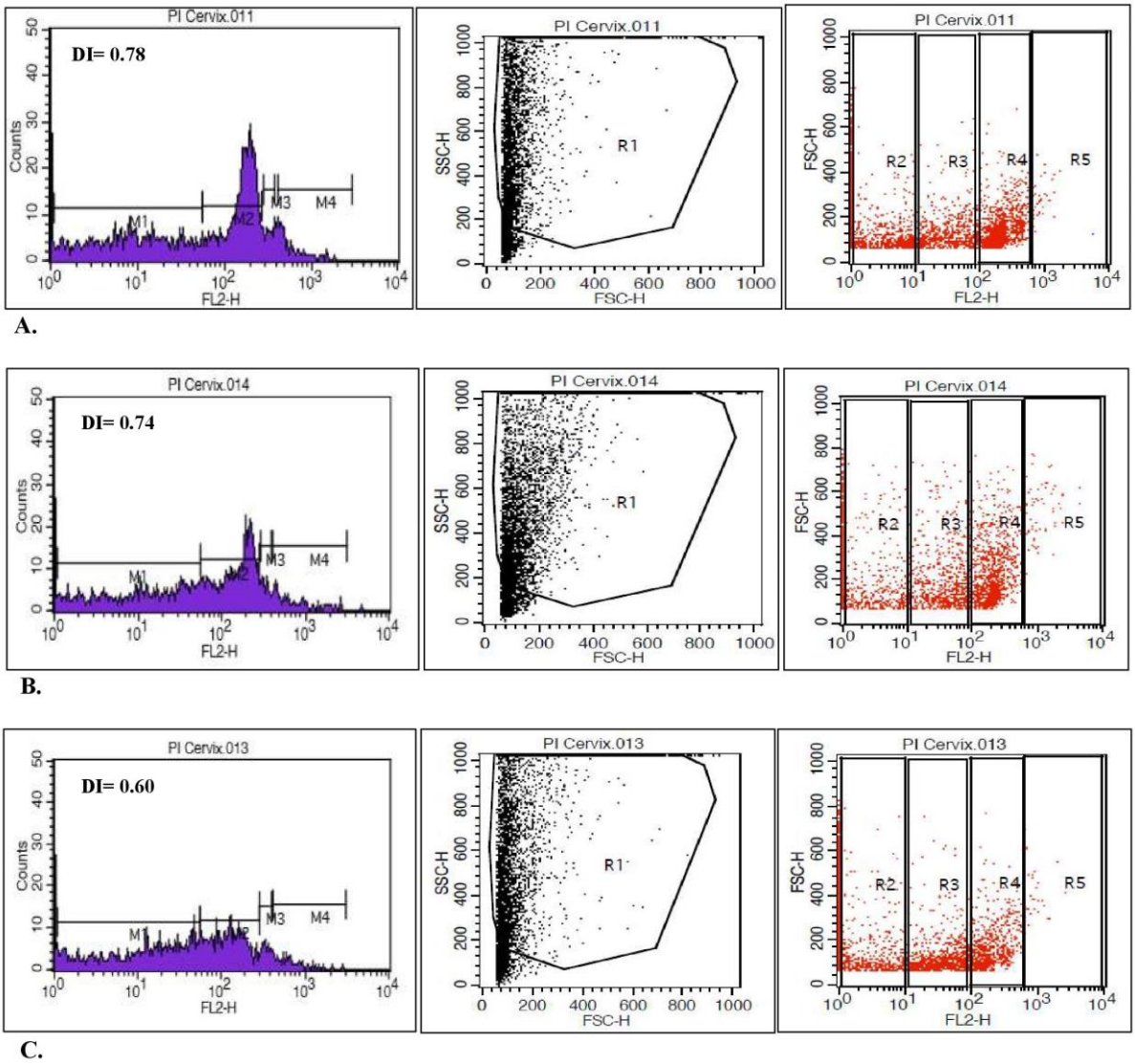

Figure 13. A. Histogram and Dot plot of CIN I showing aneuploid DNA content (DI - 0.78); B. Histogram and Dot plot of CIN II showing aneuploid DNA content (DI- 0.74); C. Histogram and Dot plot of CIN III showing aneuploid DNA content (DI- 0.60)

4. Discussion

p16 overexpression has been considered as a surrogate marker for high risk HPVs and discriminates it from non -integrated low grade HPV infection (I. Lesnikova, M. Lidang, S. Hamilton-Dutoit, et al., 2009; Sarma, U., Biswas, I., Das, A., Das, G. C., Saikia, C., & Sarma, B., 2017). Thus, it is considered as specific and sensitive biomarker of cervical pre neoplastic and non-neoplastic lesions and high-risk HPV association.

In this study, the high-risk HPV (HR HPV) association was studied, by western -Blot Analysis. The study shows that 72% cases of pre neoplastic lesions expressed HR HPV association. Two cases of Koilocytotic changes were also found to be positive for HR HPV infection. These two cases were negative for p16 immunohistochemistry. The above fact shows the higher sensitivity and specificity of Western Blot analysis over Immunohistochemistry.

Many CIN III cases expected to regress spontaneously (I. Lesnikova, M. Lidang, S. Hamilton-Dutoit, et al., 2009). The rate of regression of CIN III is almost threefold in comparison to the progression of invasive carcinoma. Therefore, the decreased conversion of p16 in high grade cervical lesions justifies the theory of regression. On the other hand, many research articles expressed view that the p16 expression increases progressively with change of histopathological grade such as CIN I has the lowest expression and CIN III has the highest (Tsoumpou, I., et al., 2009; von Knebel Doeberitz, et al., 2012; Wu, J., et al., 2014), which subsequently progress to carcinoma cervix.

Ki67, a cell cycle associated protein, is necessary for cellular proliferation and is also associated with ribosomal RNA transcription. It is expressed in all active phases of the cell cycle except resting cells (Go). According to many studies Ki 67 considered as gold standard for proliferative marker to measure the proliferative capacity of almost all malignancies (L.L. Mee, H.Z. Ahmad, D. Mohd, et al., 2008; K. Raju, S. S. Punnayanapalya & N. Mariyappa, 2015).

Normal cervical squamous epithelium shows proliferation within physiological limit and is expressed in paranasal cells. However, with increasing grades of cervical lesions, Ki67 expression increases which may be due to inactivation of p53 and Rb by E6 and E7 viral oncoproteins of HPV respectively (W. Feng, J. Xian, Z. Zhang, et al., 2007; Hwang, S. J., & Shroyer, K. R., 2012) and Ki67 expression becomes strong and more diffused involving the whole length of the epithelial layer (W. Feng, J. Xian, Z. Zhang, et al., 2007).

In the present study, Ki67 expression was highly significant and showed positivity in 73% of the cases which was similar to the finding of “Vasilescu et al., (2009)” (76.92%) and “Godoy et al., (2014)” (72.6%). Most of the studies reported that Ki67 expression increased with increasing grades of lesion (C. Isacson, T. D. Kessis, L. Hedrick, et al., 1996; J.M. Kim, D.M. Shin, A El-Naggar, et al., 2001; M.L. Looi, A. Z. Dali, S. A. Ali. et al., 2008; K. Gupta, K. Alam, V. Maheshwari, et al., 2013). This study also found that Ki67 expression increased from CIN I to CIN II but the expression slightly reduced in CIN III (CIN I 58.3%, CIN II 83.3%, CIN III 50%), which was again comparable with the study conducted by “Nam et al.” (E.J. Nam, J.W. Kim, J.W. Hong, et al., 2008).

This decrease in expression may be due to increase in host defence mechanism. It was also found that the strong intensity of Ki67 positivity was significantly higher than moderate and mild intensity, and also the Ki67 expression was significant in all layers of squamous epithelium for positive (CIN) cases similar to the findings of “Feng et al., (2007) & Looi et al.” (2008). Study of “Looi et al.” (2008), also stated that overexpression of Ki67 confirms the progression and aggressive behaviour of dysplastic and tumour cells. Whereas “Carreras et al.”, (2007) in their study has shown that Ki67 expression is useful in distinguishing the different grades of dysplasia but is unable to predict their behaviour.

In 1869, “Friedrich Miescher”, a Swiss physiological chemist, first isolated and identified DNA. The basal genome size of an organism is defined as the content of DNA, which is measured by weight or number of base pairs in a single copy of the entire sequence of DNA found within cells of that organism (T. R. Gregory, P.D.N. Hebert, 1999). It is the most frequently measured entity of the cell (Z. Darzynkiewicz, 2011) and its quantification serves to assess DNA ploidy level, cell position in the cell cycle and may also reveal the presence of apoptotic cells.

DNA content measured by cytometry is termed as DNA ploidy or DNA index (T.R. Gregory, P.D.N. Hebert, 1999; Z. Darzynkiewicz, 2011).

Persistent infection with high-risk HPVs is considered to be the strongest independent risk factor for cervical pre-neoplastic lesions and cervical cancer and chromosomal aberrations have been reported in pre-neoplastic lesions and cervical cancer (M. Singh, S. Mehrotra, N. Kalra, et al., 2008). Chromosomal aneuploidy is considered as an early key event in tumorigenesis caused by genomic instability (Melsheimer, P., et al., 2004; M. Singh, S. Mehrotra, N. Kalra, et al., 2008). Thus, DNA ploidy or DNA content assessment by flow cytometry technique can be used as a significant prognostic marker in cervical pre-neoplastic lesion and cervical cancer as aneuploid cell clones are more likely to survive and progress to more advanced dysplasia as compared to diploid cell clones. High-grade dysplasia, along with aneuploidy, has a 66% risk of developing cancer within five years

(J.M. Dunn, G.D. Mackenzie, D. Oukrif, et al., 2010).

Another study has reported the incidence of aneuploidy to vary between 14% and 75.5% in CIN I, 55% and 64.3% in CIN II and 83.3% and 95.2% in CIN III (M. Singh, S. Mehrotra, N. Kalra, et al., 2008; J.M. Dunn, G.D. Mackenzie, D. Oukrif, et al., 2010) in a study have shown that aneuploid DNA content could be used as a biomarker of progressive cervical changes toward malignancy and have found progressive increase in aneuploidy with the increase in severity of the lesion (49.36% for mild dysplasia, 77.77% for moderate dysplasia versus 91.66% for severe dysplasia) (M. Singh, S. Mehrotra, N. Kalra, et al., 2008; D. Demirel, N. Akyürek & I. Ramzy, 2013) in their study also reported abnormal DNA content in 65% of cases with cervical pre-cancerous lesions (D. Demirel, N. Akyürek & I. Ramzy, 2013). However, Melsheimer et al., 2004 reported the percentage of aneuploidy ranged from 20% in low-grade lesions (CIN I) to 32% in high-grade lesions, but they also stated that DNA aneuploidy was significantly linked to the degree of neoplastic progression (Melsheimer, P., et al., 2004). DNA ploidy can be used as a valid clinical test used in the assessment of prognosis of the cancer patients. (C.E. Duarte, et al., 2014; Aziz, D. C., & Peter, J. B., 1991).

