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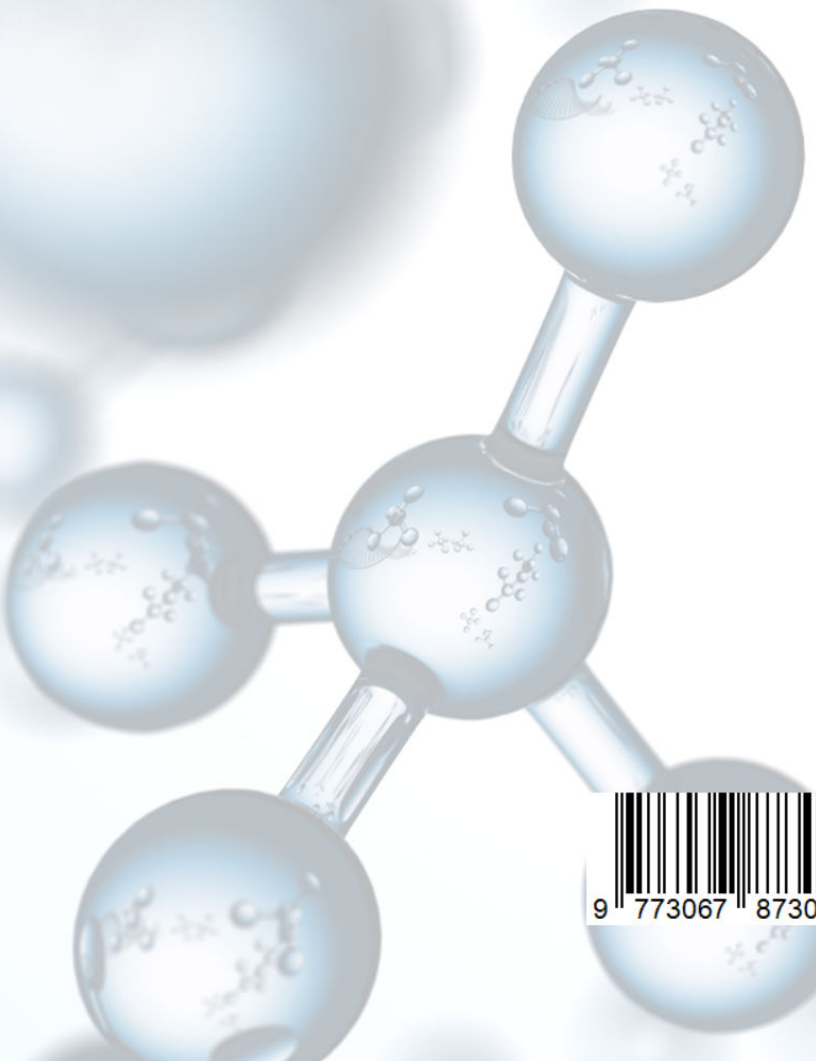

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Index

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Original Article

Development and Preliminary Evaluation of a Behavioral Lifestyle Assessment Tool: A Methodological Case Study with Graduate-Level Women

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ABSTRACT

Introduction: Obesity in adults remains a pressing public health issue in the United States, closely linked to modifiable behaviors such as diet, physical activity, stress, sleep, and substance use. This study aimed to develop and pilot a brief, multidimensional assessment tool to identify lifestyle risk factors associated with obesity and inform future prevention efforts.
Methods: A 30-item Behavioral Lifestyle Risk Assessment (BLRA) survey was developed to measure six domains: physical activity, diet, screen time, sleep, stress, and substance use. The survey was administered online via Microsoft Forms to a purposive sample of six adults. The small sample size was intentionally selected based on cognitive interviewing methodology, which recommends 5–10 participants for early-stage instrument clarity and feasibility testing. Descriptive statistics were used to summarize behavioral patterns and survey usability.
Results: All six participants (100% female, aged 18–33) completed the survey without missing data or technical issues. Half reported low physical activity (1–2 days/week), 33% consumed fewer than two servings of fruits and vegetables daily, and another 33% had screen time exceeding eight hours daily. All participants reported moderate to high stress; 83% consumed alcohol, and 17% reported tobacco use. The average survey completion time was 6.8 minutes (SD = 0.6), with positive feedback on clarity and flow.
Conclusion: Pilot findings suggest the BLRA is a feasible and user-friendly tool for assessing obesity-related behavioral risks. Broader testing in larger, diverse populations is recommended to validate its public health application.