In this study, 57% of the cases showed aneuploid DNA content, and the remaining 43% had diploid DNA content. The results of this study also found a progressive increase in aneuploidy from CIN I (50.0%) to CIN II (66.7%) to CIN III (70.3%) which was similar with the findings of “Singh et al., (2008) & Demirel et al., (2013)”. “Duesberg et al, (2004)” stated that lesions with aneuploid DNA content are considered more dangerous and aggressive. “Bollmann et al., (2001)” suggested that ASCUS with abnormal DNA content represented similar biological features as HSIL and had elevated risk to develop cancer (Bollmann, R., et al., 2001). However, univariate analysis of this study showed that CIN I with aneuploid DNA content does not carry risk, but CIN II with aneuploid DNA content carries risk, whereas CIN III with aneuploid DNA content carries a significant risk for cancer conversion.

By measuring cellular DNA content, patients at risk could be identified earlier, and treatment regimens could be given selectively to improve the current poor survival rate of cervical cancer. Thus, flow cytometric analysis of DNA ploidy may provide a strategic tool for early detection of carcinoma of uterine cervix.

Therefore, the present study supports the fact that the measurements of DNA content by flow cytometry provide important information, and the presence of aneuploidy could serve as a useful biomarker for assessing risk in cervical pre-neoplastic lesions.

Apoptosis or programmed cell death involves genetically determined elimination of damaged cells with defective repair process (Kerr J. F., 2002; J.F. Kerr, et al, 1972). It is same as an inherent anti-malignant programme of the host cells during the course of carcinogenesis (S.C. Tan & R. Ankathil, 2015).

In HPV infected cells apoptosis is a part of host defense mechanism for production of new virus particles and replication (S.C. Tan & R. Ankathil, 2015). Apoptosis is also responsible for maintaining number of cells by tight regulation and tumour formation (S.C. Tan & R. Ankathil, 2015). This pathway justifies the non-conversion of most of the CIII for malignancy. Apoptosis can be a useful biomarker for evaluating proliferative activity and progressive potential of the cervical pre-neo-plastic lesions and cervical cancer.

Studies have shown that inhibition of apoptosis plays a role in carcinogenesis since evasion of apoptosis allows accumulation of cells with damaged and unchecked genetics alteration and may also lead to unbalanced proliferative activity of tumours (M.M. Garrity, L.J. Burgart, D.L. Riehle, et al., 2003).

In this study, 63% cases were positive for Tunnel assay and the rate of apoptosis progressively increased from CINI (58%), CINII (61.1%), and CINIII (83.8%). Similar results were also observed by “Chauhan et al., (2016), Mysoekar, et al., (2008), Bharadaj. et al., (2015)”.

Univariate analysis revealed that CINIII had significant relation with apoptosis. In a study by “Gupta et al.,” also reported that apoptotic index increased progressively with increased grades of dysplasia. However apoptotic index between CIN II and CIN III was not significant but apoptotic index between CIN I and CIN II, and between CIN I and CIN III were statistically significant (K. Gupta, K. Alam, V. Maheshwari, et al., 2013).

From the above discussions, it can be stated that apoptosis is a useful and reliable biomarker for risk categorization of cervical pre neoplastic lesions that have high risk for malignant conversion.

5. Conclusions

Cervical cancer causes enormous health, social and economic problem in India and around the world. Apart from HR HPV expressed in CIN’s, other lesions such as koilocytotic changes are also caused by HR HPV.

The High-risk HPV association1 and cervical cancer is a well-established fact. Preventive vaccination for it is an important step. However certain percentage of cervical adenocarcinoma are HPV independent.

Therefore, early diagnosis of pre-neoplastic lesions, risk categorization by marker study and treatment are the

most important steps needed for stopping malignant conversion. The early diagnosis and risk assessment are not only lifesaving but also reduce the treatment cost and incidence of cervical cancer.

Ethics Approval and Consent to Participate

The study design (Project) was ethically approved by the Ethical committee of Chittaranjan National Cancer Institute (CNCI), Kolkata. CNCI is an autonomous Institute under the Ministry of Health and Family Welfare, Govt. of India.

List of Abbreviation

HR-HPV, CDKN2A, Ki-67, P53, DNA-PLOIDY, FACS, TUNEL, CINS, IARC, CDC-OPD, CDS-PAGE, ELISA, HRP, DAB.

Data Availability

To guarantee the confidentiality of personal and health information, only the authors have had access to the data. The data set may be available for research upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding Statement

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Authors' Contributions

All the authors including AKR, DG, AR, contributed to either conception or design of the study, acquisition of data, or analysis and interpretation. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The corresponding author had final responsibility for the decision to submit for publication.

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Compliance with Hygiene Protocols Related to the Dress Code of Nursing Students

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Abstract

Background: Healthcare-associated infections (HAIs) affect up to 20% of hospitalized patients in developing countries. Hygiene dress code protocols play a vital role in infection prevention, particularly among nursing students, whose attire and behavior in clinical settings can significantly impact cross-contamination risks. ***Objective:*** This study aimed to evaluate the knowledge, attitudes, and practices of nursing students regarding compliance with hygiene-related dress code protocols, with a focus on uniform use, changing habits, and adherence outside healthcare settings. ***Methods:*** A descriptive, quantitative, and observational study was conducted from January to March 2024 at the Institut Supérieur Privé des Études en Sciences Infirmières (ISEPSI). A total of 200 randomly selected second- and third-year nursing students completed a structured, anonymous questionnaire. Data were analyzed using SPSS software. ***Results:*** The majority of participants were female (80.5%) with a mean age of 23.1 years. Most recognized key elements of appropriate clinical attire, including clean uniforms (93%) and no jewelry (87.5%). While 82% were aware of official dress protocols, only 56% systematically changed attire when entering and leaving clinical facilities. Notably, 49% admitted wearing uniforms outside the healthcare environment. Key barriers to adherence included lack of time (82.05%), inadequate changing facilities (69.23%), lack of spare uniforms (61.53%), and work pressure (51.28%). Only 46% ensured they avoided touching non-sterile areas of their attire after hand hygiene. Students strongly supported increased awareness efforts, practical training, access to extra uniforms, and improved changing infrastructure. ***Conclusion:*** Despite good awareness of hygiene protocols, nursing students face several obstacles in implementation, primarily due to systemic and logistical challenges. Reinforcing hands-on training, improving facilities, and enhancing supervisory support are essential to promote compliance, reduce infection risks, and strengthen professional conduct in clinical environments.

Keywords: nursing students, hygiene dress code, infection prevention, uniform protocol, healthcare-associated infections, clinical practice compliance

1. Introduction

Healthcare-associated infections (HAIs) affect 5–10% of hospitalized patients in developed countries and up to 20% in developing regions, representing a major global health concern (Allegranzi, B., et al., 2011). Hospital hygiene protocols play a central role in preventing these infections, and the attire of healthcare workers, including nursing students, is a critical component of infection control (Otter, J.A., et al., 2013; Spruce, L., 2017). Official guidelines recommend clean uniforms, closed shoes, tied hair, no jewelry, and visible ID badges (Loveday, H.P., et al., 2014), along with changing attire when entering or leaving healthcare facilities to reduce

pathogen transmission (Gillespie, B.M., et al., 2015). However, studies report gaps between these recommendations and the practices of nursing students, often due to insufficient training or awareness (Wilson, J.A., et al., 2007). Wearing uniforms outside clinical settings, especially on public transport, increases contamination risks (Mitchell, A., et al., 2015). Infrequent changing or improper laundering of uniforms can further lead to pathogen buildup (Edmonds, S.L., et al., 2013), underscoring the need to reinforce compliance with hygiene dress protocols. The objective of this study is to assess the knowledge, attitudes, and practices of nursing students regarding compliance with hygiene-related dress code protocols. More specifically, this study aims to analyze the conditions under which students change their uniforms, as well as their behaviors concerning wearing the uniform outside healthcare facilities and in public transportation.

2. Methodology

A quantitative, descriptive, and observational study was conducted between January and March 2024 at the Institut Supérieur Privé des Études en Sciences Infirmières (ISEPSI). A random sample of 200 second- and third-year nursing students was surveyed using a structured and anonymous questionnaire consisting of 30 questions divided into five sections: sociodemographic data, knowledge, practices, attitudes, and suggestions regarding hygiene dress protocols. Data were analyzed using SPSS software with descriptive and comparative statistical methods.

3. Results

The population study was predominantly female (80.5%; sex ratio 0.24), with a mean age of 23.1 years and 93% over 20 years old. Academically, 19% were in first year, 43.5% in second year, and 37.5% in third year. According to the respondents, the essential elements of appropriate clinical attire include a clean uniform (93%), absence of jewelry (87.5%), tied hair (77.5%), clean surgical scrubs (65%), and a visible identification badge (55.5%) (Table 1**)**.

The majority of students (82%) reported being aware of official recommendations or institution-specific protocols regarding clinical attire. Figure 1 and nearly three-quarters (74%) reported being aware of the sanctions or reminders applied in cases of non-compliance with dress protocols. Approximately 49% of students reported leaving the healthcare facility in their clinical attire to return home. About three-quarters of nursing students (72%) identified hands-on training during clinical placements as essential for better adherence to dress protocols, while over half (58%) suggested the use of explanatory videos. The main difficulties encountered in complying with these protocols are, according to the majority of the surveyed students, the lack of time (82.05%), inadequate facilities for changing clothes (69.23%), the lack of equipment or spare uniforms (61.53%), and work pressure (51.28%). According to the results obtained, it is observed that nearly three-quarters of the students surveyed, or 74%, stated that there are appropriate facilities for changing their clothing in the institution where they carry out their internships. More than half of the students surveyed, or 56%, reported that they always systematically change their clothing when entering and leaving the healthcare facility. Half of the students surveyed, or 50%, reported that they change their work attire once per day during a working day. This figure clearly shows that nearly half of the nursing students, or 46%, stated that after washing or disinfecting their hands, they sometimes make sure not to touch non-sterile areas of their clothing.

Table 1. Distribution of Respondents by Essential Clothing Elements in Healthcare Settings (Hospitals or Others)

Clothing Element	Frequency	Percentage (%)
Clean lab coat	186	93
Clean surgical scrubs	130	65
Hair tied back	155	77.5
No jewelry	175	87.5
Closed shoes	96	48
Visible identification badge	111	55.5
Clean gloves	2	1
Clean trousers	1	0.5
Discreet makeup	1	0.5
No nail polish	1	0.5
Bib apron	2	1

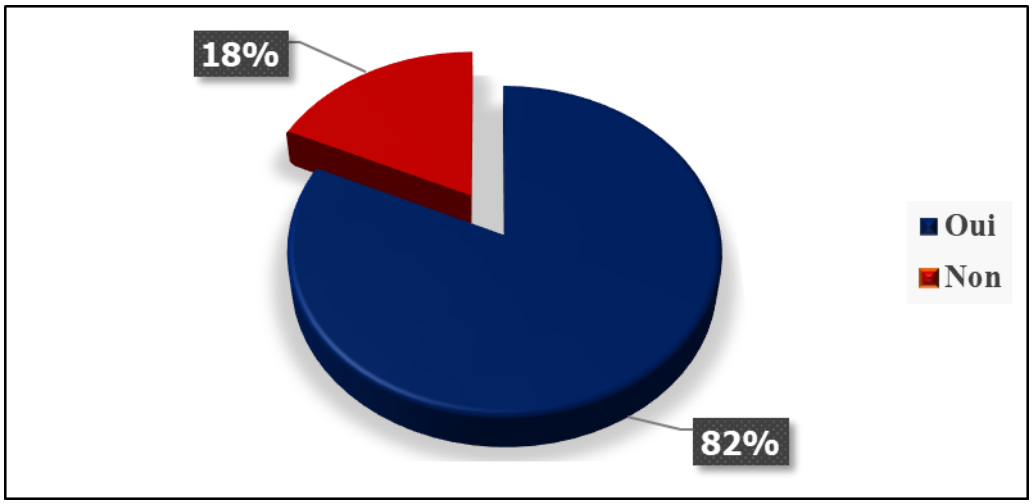

Figure 1. Distribution of respondents according to their knowledge of official recommendations or institution-specific protocols regarding dress code

4. Discussion

Our sample was predominantly female (80.5%; sex ratio 0.24), reflecting the ongoing feminization of the nursing profession (Lelièvre B & Chaffron S., 2019). The average age was 23.1 years, consistent with trends among nursing students (Chibane F, Boulaghmen A & Harzallah D., 2020). Most participants were in their second or third year, suggesting greater exposure to hygiene protocols. However, 64.5% reported not receiving formal training on dress code hygiene, echoing previous findings on insufficient curricular emphasis (Kammoun M, Ben Rejeb M & Lajmi K., 2021). As a result, although students identified key elements such as clean coats (93%), no jewelry (87.5%), and tied hair (77.5%), other crucial aspects like closed shoes (48%) and visible badges (55.5%) were less recognized (Duguet A, Alby F & Chapuis C., 2018; Martin G, Hermans L & Delsaux N., 2019). While 71.5% acknowledged the importance of attire in infection prevention, 69.5% mistakenly believed that clean clothing alone is sufficient — overlooking essential practices such as hand hygiene, cited by only 36% (World Health Organization, 2011; Ben Ayed H, Ben Hmida M, Daoud F, Fendri J & Abdelhedi S., 2020). This partial understanding underscores the need to strengthen education on standard precautions, combining attire and hygiene behaviors. A majority (82%) were aware of institutional clothing protocols, and 74% reported knowing the associated sanctions, factors that promote better compliance according to previous studies (Kebede A, Gerensea H & Meles K., 2017). Similarly, 74% noted the availability of changing rooms, which facilitate proper practice (Al-Khawaldeh OA, Al-Hussami M & Darawad MW., 2019). However, only 56% reported systematically changing their attire at the beginning and end of shifts, and 49% admitted to wearing uniforms outside healthcare settings, which increases contamination risk (Dagne H, Andualem Z, Dagnew B, Gizaw A & Adane T., 2021; World Health Organization, 2009). Moreover, 55% wore uniforms in break areas, contravening international guidelines (Al-Khawaldeh OA et al., 2019).

Regarding uniform maintenance, half of the students (50%) changed their uniforms daily, 35% twice daily, but 13% did so infrequently — contrary to official guidelines recommending changes after each shift or when soiled (Kebede A et al., 2017; Dagne H et al., 2021). While 83% avoided accessories (Loveday HP et al., 2014; Girou E, Oppein F., 2001), only 46% refrained from touching non-sterile areas after hand hygiene, a critical yet often neglected practice. These results stress the need for practical, ongoing training starting from the first year (WHO, 2004). Though 61% found it easy to follow dress protocols during placements, key barriers included time constraints (82.05%), inadequate facilities (69.23%), and a lack of spare uniforms (61.53%). Work pressure (51.28%) and, to a lesser extent, uniform discomfort (7.69%) also impacted adherence (Le Texier R, L'Her E & Mallédant Y., 2016).

Suggestions for improvement included better changing rooms (14%) and more training (7.5%), both aligned with existing recommendations. Only 52% of students made suggestions, possibly reflecting a lack of awareness of the issue. Furthermore, 53% perceived supervisors as tolerating non-compliance, indicating a need for stronger role modeling (Lee E & Cho S., 2016). Alarmingly, 52% had witnessed or heard of infections linked to non-compliance with clothing protocols, underlining the associated risks. Finally, 86% of students favored more frequent awareness efforts, confirming previous findings on their effectiveness. The most recommended methods were interactive training (52%), educational sanctions (47%), and informative posters (33%). Around 72% of students highlighted the value of hands-on training during placements, with 58% favoring instructional videos

over less effective written manuals (23.5%) (Lee E & Cho S., 2016). A large majority (85%) supported the provision of extra uniforms, and many suggested improving changing facilities (58.5%) or upgrading existing ones (41.5%) (Tonna J & Williams E., 2015). Regular evaluations were supported by 92% of students as a way to improve adherence, in line with findings from Tonna et al. (2015). Lastly, 70.5% supported frequent reminders from supervisors, and 49.5% advocated for stricter policies with sanctions, which should be balanced by positive reinforcement strategies (Watson P, O'Neill J & Roberts J., 2020).

Adherence to dress code protocols is essential for infection prevention, patient safety, and the professional image of nurses. This study revealed gaps in practical training, inadequate facilities, and limited institutional support as major barriers among nursing students. Improved hands-on training, better infrastructure (changing rooms and spare uniforms), regular supervision, and awareness campaigns were strongly recommended. Strengthening these areas can promote long-term compliance and enhance care quality.

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A Longitudinal Study on the Perioperative Period of Cardiac Surgery — Multiple Interactions and Effects of Heparin Pleiotropy and Immune Microenvironment

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Abstract

Objective: To investigate the differences and trends of multiple monitoring indicators during the peri-cardiopulmonary bypass (CPB) period in patients undergoing heart transplantation (HTx) and ventricular assist device (VAD) implantation, and to explore the interaction and impact of unfractionated heparin (UFH) on the immune microenvironment under short-term, high-dose UFH administration. ***Methods:*** This prospective study enrolled 15 HTx and 19 VAD recipients between 2023 and 2024. Monitoring was conducted at five observation points: pre-CPB, 30 min post-CPB, before shutdown, 10 min post-heparin neutralization, and 30 min post-heparin neutralization. Coagulation function (TAT, PIC, D-dimer, etc.), inflammatory markers (IL-6, etc.), and endothelial injury markers (TM, etc.) were assessed, with Anti-Xa not monitored pre-CPB. Biochemical indicators, including liver and kidney function (ALT, AST, Cr, etc.), blood glucose, and lipid profiles (TC, TG, etc.), were measured only at pre-CPB, before shutdown, and 30 min post-heparin neutralization. Intergroup differences and trend changes were analyzed. ***Results:*** Significant differences were observed between the two groups in age, BMI, CPB duration, heparin dose, and aortic cross-clamp time, whereas no significant differences were found in priming volume or protamine sulfate (PS) dosage. Pre-CPB, PIC ($P=0.019$) and CK ($P=0.044$) were significantly higher in the HTx group, while TP ($P=0.018$) and CHE ($P=0.023$) were significantly higher in the VAD group. No significant intergroup differences were observed 30 min post-CPB. At the before shutdown timepoint, TAT ($P=0.027$), ALT ($P=0.048$), AST ($P<0.001$), TP/ALB ($P<0.001$), TBA ($P=0.006$), CK ($P<0.001$), HBP ($P=0.038$), and IL-6 ($P<0.001$) were significantly higher in the HTx group. At 10 min post-UFH neutralization, PIC ($P=0.027$), IL-6 ($P=0.001$), and PT ($P=0.047$) were significantly higher in the HTx group. At 30 min post-UFH neutralization, AST ($P<0.001$), TBA ($P=0.027$), CK ($P=0.005$), and IL-6 ($P<0.001$) remained significantly elevated in the HTx group. Trend analysis revealed differing dynamic patterns between HTx and VAD groups for ALP, D-dimer (DD), HBP, LDL, PCT, TM, TBA, and tPAIC, while other indicators exhibited similar trends. UFH, APTT, and PT peaked during CPB and declined post-heparin neutralization, whereas DD (HTx group) peaked 10 min after heparin neutralization before declining. ALB, ATIII, FIB, and HDL reached their nadir during CPB. CK, AST, and IL-6 showed time-dependent increases, while CREA (HTx group) and HDL (HTx group) decreased over time. PCT (HTx group) displayed fluctuating changes. ***Conclusion:*** During the peri-CPB period in cardiac surgery, the pleiotropic effects and unique pharmacokinetics of UFH lead to complex and significant interactions with the immune microenvironment. High-dose UFH exerts a positive regulatory effect on immune homeostasis during CPB, while the immune microenvironment also influences UFH's anticoagulant efficacy through multiple

pathways. Additionally, the rapid clearance of UFH by PS may trigger a “second hit” on the immune microenvironment via inflammatory mechanisms.

Keywords: cardiopulmonary bypass (CPB), heart transplantation (HTx), ventricular assist device (VAD), unfractionated heparin (UFH), immune microenvironment, coagulation-inflammation interaction

1. Introduction

Cardiopulmonary bypass (CPB) serves as the cornerstone technology in cardiac surgery, with its management quality directly determining patient prognosis (Eltzschig, H.K., B. Zwissler, & T.W. Felbinger, 2003). CPB can induce multiple pathophysiological disorders, including abnormal shear stress caused by non-physiological blood flow (Jiang, Q., J. Sun, L. Xu, X. Chang, L. Sun, Y. Zhen, & Z. Guo, 2021), cascade activation of systemic inflammatory response syndrome (SIRS) (Evora, P.R., C. Bottura, L. Arcêncio, A.A. Albuquerque, P.M. Évora, & A.J. Rodrigues, 2016), imbalance in the coagulation-fibrinolysis system (Bartoszko, J., & K. Karkouti, 2021), oxidative stress (Zakkar, M., G. Guida, M.S. Suleiman, & G.D. Angelini, 2015), and ischemia-reperfusion injury (Salameh, A., & S. Dhein, 2015). These disorders intertwine to form a vicious cycle, ultimately leading to CPB-associated liver and kidney injuries during cardiac surgery (Kulthinee, S., M. Warhoover, L. Puis, L.G. Navar, & E.Y. Gohar, 2024; Wang, X.D., Z.Z. Zhao, X.Y. Yang, R. Bao, Y.Y. Wang, Y. Lan, Z.Y. Quan, J.F. Wang, & J.J. Bian, 2024).

Unfractionated heparin (UFH), as the fundamental anticoagulant for CPB, at present, it is still considered the “gold standard” for CPB anticoagulation (Chen, Y., P.H.Y. Phoon, & N.C. Hwang, 2022; Frederiksen, J.W., 2000; Erdoes, G., I. Birschmann, M. Nagler, & A. Koster, 2021), possesses mechanisms of action that extend far beyond traditional understanding. UFH not only enhances antithrombin (AT)-mediated inhibition of thrombin (IIa) and factor Xa through binding with AT, but also exhibits complex immunomodulatory effects. These include suppressing complement activation, modulating neutrophil-endothelial cell adhesion, and reducing von Willebrand factor (vWF) release, thereby significantly attenuating CPB-related inflammatory activation, protecting endothelial tissue, and regulating coagulation function (Beurskens, D.M.H., J.P. Huckriede, R. Schrijver, H.C. Hemker, C.P. Reutelingsperger, & G.A.F. Nicolaes, 2020; Weiler, J.M., R.E. Edens, R.J. Linhardt, & D.P. Kapelanski, 1992; Spiess, B.D., 2017). Conversely, the anticoagulant efficacy of UFH can be influenced by various factors in the immune microenvironment, including direct interactions between UFH and inflammatory mediators, as well as indirect effects resulting from insufficient anticoagulation due to cross-talk mechanisms between inflammation and coagulation networks (Maier, C.L., J.M. Connors, & J.H. Levy, 2024). Therefore, it warrants investigation to determine whether and to what extent changes in immune microenvironment homeostasis during the peri-CPB period of cardiac surgery affect UFH’s anticoagulant efficacy.