1. Introduction

Introduction Obesity is a significant public health concern of the 21st century, with serious consequences for human health, healthcare systems, and global economic stability. Obesity, acknowledged by the World Health Organization (WHO) as a significant risk factor for several chronic illnesses, has reached epidemic levels globally [1]. The condition contributes to the increasing prevalence of non-communicable illnesses, including type 2 diabetes, cardiovascular disease, and certain malignancies, while also intensifying healthcare disparities, social stigmatization, and economic productivity declines [2]. In 2022, over one billion individuals worldwide were deemed obese, a number that has more than quadrupled since 1990, highlighting the need for extensive preventative and intervention programs [3]. In the United States, obesity impacts over 42% of individuals and strongly contributes to the primary causes of avoidable early mortality [4]. Obesity is a complex, multifaceted problem that requires coordinated efforts across healthcare, education, urban planning, food systems, and social frameworks. Understanding the behavioral motivators and sociocultural elements that drive this epidemic is essential for creating effective, fair public health solutions. Obesity is clinically defined as a chronic condition marked by the excessive buildup of body fat that negatively affects health. The predominant instrument for categorizing obesity is the Body Mass Index (BMI), computed by dividing an individual's weight in kilograms by the square of their height in meters [5]. According to guidelines from the Centers for Disease Control and Prevention (CDC), adults with a BMI of 30.0 or higher are classified as obese [4]. Obesity is further stratified into three classes based on severity: Class 1 (BMI 30.0–34.9), Class 2 (BMI 35.0–39.9), and Class 3 (BMI 40.0), the latter commonly referred to as severe or morbid obesity. BMI is a valuable population-level screening tool but does not distinguish between fat and lean mass. Thus, clinical judgment using other health markers is essential. However, BMI criteria are extensively used in public health research and policy to standardize monitoring, risk stratification, and obesity prevention and treatment [6].

Complex interactions between biological, behavioral, and environmental variables cause obesity. Modifiable risk factors drive the worldwide obesity pandemic in each of them. Poor dietary habits, such as eating too many ultra-processed foods, sugary drinks, and calories, and insufficient fiber, fruits, and vegetables, are major causes. Risk is further compounded by physical inactivity driven by sedentary lifestyles and technology use in urban living [7].

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Research emerges and underscores that sleep disturbances predict obesity independently, and specifically inadequate duration and poor quality mediate such prediction through hormonal dysregulation affecting appetite and metabolism. Chronic psychological stress has also been linked. This influences neuroendocrine pathways, promoting emotional eating and decreasing physical activity. Poor nutrition is connected to excessive screen time on devices and reduced physical exercise. People utilize drugs, particularly if they drink too much and smoke. These behaviors relate to metabolic dysfunction and adiposity [8]. Genes predispose bodies to metabolize at some basal rate and store fat readily to regulate satiety. Hormonal changes and reduced lean body mass enhance susceptibility with age. Sex-based variations affect obesity trends, such as hormonal changes during pregnancy or menopause and women's greater fat percentages. Medical conditions like hypothyroidism, Cushing's syndrome, and polycystic ovary syndrome (PCOS) elevate obesity risk independently of lifestyle behaviors [9].