In addition, in extracorporeal circulation applications, sulfate protamine (PS) achieves precise neutralization of UFH for controllable anticoagulant management and provides the possibility for individualized weaning time selection. This is the key reason why unfractionated heparin remains the most widely used anticoagulant in extracorporeal circulation (Boer, C., M.I. Meesters, D. Veerhoek, & A.B.A. Vonk, 2018; Foubert, R., G. Van Vaerenbergh, G. Cammu, S. Buys, N. De Mey, P. Lecomte, S. Bouchez, S. Rex, & L. Foubert, 2024). The use of PS further leads to the unique pharmacokinetic characteristics of UFH in CPB — short-term high-dose infusion and rapid neutralization, which results in a significantly different impact of UFH on the immune microenvironment of patients during CPB compared to other clinical applications of UFH. This may further increase the complexity of dynamic changes in the patient’s microenvironment during extracorporeal circulation.

The treatment of end-stage heart failure (ESHF) continues to pose significant challenges in modern cardiovascular medicine. As two pivotal therapeutic approaches that overcome the limitations of pharmacological treatment, heart transplantation (HTx) and ventricular assist device (VAD) implantation significantly improve patient outcomes through fundamentally distinct mechanisms (Miller, L., E. Birks, M. Guglin, H. Lamba, & O.H. Frazier, 2019; Habal, M.V., & A.R. Garan, 2017). During the perioperative period, HTx causes more severe inflammatory activation, coagulation disorders, and tissue damage compared to VAD. This difference stems from the greater surgical trauma associated with HTx, which involves donor heart procurement, complete cardiac excision, and anastomosis — procedures that markedly activate systemic inflammatory responses (Napoli, F., R. Aleman, N. Zadneulitca, J. Navia, & N.A. Brozzi, 2024; Boeken, U., P. Feindt, M. Micek, T. Petzold, H.D. Schulte, & E. Gams, 2000). In addition, HTx relies on CPB as perioperative life support and involves a longer duration of CPB, which leads to prolonged blood contact time with artificial materials, more pronounced complement activation, platelet consumption, and fibrinolysis imbalance. VAD, on the other hand, can undergo minimally invasive surgery and use partially bypass CPB or not require CPB to complete the surgery, which significantly shortens the CPB bypass time of VAD surgery (Cheung, A., J.L. Soon, J. Bashir, A. Kaan, & A. Ignaszewski, 2014; Lewin, D., G. Nersesian, L. Roehrich, M. Mueller, J. Mulzer, J. Stein, M. Kukucka, C. Starck, F. Schoenrath, V. Falk, S. Ott, & E.V. Potapov, 2022).

Given that HTx and VAD recipients share similar underlying diseases but undergo surgical protocols with significant differences in trauma extent, CPB duration, and bypass strategies, we aim to investigate the mutual interactions between UFH and the immune microenvironment during cardiac surgery CPB by observing multiple dimensions of biomarkers in both groups, including coagulation, inflammation, tissue injury, and organ function during the peri-CPB period, combined with monitoring of plasma UFH concentrations. This approach will yield more comprehensive conclusions regarding immune microenvironment-based heparin resistance and further expand research findings on CPB-associated UFH resistance. Moreover, we particularly focus on the unique pharmacokinetic characteristics of CPB-related UFH application — short-term high-dose administration and rapid clearance. By integrating UFH's pleiotropic drug effects, we further discuss the potential impact of rapid UFH clearance at CPB weaning on the shock-like effects to recipients' immune microenvironments.

2. Materials and Methods

2.1 Study Subjects

The study subjects consisted of patients who underwent heart transplantation (HTx) or ventricular assist device (VAD) implantation at our institution between 2023 and 2024. All HTx recipients required cardiopulmonary bypass (CPB) support, while the need for CPB in VAD recipients was determined based on preoperative evaluation criteria including severe cardiogenic shock (cardiac index [CI] <2.0 L/min/m² or systolic blood pressure <80 mmHg), right heart failure (tricuspid annular plane systolic excursion [TAPSE] <1.4 cm, pulmonary vascular resistance [PVR] >4 Wood units), or requirement for concomitant cardiac surgery. Additional factors considered in CPB decision-making included end-organ dysfunction (e.g., lactate >4 mmol/L or coagulation abnormalities), imaging findings of left ventricular thrombus or aortic pathology, and team emergency response capabilities (such as ECMO backup). The final decision was made through multidisciplinary discussion, weighing the benefits of CPB support against associated risks on an individualized basis. We recorded baseline information for all subjects including age, BMI, total CPB time (min), CPB flow time (min), aortic cross-clamp time (min), priming volume (ml/kg), heparin dose (mg/kg), and protamine sulfate (PS) dose (mg/kg).

2.2 CPB Management

For HTx, full-flow bypass was established via ascending aortic cannulation (2-3 cm from the aortic root) and bicaval venous cannulation (separate superior and inferior vena cava cannulation or single right atrial cannulation). After heparinization (ACT >480 seconds), the aorta was clamped and cardioplegia (either 4:1 blood cardioplegia or HTK solution) was administered, followed by recipient heart excision and donor heart anastomosis. For VAD implantation (e.g., left ventricular assist), partial bypass was employed using aortic cannulation for inflow and left atrial appendage or left atrial cannulation for drainage, preserving native heart function. During bypass, hemodynamics (MAP 50-70 mmHg, CVP, SvO_2 >65%), oxygenation (PaO_2, $PaCO_2$, lactate), temperature (28-32°C for HTx, normothermia or mild hypothermia for VAD), and coagulation (ACT, platelets) were monitored, with transesophageal echocardiography (TEE) used to assess cardiac filling and VAD positioning. For HTx weaning, rewarming to >36°C, stable sinus rhythm, CI >2.2 L/min/m², and absence of severe acidosis (pH >7.3) were ensured before gradual flow reduction and decannulation. VAD weaning required stable device flow (2.5-5 L/min) and absence of left ventricular over-decompression (confirmed by TEE) before gradual CPB separation.

The initial UFH dose was fixed at 3.5 mg/kg, with 40 mg UFH added to the CPB circuit prime (16 mg for patients under 14 years). If ACT failed to reach 480 seconds despite normal antithrombin (AT) activity, additional UFH (50-100 U/kg, with possible adjustments beyond this range based on individual variation and ACT results) was administered, followed by repeat ACT testing after 5 minutes to confirm target attainment. If ACT remained subtherapeutic with reduced AT activity, fresh frozen plasma (FFP) or ATIII concentrate was administered before further UFH dosing. PS neutralization was administered at a 1:1 to 1.3:1 ratio to total heparin dose after complete weaning and surgical hemostasis. Post-PS administration, ACT was measured at 5-10 minutes and subsequently every 30 minutes for 2 hours, with therapeutic targets of 120-150 seconds (consecutive measurements varying <10%) and no clinical bleeding. For ACT >150 seconds with active bleeding (after excluding surgical causes), additional PS (0.25-0.5 mg/kg) was given. For ACT >180 seconds without bleeding, repeat testing was performed within 30 minutes. Mild ACT elevation (150-180 seconds) without bleeding tendency required confirmation of persistent elevation before PS supplementation (0.25-0.5 mg/kg per 50 seconds above target, or 0.5-1 mg/kg empirically for active bleeding), with repeat ACT testing 5-10 minutes post-adjustment and total PS dose not exceeding 3 mg/kg.

All CPB procedures used the Stockert S5 system, with Medtronic BB541 oxygenators for adults and CAPIOX FX15 for patients under 18 years.

2.3 Observation Timepoints and Sampling Protocol

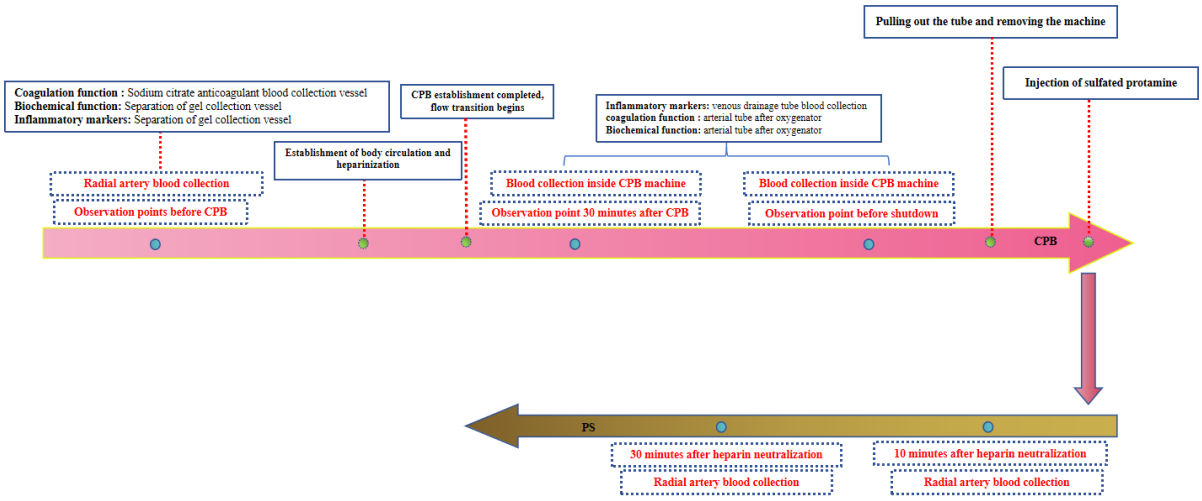

Figure 1. Experimental procedure. The specific location of each observation point during the CPB period, the sampling position and sampling method of each observation point.

This longitudinal peri-CPB study employed a standardized multi-timepoint sampling protocol across five observation points: pre-CPB (radial artery sampling before incision), 30 minutes post-CPB initiation (circuit sampling), before shutdown (circuit sampling 5-10 minutes before decannulation), and 10/30 minutes post-heparin neutralization (radial artery sampling). Coagulation tests used citrate-anticoagulated tubes (2 mL whole blood), while inflammatory markers and biochemistry used serum separator tubes (2 mL pre-CPB and after heparin neutralization; 1 mL x 2 at 30 min post-CPB and before shutdown). To avoid artifact, coagulation samples were drawn from the oxygenator's arterial line (minimizing contact activation), inflammatory samples from venous drainage (reflecting tissue cytokine release), and biochemistry samples from the arterial line (representing end-organ perfusion). All samples were processed immediately (Figure 1).

2.4 Analytical Measurements

Thirty-four peri-CPB biomarkers were longitudinally monitored across coagulation (PT, APTT, FIB, D-dimer, FDP, AT, Anti-Xa, TAT, PIC, TM, tPAIC), inflammation (IL-6, PCT, HBP), liver function (ALT, AST, TBIL, DBIL, TP, ALB, TBA, CHE, GGT, ALP), renal function (UREA, CREA, UA), lipids (TG, TC, HDL, LDL), glucose (GLU), and others (AMY, CK). Select markers had dual clinical significance (e.g., TM/tPAIC/HBP for endothelial injury; AST for hepatic/myocardial damage). Coagulation/inflammatory markers were measured at all five timepoints; biochemistry only at pre-CPB, before shutdown, and 30 minutes after heparin neutralization.

2.5 Detection Technology and Instruments

Due to intraoperative space constraints, a centrifugal microfluidic platform was selected for its compact size, multi-analyte capacity (enabling simultaneous measurement of numerous parameters from minimal whole blood volumes without preprocessing), and methodological alignment with conventional laboratory techniques (optical detection for coagulation/biochemistry; magnetic particle chemiluminescence for immunoassays). Preloaded reagent discs limited test menu flexibility. All instruments (MC550 coagulation analyzer, MI600 chemiluminescence analyzer, MS200 biochemistry analyzer) and consumables were from Zhejiang Pustar Biotechnology.

2.6 Statistical Analysis

Data were analyzed using SPSS (v27.0). Normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) determined parametric (mean ± SD) or nonparametric (median [IQR]) descriptive statistics. Between-group comparisons used independent t-tests (normal distribution, equal variance), Welch's t-tests (normal, unequal variance), or Mann-Whitney U tests (non-normal). Timepoint-specific HTx-VAD comparisons were performed, with dynamic trends visualized via observation point vs. mean value line graphs.

3. Results

3.1 Comparison Between HTx and VAD Groups

This study included a total of 34 subjects, with 19 in the VAD group (15 LVAD and 4 BiVAD recipients) and 15 in the HTx group. We recorded basic information including age, BMI, total CPB time, CPB flow time, aortic cross-clamp time, and weight-adjusted doses of UFH, priming solution, and PS. Statistical analysis revealed

significant between-group differences in age (P=0.017) and BMI (P=0.005), with higher values in the VAD group. Regarding CPB parameters, the HTx group showed significantly longer total CPB time (P=0.012), CPB flow time (P<0.001), aortic cross-clamp time (P<0.001), and higher heparin dose (P=0.030), while PS dose (P=0.336) and priming volume (P=0.408) showed no significant differences (Table 1).

At the observation point before CPB, CHE (P=0.023) and TP (P=0.018) were significantly higher in the VAD group, whereas PIC (P=0.019) and CK (P=0.044) were significantly higher in the HTx group (Table2), with no other significant differences observed (STable1).

Subgroup analysis of BiVAD versus LVAD recipients revealed multiple significant differences in post-weaning biochemical markers but no differences in coagulation or inflammatory markers throughout the observation period. Due to limited sample size (The sample size of BiVAD group is too low), further analysis was not performed (STable 6).

At the observation point 30 minutes after CPB, coagulation and inflammatory markers showed no significant intergroup differences, indicating comparable coagulation/fibrinolysis and inflammatory status between groups during early CPB (STable 2). Biochemical markers were not assessed at this timepoint.

At the observation point before shutdown (5-10 minutes before decannulation), demonstrated significantly higher values in the HTx group for TAT (P=0.027), ALT (P=0.048), AST (P<0.001), TP/ALB ratio (P<0.001), TBA (P=0.006), CK (P<0.001), HBP (P=0.038), and IL-6 (P<0.001) (Table1). Other test results did not show significant differences (STable3).

Following heparin neutralization with PS, assessments at 10 minutes after heparin neutralization significantly higher values in the HTx group for PIC (P=0.027), IL-6 (P=0.001), and PT (P=0.047) (Table 1), other test results did not show significant differences (STable4). At 30 minutes after heparin neutralization, AST (P<0.001), TBA (P=0.027), CK (P=0.005), and IL-6 (P<0.001) remained significantly elevated in the HTx group (Table 1), other test results did not show significant differences (STable5).

3.2 Temporal Changes in Measured Parameters Between HTx and VAD Groups

To more intuitively observe and compare the dynamic changes between the HTx and VAD groups at each observation timepoint, we calculated the mean values of each measured parameter across subjects at respective timepoints and plotted them on coordinate axes. This generated dynamic trend curves with the y-axis representing the mean values of test results and the x-axis representing the chronological sequence of observation timepoints. However, considering the presence of extreme outliers in some parameters and non-normal distribution of data sets, we also created box plots within the same coordinate system to better reflect the distribution characteristics of test results between the two groups. In both the line graphs and box plots, the same parameters from HTx and VAD groups are presented in identical coordinate systems to facilitate direct comparison.

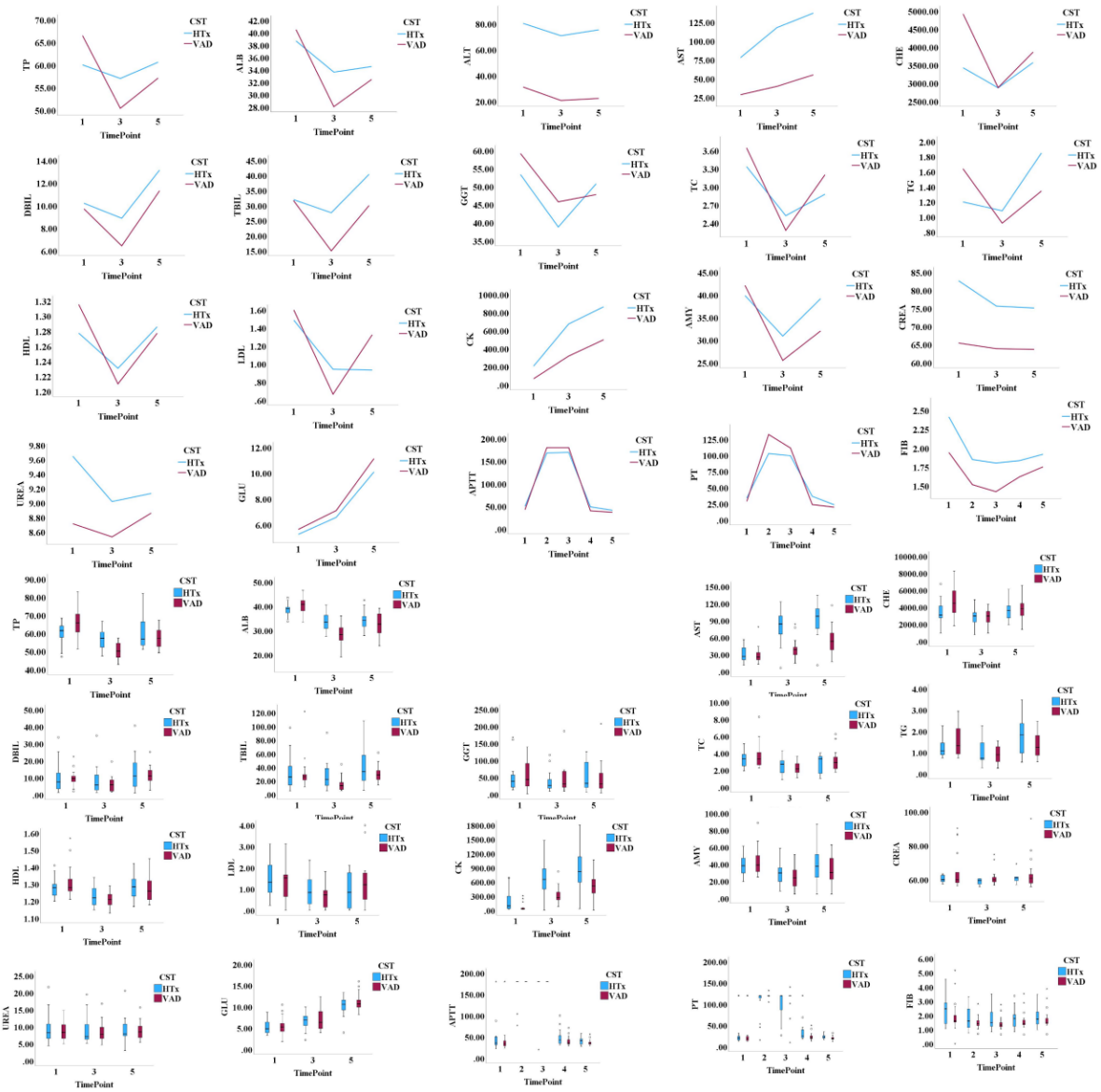

Figure 2.

Line chart and box chart of detection items with differences in observation point trends. Each point in the line chart is the average data of the detection results for the same item (HTx and VAD) in the group, while the box plot shows the overall distribution of the data. CST represents the type of cardiac surgery. TimePioneer is the time point corresponding to each observation point. On the left side of the coordinate axis are the corresponding detection items.

Parameters including TP, ALB, ALT, AST, CHE, DBIL, TBIL, GGT, TC, TG, HDL, LDL, CK, AMY, CREA, UREA, GLU, APTT, PT, and FIB demonstrated similar trends between groups. Most biochemical markers (TP, ALB, CHE, DBIL, TBIL, GGT, TC, TG, HDL, LDL, AMY, UREA) reached nadir values at the before shutdown timepoint (except ALT which showed minimal change). AST, GLU, and CK exhibited progressive increases across three timepoints, while CREA progressively decreased. Coagulation parameters APTT and PT peaked during CPB (30min post-CPB and before shutdown), whereas FIB reached its nadir during CPB (Figure 2).