Along with biological and behavioral variables, social determinants of health (SDOH) significantly impact obesity risk and prevalence. Social class affects food, exercise, healthcare, and health literacy. For low-income and uneducated individuals, food deserts and unsafe locales hinder access to inexpensive, healthful food and leisure. Home walkability and green places strongly affect an active lifestyle [10]. Systemic racism, employment insecurity, and inadequate healthcare access worsen obesity-related health disparities [11], particularly among impoverished people [12]. Obesity is a pressing concern in public health and clinical practice because of the wide-ranging negative effects it may have on people's physical, mental, and social health, both in the short and long term. Obesity is physically associated with an increased risk of developing many chronic conditions. These include type 2 diabetes mellitus, hypertension (high blood pressure), dyslipidemia (a medical condition characterized by abnormal lipid levels in the blood), coronary artery disease, ischemic stroke, numerous cancers (including endometrial, colorectal, and breast cancer), osteoarthritis (a degenerative joint condition) and obstructive sleep apnea (airway blockage caused by fat deposition) [13]. When these diseases occur together, it's known as metabolic syndrome, and it may shorten a person's life expectancy by 8-10 years in extreme cases [14]. Beyond physical health, obesity has major mental health repercussions. Interiorized stigma, social isolation, and weight-based discrimination make overweight people more likely to suffer depression, anxiety, eating disorders, and low self-esteem. Mental illness may compromise quality of life and make weight loss and medical treatment harder. Hospitals, schools, and organizations that discriminate against obese people diminish incomes, employment, education, and social mobility. These effects compromise health and raise healthcare costs, productivity, and inequality. [15].

Globally, the prevalence of obesity has been rising at an alarming rate. In 2022, more than 1 billion people worldwide were living with obesity, a figure that has more than doubled since 1990. In the United States, recent data indicate that approximately 40.3% of adults aged 20 and over are classified as obese [3]. The prevalence is slightly higher among women (41.3%) than men (39.2%). Age-wise, adults aged 40–59 years exhibit the highest obesity rates at 46.4]%, followed by those aged 60 and over at 38.9%, and adults aged 20–39 years at 35.5%. Focusing on Kentucky, the state has one of the highest adult obesity rates in the nation[16]. According to the CDC's Behavioral Risk Factor Surveillance System, the prevalence of adult obesity in Kentucky was 37.7% in 2022, placing it among the top states with obesity rates exceeding 35% [4, 17]. Inactivity, a diet high in energy-dense, nutrient-poor foods, poor sleep, chronic psychological stress, excessive screen time, and drug use—including alcohol and tobacco—all raise the risk of obesity [18, 19]. Moreover, these factors frequently cluster together, creating synergistic effects that amplify obesity risk far beyond the impact of any single behavior. Behavior is formed and restrained by social and environmental variables such as food deserts, limited recreational space, and limited access to inexpensive, excellent healthcare[20]. Substance use, particularly alcohol and tobacco consumption, is increasingly recognized as a contributing factor to obesity through both behavioral and physiological mechanisms [21]. These include increased caloric intake, appetite dysregulation, poor sleep quality, and reduced impulse control. Targeting behavioral risk factors allows for both individualized approaches, such as counseling and behavior modification, and population-level strategies, including urban planning, policy reform, and community-based programs [22].

Over the last four decades, obesity has increasingly become a multifaceted health concern among U.S. adults. Over 42% of Americans are obese, a number that continues to rise despite public health initiatives [4]. Research, including the National Health and Nutrition Examination Survey (NHANES), has yielded comprehensive surveillance data that elucidate these behavioral trends on a national scale [23]. Recent literature continues to emphasize the complex interplay between behavioral, psychosocial, and environmental factors in the development and persistence of adult obesity. National surveillance reports, such as the CDC's Behavioral Risk Factor Surveillance System (BRFSS) and NHANES studies, have consistently identified low physical activity, poor dietary quality, inadequate sleep, high levels of perceived stress, and excessive screen time as dominant behavioral risk factors [23, 24]. Recent large-scale meta-analyses have also pointed to screen time, particularly among adults aged 18–49, as an increasingly significant independent risk factor beyond its association with reduced physical activity [25]. Although these studies provide valuable epidemiologic evidence, concise, integrated survey instruments that holistically assess these overlapping behavioral domains practically and ethically for rapid community-level surveillance and intervention planning are scarce [26].