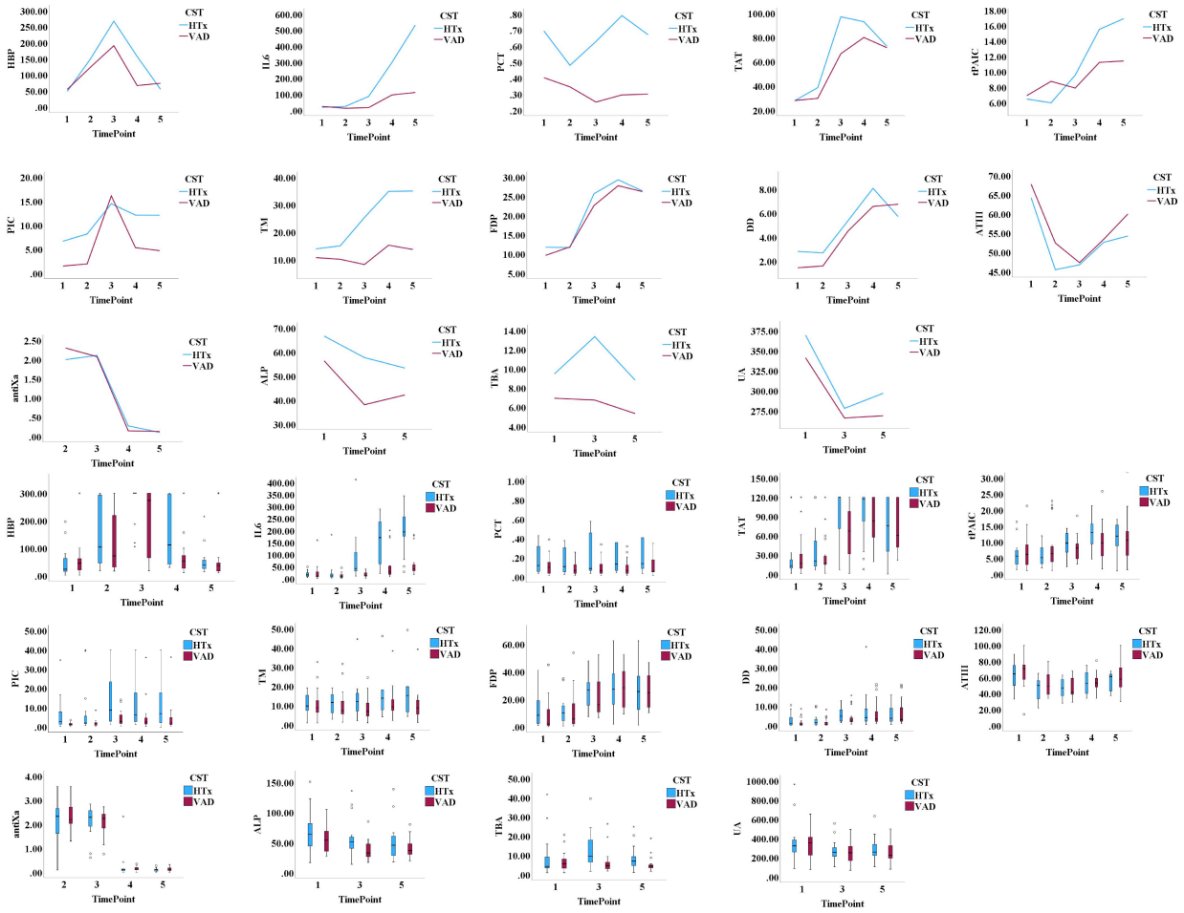

Figure 3. Line and box plots of detection items with relatively consistent trends at each observation point.

IL-6 dynamics differed markedly: VAD recipients showed CPB-associated suppression (levels below baseline during CPB), while HTx recipients demonstrated progressive elevation. Both groups showed after heparin neutralization increases, with more pronounced elevation in HTx (Figure 3).

HBP peaked during CPB in both groups (maximum assay limit: 300 ng/ml), but after heparin neutralization patterns diverged - HTx showed continuous decline while VAD exhibited mild rebound at 30 minutes (Figure 3).

PCT dynamics varied: HTx reached nadir at 30min post-CPB then rose before declining after heparin neutralization; VAD nadired before shutdown with partial recovery after heparin neutralization (Figure 3).

TAT trajectories differed: HTx peaked before shutdown then declined after heparin neutralization; VAD peaked at 10min after heparin neutralization before declining (Figure 3).

tPAIC patterns diverged: HTx nadired at 30min post-CPB then rose continuously; VAD rose initially before nadiring before shutdown then rising again (Figure 3).

While PIC trends were generally similar, VAD showed abrupt before shutdown elevation exceeding HTx levels (Figure 3).

TM dynamics differed: HTx rose continuously until stabilizing after heparin neutralization; VAD declined before shutdown before peaking at 10min after heparin neutralization (Figure 3).

FDP patterns were generally concordant except for HTx showing CPB-associated suppression at 30min (Figure 3).

DD trajectories diverged: HTx nadired at 30min post-CPB then rose until neutralization; VAD rose continuously (Figure 3).

AT activity declined during CPB in both groups but reached nadir earlier in HTx (30min post-CPB) versus VAD (before shutdown) (Figure 3).

Anti-Xa (reflecting UFH concentration) peaked earlier in VAD (30min post-CPB) versus HTx (before shutdown), with faster after heparin neutralization decline in VAD (Figure 3).

ALP decreased continuously in HTx versus VAD which nadired before shutdown then recovered (Figure 3).

TBA peaked before shutdown in both groups but with more pronounced elevation in HTx (Figure 3).

UA nadired before shutdown in both groups, with VAD showing stable after heparin neutralization levels (Figure 3).

These temporal analyses comprehensively demonstrate the dynamic changes of various parameters during HTx and VAD procedures, reflecting the real-time alterations in subjects' internal environment and organ function. By superimposing the curves, we can further examine the differences in trend patterns between the two groups of subjects.

4. Discussion

Based on the baseline characteristics of the subjects, although significant differences existed in age and BMI between the two groups, among all parameters measured at the pre-CPB (preoperative) timepoint, only CHE and TP showed significantly higher values in the VAD group compared to HTx, while PIC and CK were significantly higher in the HTx group. All other parameters showed no significant differences, indicating relatively comparable coagulation function, inflammatory status, and tissue damage levels between the two groups before CPB. The absence of significant difference in weight-adjusted priming volume further suggests similar degrees of hemodilution effects. Data analysis revealed that the HTx group maintained consistently higher TAT levels throughout the peri-CPB period, with significantly higher values at before shutdown compared to VAD, and IL-6 levels were persistently higher in HTx, becoming significantly elevated after the before shutdown timepoint. These findings suggest stronger inflammatory and coagulation activation in HTx recipients. Interestingly, while the fibrinolytic system appeared more activated in HTx, the formation of fibrinolytic products (DD, FDP) was not markedly evident during CPB due to UFH administration (no significant intergroup differences), and PIC generation also showed no significant difference during CPB.

Our initial observation was that the HTx group received significantly more UFH than VAD, yet both groups required nearly equivalent PS doses for neutralization. Anti-Xa measurements revealed comparable plasma UFH concentrations between groups throughout CPB, with VAD even showing slightly higher levels at 30 minutes post-CPB initiation. This suggests immediate UFH loss in HTx recipients upon initial administration. Therefore, a thought-provoking question arises, which is why the HTx group used more UFH compared to the VAD group, while the two groups of subjects used almost equal amounts of PS to neutralize UFH, and within 30 minutes after UFH neutralization, the plasma UFH levels of the two groups of subjects were almost the same, indicating that UFH was also effectively cleared in the HTx group. Has this additional UFH really been effectively cleared?

Assuming that the UFH of both groups of subjects is effectively cleared, we believe that there may be three factors leading to an increase in UFH consumption in the HTx group: prolonged CPB duration, accelerated heparin metabolism due to hepatic injury, and inflammatory mediator-mediated UFH "sequestration" in the immune microenvironment. While HTx did involve significantly longer CPB times, the negative correlation between UFH metabolism and dosage, combined with continuous infusion during CPB, ensures relatively stable circulating UFH levels. Therefore, time difference alone cannot fully account for the observed UFH depletion.

Biochemical analysis showed no marked differences in lipid profiles, glucose, or renal function between groups. However, the HTx group demonstrated significantly higher ALT, AST, TP/ALB ratio, TBA, and CK at before shutdown. ALT, AST, and CK are sensitive markers for parenchymal injury in liver and heart, while TP, ALB, and TBA reflect hepatic synthetic function. The progressive rise in CK and AST aligns with expected cardiac injury patterns during surgery, whereas the more stable ALT trend suggests hepatic injury may reach its peak earlier during CPB. Notably, HTx showed both greater hepatic injury and better preserved synthetic function compared to VAD — an apparent paradox. We propose that while HTx's longer CPB duration and procedural complexity cause more severe hepatic and cardiac injury, the liver's strong compensatory capacity enables it to enter an acute-phase response under prolonged stress. This fundamentally indicates more significant organ dysfunction in HTx despite apparent synthetic function preservation.

As UFH clearance primarily occurs via heparinase in the reticuloendothelial system rather than hepatic enzymes, acute-phase liver changes may variably affect UFH metabolism through alterations in hepatic hemodynamics and Kupffer cell activation by inflammatory mechanisms. While enhanced protein synthesis in HTx might suggest faster UFH clearance, the nonlinear pharmacokinetics of heparin metabolism in acute liver injury precludes definitive conclusions about its predominant role in UFH depletion.

We posit that heightened inflammatory activation and endothelial injury in HTx represent the primary mechanisms for UFH "disappearance." Although UFH's sulfate groups exhibit higher affinity for AT ($Kd \approx 10^{-9}$ M) than inflammatory mediators (e.g., HMGB1 with $Kd \approx 10^{-6}$ M), acute inflammation dramatically increases cytokine concentrations. According to mass action principles, this "quantity advantage" enables competitive occupation of UFH binding sites, reducing AT binding efficiency and anticoagulant efficacy. Simultaneously, endothelial heparan sulfate (HS) can electrostatically bind circulating UFH ($Kd \approx 10^{-7}$–10^{-6} M). While normally

forming a “heparin reservoir,” injured endothelium sheds HS, accelerating heparin clearance through three mechanisms: direct removal, ATIII binding interference, and procoagulant microenvironment formation. Our data shows significantly higher IL-6 in HTx, while endothelial injury markers (HBP, TM, tPAIC) demonstrated consistently (though not always significantly) higher trends in HTx — all evidence supporting UFH sequestration by inflammation and endothelial damage.

There appears to be a remaining gap in the logical chain that needs to be addressed. Assuming that the UFH in the HTx group was indeed sequestered by inflammatory mediators, this portion of UFH did not vanish — rather, after neutralization by protamine sulfate (PS), it remained in circulation. However, why was this residual UFH not detected via Anti-Xa assay? We hypothesize that this fraction of UFH may predominantly exert non-anticoagulant pharmacological effects, such as modulation of inflammatory pathways. Since the Anti-Xa assay is based on the anticoagulant properties of UFH, the assay’s inability to detect this fraction could be attributed to the suppression of UFH’s anticoagulant activity by abundant inflammatory mediators. Consequently, this additional UFH may continue to exert limited non-anticoagulant effects and is gradually metabolized until elimination. Subsequent observations revealed no heparin rebound-like symptoms in the HTx group, suggesting that the UFH in this cohort was effectively cleared. The excess UFH appears to have been masked by various antagonistic mechanisms against its anticoagulant function. Furthermore, dynamic changes in inflammatory mediators seem to support this hypothesis: following PS-mediated UFH clearance, a surge of inflammatory mediators re-entered systemic circulation (In the following discussion, there will be further elaboration). However, if we further assume that this situation does exist, we may need to consider whether using zero balance ultrafiltration (ZBUF) and Modified Ultrafiltration (MUF) before CPB shutdown to clear a large amount of inflammatory factors, while still using PS at a dose lower than the total UFH dosage, will result in insufficient heparin clearance, especially in pediatric cardiac surgery where higher CPB flow rates lead to more thorough clearance of inflammatory mediators.

Based on this consideration, it is possible to further evaluate the optimal dose of PS for UFH clearance by combining the degree of ultrafiltration with the concentration of inflammatory mediators in plasma. This may be a valuable research direction for optimizing PS dosage. This may be a valuable research direction for optimizing PS dosage.

Given UFH’s unique peri-CPB pharmacokinetics (bolus + continuous infusion with rapid neutralization), we specifically analyzed IL-6 dynamics. From 30min post-CPB to before shutdown, IL-6 elevation was minimal, with VAD even showing slight decline. However, post-PS neutralization triggered dramatic IL-6 surges, particularly in HTx. This suggests UFH’s direct cytokine binding reduces plasma inflammatory mediator concentrations, exerting anti-inflammatory effects that protect endothelial integrity and modulate coagulation. Although UFH suppresses IL-6 during CPB, ongoing production leads to cytokine accumulation, which floods circulation upon UFH clearance by PS.

HBP, released by neutrophils during tissue injury/inflammation, showed characteristic peri-CPB elevation followed by after heparin neutralization decline to pre-UFH levels. While previous studies attributed this pattern to UFH-neutrophil interactions, we propose an endothelial mechanism: At resting state, HBP binds activated endothelium via high-affinity β-integrins (Kd≈nM). During CPB, bolus UFH (reaching 30-60 μM) competitively displaces endothelial-bound HBP through sheer concentration advantage despite lower affinity (Kd≈μM). This explains both the dramatic HBP surge during UFH administration and rapid after heparin neutralization decline as PS clears UFH, allowing HBP re-binding to endothelium. While HBP-endothelial reattachment may exacerbate barrier damage, our TM/tPAIC data showed no significant after heparin neutralization spikes, though delayed endothelial injury markers cannot be excluded.

Collectively, surgical trauma and CPB constitute the “first hit” to immune homeostasis, while PS-mediated UFH clearance and consequent inflammatory mediator redistribution may represent a “second hit” to the immune microenvironment — a phenomenon warranting further investigation.

5. Conclusions

It is evident that during CPB, the pleiotropic effects of UFH and its unique pharmacokinetic characteristics (short-term high-dose infusion + rapid neutralization) interact with the patient’s immune microenvironment in a complex and significant manner. First, high-dose UFH in circulation can regulate immune microenvironment homeostasis by modulating coagulation function, inflammatory responses, and mitigating tissue damage. On the other hand, inflammatory mediators and endothelial cell injury within the immune microenvironment, under the influence of UFH’s non-anticoagulant effects, further impact UFH’s anticoagulant efficacy. This reminds us that when addressing UFH resistance during the peri-CPB period, it is essential to consider not only factors such as AT deficiency but also the broader immune microenvironment, including the degree of inflammation, endothelial injury, and circulatory dysfunction, to comprehensively understand the causes of UFH resistance. Furthermore, the clearance dose of PS on UFH may also need to be comprehensively evaluated in conjunction with the plasma

levels of inflammatory mediators. Additionally, the rapid clearance of UFH by PS may lead to the redistribution of inflammatory mediators in circulation. Furthermore, the loss of UFH's protective effects on endothelial tissue raises the question of whether PS-mediated neutralization of UFH could result in a "second hit" to the immune microenvironment — a topic highly worthy of further investigation.

6. Limitations

The primary limitation of this study lies in its small sample size, which may introduce uncertainty to the findings. Due to experimental constraints, our selection of biomarkers was limited, thereby restricting the comprehensiveness and sensitivity of our results. For instance, in studying inflammatory mechanisms, we initially aimed to assess multiple levels, including inflammatory initiators and inhibitors, rather than relying solely on IL-6 measurements. Additionally, CK and AST lack specificity for myocardial injury. Incorporating markers such as cTnI, BNP, and others could have provided a clearer distinction in myocardial damage between the two surgical approaches. Therefore, in future research, we hope to conduct more longitudinal studies with larger sample sizes and a broader range of detection indicators to enhance the rigor of our findings.

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Appendix

Table 1. Comparison of Basic information characteristics results of subjects

Basic information	**VAD**	**HTx**	**P**
Number	19 (L 15, B 4)	15	-
Age (year)	58 (48,62)	45 (14,54)	0.017*
BMI	85 (74,105)	19.39±4.59	0.005**
Total extracorporeal circulation time (min)	85 (74,105)	111 (99,118)	0.012*
Heparin dosage (mg/kg)	4.7 (4.25,5.21)	5.24 (4.64,6.17)	0.030*
Pre flushing solution (ml/kg)	18.38 (15.00,19.35)	20.75 (18.93, 29.61)	0.408
Ascending aorta occlusion time (min)	37 (30,50)	24 (21,31)	<0.001***
Extracorporeal circulation time (min)	49.32±30.24	80.47±14.88	<0.001***
Dosage of sulfated fish protein (mg/kg)	4.44 (4.23, 4.67)	4.46 (4.29, 5.38)	0.336

In the VAD group, L represents LVAD and B represents BiVAD; Data columns that follow a normal distribution are expressed as Mean ± SD, while data that do not follow a normal distribution are expressed as median (lower quartile, upper quartile); For P-values, they are annotated based on the degree of statistical significance*, p<0.05, **p<0.01, ***p<0.001.

Table 2. The detection results show significant differences between two sets of data at all observation points

Observation Point	**Item**	**VAD**	**HTx**	**P**
Before CPB	PIC (μg/ml)	1.52±0.97	2.83 (1.41,9.45)	0.019*
	TP (g/L)	66.49±8.04	60.03±6.30	0.018*
	CK (U/L)	29 (25.55,50.70)	94.00 (29.40,387.00)	0.044*
	CHE(U/L)	4916.53±1949.39	3430.87±1492.00	0.023*
Before shutdown	TAT (ng/ml)	67.31 (29.27,110.78)	120.00 (65.84,120.00)	0.027*
	ALT (U/L)	14.95 (10.25,28.38)	25.8 (15.00,44.10)	0.048*
	AST (U/L)	38.90 (28.96,43.68)	84 (63.7,101.00)	<0.001***
	TP (g/L)	50.25 (46.65,54.65)	57.02±5.67	<0.001***
	ALB (g/L)	28.07±4.29	33.65±3.83	<0.001***
	TBA (μmol/L)	4.78 (3.08,6.59)	9.61 (6.26,21.8)	0.006**
	CK (U/L)	273.50 (222.25,389.00)	674.17±360.80	<0.001***
	IL-6 (pg/ml)	17.32±9.95	42.57 (29.90,152.92)	<0.001***

10 mins after heparin neutralization	PIC (μg/ml)	2.65 (1.49,4.86)	7.11 (2.74,19.90)	0.027*
	IL-6 (pg/ml)	28.36 (16.76,54.35)	174.75 (61.74,254.75)	0.001**
	PT (s)	21.30 (18.60,27.60)	27.00 (21.40,39.90)	0.047*
30 mins after heparin neutralization	PIC (μg/ml)	2.06 (0.85,5.95)	8.71 (2.23,17.91)	0.036*
	AST (U/L)	53.65 (37.95,68.93)	102 (76.40,125.00)	<0.001***
	TBA (μmol/L)	4.45 (3.31,5.22)	7.53 (4.77,14.80)	0.027*
	CK (U/L)	499.81±247.66	865.68±450.99	0.005**
	IL-6 (pg/ml)	39.98 (31.77,66.45)	196.89 (177.27,290.18)	<0.001***

STable 1.

Before CPB	**VAD**	**HTx**	**P**
TM (U/ml)	8.86 (6.26,12.84)	9.81 (6.85,17.23)	0.471
TAT (ng/ml)	16.76 (8.79,40.11)	13.17 (9.41,24.79)	0.811
PIC (μg/ml)	1.52±0.97	2.83 (1.41,9.45)	0.019*
tPAIC (ng/ml)	6.23 (2.69,9.55)	5.68 (2.97,7.67)	0.864
PT (s)	18.6 (14.6,25.1)	20.80 (15.85,26.43)	0.377
APTT (s)	33.8 (28.6,43.9)	36.1 (30.98,50.70)	0.397
FIB (g/L)	1.6 (1.53,2.15)	2.42±1.09	0.304
D-Dmier (μg/ml)	0.33 (0.24,2.24)	1.20 (0.28,4.56)	0.212
ATIII (%)	67.74±19.78	64.21±16.33	0.553
FDP (μg/ml)	2.01 (0.91,20.55)	8.35 (2.15,19.98)	0.226
ALT (U/L)	31.16±20.46	13.50 (11.70,41.50)	0.433
AST (U/L)	25.9 (19.1,35.05)	27.00 (20.80,42.20)	0.455
ALP (U/L)	56.37±23.84	66.65±36.82	0.455
GGT (U/L)	59.15±41.19	38.80 (19.60,59.60)	0.526
TP (g/L)	66.49±8.04	60.03±6.30	0.018*
ALB (g/L)	40.51±3.50	38.68±2.62	0.109
DBIL (μmol/L)	9.5 (6.55,11.4)	7.62 (2.99,13.50)	0.576
TBIL (μmol/L)	26.2 (19.85,32.65)	25.70 (9.99,41.90)	0.941
TBA (μmol/L)	5.68 (3.0,8.18)	4.31 (3.09,9.38)	0.970
GLU (mmol/L)	5.66±2.21	5.27±1.58	0.580
CK (U/L)	29 (25.55,50.70)	94.00 (29.40,387.00)	0.044*
TC (mmol/L)	3.3 (2.56,4.17)	3.34±1.02	0.823
TG (mmol/L)	1.64±0.75	1.08 (0.90,1.49)	0.132
HDL (mmol/L)	1.28 (1.25,1.35)	1.28±0.62	0.350
LDL (mmol/L)	1.54 (0.65,1.87)	1.49±0.90	0.941
CHE(U/L)	4916.53±1949.39	3430.87±1492.00	0.023*
AMY (U/L)	39.3 (30.56,50.55)	39.87±13.77	0.710
UA (μmol/L)	341.25±155.95	323 (258,397)	0.882
UREA (mmol/L)	8.71±2.56	8.25 (6.10,11.70)	0.970
CREA (μmol/L)	60 (58.15, 70.05)	60.10 (58.80,63.50)	0.628
HBP (ng/ml)	45.6 (19.49,63.01)	23.51 (15.51,75.35)	0.560
PCT (ng/ml)	0.1 (0.04,0.16)	0.12 (0.06,0.43)	0.256
IL-6 (pg/ml)	11.84 (7.23,33.61)	16.16 (8.91,29.94)	0.811

The comparison results of all detection data at the observation points before CPB are described by the mean ± SD for data columns that conform to normal distribution, and the median (lower quartile, upper quartile) for data columns that do not conform to normal distribution; The results of the significant difference analysis are represented by the P-value. For data that follows a normal distribution and has homogeneous variance, independent sample t-test is used for analysis; If the data follows a normal distribution but with uneven variance, Welch's t-test is used; For data that does not follow a normal distribution, Mann Whitney U test is uniformly used. If the P value is less than 0.05, it indicates a significant difference and is marked with an asterisk (*). The marking rule is p<0.05**, p<0.01, ***p<0.001. (The following tables all adopt this standard.)

STable 2. Comparison of data at observation points 30 minutes after body circulation

30 minutes after CPB	VAD	HTx	P
TM (U/ml)	7.4 (5.36,12.32)	11.69 (6.06,16.20)	0.336
tPAIC (ng/ml)	6.4 (3.88,8.99)	5.16 (3.19,8.40)	0.451
TAT (ng/ml)	17.44 (14.32,30.25)	20.56 (12.16,60.39)	0.681
PIC (μg/ml)	1.58 (0.82,2.37)	2.12 (1.71,8.38)	0.071
PCT (ng/ml)	0.07 (0.04,0.13)	0.11 (0.04,0.38)	0.190
IL-6 (pg/ml)	7.49 (5.35,17.69)	11.48 (6.13,22.31)	0.391
PT (s)	120.00	120.00	-
APTT (s)	180.00	180.00	-
FIB (g/L)	1.42 (1.28,1.75)	1.85±0.82	0.141
ATIII (%)	52.47±14.66	50 (33,56)	0.256
DD (μg/ml)	0.70 (0.29,1.78)	1.45 (0.5,3.42)	0.242
FDP (μg/ml)	5.65 (1.98,20.8)	10.05 (4.51,15.57)	0.471
Anti-Xa (U/ml)	2.30±0.61	2.00±0.96	0.173
HBP (ng/ml)	124.44±112.58	104.00 (42.16,300)	0.451

STable 3. Comparison of data from observation points before shutdown

Before shutdown	VAD	HTx	P
TM (U/ml)	6.95 (4.49,12.46)	12.17 (6.95,8.02)	0.051
TAT (ng/ml)	67.31 (29.27,110.78)	120.00 (65.84,120.00)	0.027*
PIC (μg/ml)	3.01 (1.86,6.84)	14.49±14.06	0.060
tPAIC (ng/ml)	8.21 (4.83,9.68)	9.58±3.68	0.214
PT (s)	120	120	-
APTT (s)	180	180	-
FIB (g/L)	1.32 (1.17,1.54)	1.80±0.78	0.202
D-Dmier (μg/ml)	2.79 (1.77, 4.82)	5.37±3.74	0.354
ATIII (%)	42.00 (39.00,60.00)	47.00 (36.00,58.00)	0.811
FDP (μg/ml)	22.63±14.78	25.71±12.49	0.524
ALT (U/L)	14.95 (10.25,28.38)	25.8 (15.00,44.10)	0.048*
AST (U/L)	38.90 (28.96,43.68)	84 (63.7,101.00)	<0.001***
ALP (U/L)	32.70 (26.48,48.15)	51.50 (39.10,62.80)	0.051
GGT (U/L)	30.70 (20.65,68.00)	26.80 (14.9,48.1)	0.532
TP (g/L)	50.25 (46.65,54.65)	57.02±5.67	<0.001***
ALB (g/L)	28.07±4.29	33.65±3.83	<0.001***
DBIL (μmol/L)	5.94 (2.20,8.91)	5.81 (1.91,12.6)	0.789
TBIL (μmol/L)	12.95 (7.46,18.28)	22.5 (9.76,39.1)	0.052

TBA (μmol/L)	4.78 (3.08,6.59)	9.61 (6.26,21.8)	0.006**
GLU (mmol/L)	6.37 (4.91,9.09)	6.58±2.19	0.530
CK (U/L)	273.50 (222.25,389.00)	674.17±360.80	<0.001***
TC (mmol/L)	2.28±0.74	2.52±1.02	0.434
TG (mmol/L)	0.90 (0.56,1.28)	1.08±0.61	0.355
HDL (mmol/L)	1.21 (1.18,1.25)	1.23±0.06	0.298
LDL (mmol/L)	0.67±0.60	0.94±0.77	0.256
CHE(U/L)	2972.00 (2121.75,3548.00)	2876.87±980.73	0.987
AMY(U/L)	24.15 (14.48,34.20)	30.91±14.38	0.268
UA (μmol/L)	266.27±115.04	256.00 (207.00,307.00)	0.766
UREA (mmol/L)	7.83 (6.48,10.14)	7.13 (6.23,11.2)	0.762
CREA (μmol/L)	60.00 (58.63,61.95)	59.70 (56.50,60.60)	0.486
HBP (ng/ml)	273.89 (40.78,300.00)	300	0.038
PCT (ng/ml)	0.70 (0.04,0.14)	0.90 (0.07,0.58)	0.066
IL-6 (pg/ml)	17.32±9.95	42.57 (29.90,152.92)	<0.001***
Anti-Xa (U/ml)	2.24 (1.83,2.42)	2.29 (1.76,2.57)	0.632

STable 4. Comparison of data at the observation point 10 minutes after heparin neutralization

10 minutes after heparin neutralization	**VAD**	**HTx**	**P**
TM (U/ml)	9.33 (7.24,13.17)	14.59 (7.83,18.51)	0.167
tPAIC (ng/ml)	9.75 (5.45,13.12)	13.45 (9.53,16.20)	0.096
TAT (ng/ml)	83.28 (54.04,120)	120 (82.68,120)	0.286
PIC (μg/ml)	2.65 (1.49,4.86)	7.11 (2.74,19.90)	0.027*
PCT (ng/ml)	0.07 (0.04,0.13)	0.13 (0.07,0.36)	0.051
IL-6 (pg/ml)	28.36 (16.76,54.35)	174.75 (61.74,254.75)	0.001**
PT (s)	21.30 (18.60,27.60)	27.00 (21.40,39.90)	0.047*
APTT (s)	35.70 (33.00,45.40)	41.40 (34.90,53.00)	0.157
FIB (g/L)	1.43 (1.32,1.68)	1.83±0.72	0.380
ATIII (%)	53.00 (47.00,60.00)	46.00 (40.00,66.00)	0.656
DD (μg/ml)	3.39 (2.02,9.14)	4.65 (2.34,8.64)	0.681
FDP (μg/ml)	28.42 (13.24,40.69)	28.41±16.90	0.905
Anti-Xa (U/ml)	0.15±0.10	0.12 (0.07,0.14)	0.319
HBP (ng/ml)	53.36 (17.27,74.88)	108.47 (38.89,297.37)	0.137

STable 5. Comparison of data at the observation point 30 minutes after heparin neutralization

30 minutes after heparin neutralization	**MCS**	**HTx**	**P**
TM (U/ml)	8.76 (5.06,14.17)	15.56 (6.72,19.84)	0.089
TAT (ng/ml)	60.74 (40.99,120)	79.50 (35.80,120.00)	0.732
PIC (μg/ml)	2.06 (0.85,5.95)	8.71 (2.23,17.91)	0.036*
tPAIC (ng/ml)	10.57 (5.52,13.96)	12.00 (8.84,16.37)	0.945
PT (s)	19.00 (17.40,22.60)	23.40 (20.20,16.10)	0.056
APTT (s)	34.90 (32.60,38.20)	41.13±9.29	0.167
FIB (g/L)	1.50 (1.42,1.87)	1.93±0.77	0.391
D-Dmier (μg/ml)	3.17 (2.19,14.18)	4.12 (2.25,8.99)	0.837

ATIII (%)	60±16.93	60.00 (43.00,64.00)	0.220
FDP (μg/ml)	24.67 (13.51,37.81)	26.11±16.42	0.979
ALT (U/L)	15.2 (8.03,34.78)	32.10 (14.40,44.40)	0.135
AST (U/L)	53.65 (37.95,68.93)	102 (76.40,125.00)	<0.001***
ALP (U/L)	42.17±17.33	44.80 (25.40,60.60)	0.708
GGT (U/L)	30.60 (17.33,66.78)	48.86±39.53	0.735
TP (g/L)	57.12±5.30	57.70 (53.50,66.40)	0.464
ALB (g/L)	32.46±4.66	34.45±4.12	0.206
DBIL (μmol/L)	11.15 (7.81,14.43)	8.74 (4.98,18.80)	0.901
TBIL (μmol/L)	28.80 (21.30,35.70)	31.80 (20.70,57.80)	0.442
TBA (μmol/L)	4.45 (3.31,5.22)	7.53 (4.77,14.80)	0.027*
GLU (mmol/L)	10.70 (9.79,11.98)	10.70 (9.28,11.60)	0.735
CK (U/L)	499.81±247.66	865.68±450.99	0.005**
TC (mmol/L)	2.92 (2.21,3.63)	3.31 (1.66,3.64)	0.682
TG (mmol/L)	1.35±0.57	1.82±1.00	0.087
HDL (mmol/L)	1.28±0.08	1.28±0.07	0.796
LDL (mmol/L)	1.21 (0.52,1.77)	0.87 (0.10,1.77)	0.464
CHE (U/L)	3863.39±1453.18	3570.60±1085.22	0.524
AMY (U/L)	30.35 (21.88,47.13)	39.1 (24.90,53.30)	0.155
UA (μmol/L)	269.02±114.55	273.00 (224.00,339.00)	0.325
UREA (mmol/L)	8.50 (6.73,10.40)	7.81 (6.60,10.80)	0.762
CREA (μmol/L)	60.90 (58.08,64.03)	61.00 (59.50,61.70)	0.901
HBP (ng/ml)	36.9 (16.19,46.92)	38.45 (21.27,56.30)	0.945
PCT (ng/ml)	0.06 (0.05,0.21)	0.13 (0.08,0.41)	0.056
IL-6 (pg/ml)	39.98 (31.77,66.45)	196.89 (177.27,290.18)	<0.001***
Anti-Xa (U/ml)	0.13±0.10	0.11 (0.05,0.13)	0.384

STable 6. Comparison of Bivad and LVAD surgeries. The numbers in the table represent the P-values of the differential analysis

BiVAD vs LVAD	**Before CPB**	**30 min after CPB**	**Before shutdown**	**10 min after PS**	**30 min after PS**
TAT	0.885	0.665	0.736	0.124	0.124
TM	0.100	0.530	0.530	0.596	0.596
PIC	0.979	1.000	0.596	0.185	0.152
tPAIC	0.307	0.053	0.530	0.411	0.736
IL6	0.062	0.080	0.515	0.885	0.411
HBP	0.885	0.530	0.185	0.469	0.262
PCT	0.665	0.185	0.262	0.469	0.469
PT	0.961	0.885	0.665	0.411	0.411
APTT	0.469	1.000	1.000	0.262	0.530
FIB	1.000	0.596	0.307	0.307	0.411
DD	0.596	0.885	0.530	0.810	0.736
FDP	0.596	0.596	0.549	0.596	0.596
ATIII	0.810	0.886	0.357	0.665	0.957

Anti-Xa	-	0.654	0.810	0.542	0.431
ALT	0.358	-	0.192	-	0.192
AST	0.045*	-	0.505	-	0.158
TBIL	0.477	-	0.001***	-	1.000
DBIL	0.549	-	0.001***	-	0.505
TP	0.085	-	0.025*	-	0.673
ALB	0.711	-	0.001***	-	0.782
GGT	0.221	-	0.001***	-	0.327
ALP	0.572	-	0.046*	-	0.654
TBA	0.045*	-	0.001***	-	0.192
CREA	0.202	-	0.001***	-	0.959
UREA	0.409	-	0.019***	-	0.721
UA	0.984	-	0.001***	-	0.586
GLU	0.822	-	0.101	-	0.878
CHE	0.429	-	0.001***	-	0.702
CK	0.296	-	0.001***	-	0.065
AMY	0.060	-	0.001***	-	0.195
TC	0.130	-	0.001***	-	0.721
TG	0.785	-	0.001***	-	0.495
HDL	0.477	-	0.001***	-	0.310
LDL	0.060	-	0.194	-	0.442