Despite these contributions, notable gaps remain in existing literature. Much of the current research relies heavily on large-scale datasets that, while powerful, often lack specificity in behavioral measurement. Variables such as physical activity and dietary intake are frequently captured through broad or general questions that may not fully reflect nuanced behavioral patterns necessary for designing targeted interventions[27]. Moreover, there is a relative lack of studies that integrate multiple behavioral domains, including screen time, stress, and substance use, into a single, comprehensive risk assessment instrument. Most available tools tend to focus on isolated behaviors, such as physical activity measured by the International Physical Activity Questionnaire (IPAQ), or emphasize clinical risk factors without integrating broader lifestyle dimensions[28]. Furthermore, few research has used brief, human-centered surveys to assess adult obesity's behavioral and lifestyle risk variables. Developing simplified, morally acceptable instruments that are behaviorally complete and feasible for quick delivery across varied adult groups is hindered by this methodological gap [29]. Few techniques incorporate physical activity, food, stress, screen time, and drug use into a single evaluation. Traditional instrument design neglects ethical issues such as participant confidentiality, responder burden, and confidence in self-reported health practices. Community-level risk monitoring and intervention planning for adult obesity need adaptive survey tools that combine rigor and usability [30].

The present pilot study addressed a specific and pressing gap within the current literature: the absence of a concise, behaviorally integrated, and practically adaptable survey instrument focused explicitly on modifiable risk factors for adult obesity. While large national surveillance systems, such as NHANES and BRFSS, provide important prevalence estimates, they often lack the behavioral specificity and participant-centered design necessary for effective use at the community level [31]. This study develops and tests a structured behavioral risk assessment tool that includes several lifestyle factors in a time-efficient, ethical, and user-friendly framework. It reduces participant tiredness, clarifies data, and improves field adaptability to connect broad surveillance research and community-based public health activities. It helps localized programs monitor and treat adult obesity in rural Kentucky, where behavioral risk clusters and access barriers cause health disparities [32]. Despite the rising prevalence of obesity and related health concerns, brief, human-centered survey tools that are behaviorally thorough and feasible for fast administration and adaptation across varied community settings are lacking. Validated instruments generally stress epidemiological breadth above usability, creating a methodological void in creating simplified, morally acceptable community-level risk monitoring tools [33].

Focusing on behavioral specificity and practicality, this initiative innovates large-scale surveillance with actionable community-based research. It also adapts the instrument to diverse, at-risk groups, particularly in disadvantaged areas like rural Kentucky, benefiting public health and behavioral epidemiology. The main goal was to create, implement, and evaluate a structured behavioral survey instrument for measuring adult obesity risk factors. The goal was to test the new tool's feasibility, usability, and clarity in a pilot population, identify key behavioral risk factors trends, and refine its structure and content for future research on larger and more diverse adult populations.

2. Methods

2.1. Study Design

This study was designed as a cross-sectional, online pilot survey to assess behavioral and lifestyle risk factors associated with adult obesity among adults residing in the United States. The primary objectives were to develop and test the feasibility, clarity, and analytic potential of a newly created behavioral risk assessment instrument targeting obesity-related factors. Despite its pilot scope, the study emphasized the methodological rigor of real-world epidemiological survey research. The study was classified as minimal risk, as it involved anonymous participation and did not collect identifiable personal information. The research was conducted entirely online to ensure participant safety, confidentiality, and accessibility.

2.2. Population Selection Criteria

The study population comprised adults aged 18 years or older with English proficiency and access to an internet-enabled device, including smartphones, tablets, laptops, or desktop computers. Eligibility criteria specifically required participants to reside within the United States to ensure cultural and behavioral relevance to national public health contexts. Participants were also required to have basic digital literacy, as the survey was administered through an online platform. Individuals who could not consent independently or indicated any cognitive limitations impairing their ability to complete a self-administered survey were excluded. Participants were six graduate-level women recruited via convenience sampling at the University of Louisville. While this homogeneous sample limits broader generalizability, it effectively provides preliminary insights into the survey instrument's clarity, usability, and administrative feasibility within this demographic group. No restrictions were placed on gender, race, ethnicity, or socioeconomic status, given the exploratory nature of the pilot study.

2.3. Sample Size and Participant Selection Rationale

The sample size of six participants was purposefully chosen based on established methodological guidelines for preliminary survey development and cognitive interviewing, which typically recommend small samples (5–10 participants) to initially assess item clarity, comprehension, and survey usability prior to extensive validation (Willis, 2005). Graduate-level women were specifically selected through convenience sampling at the University of Louisville due to their accessibility, higher likelihood of familiarity with digital survey platforms, and capacity to provide detailed, informed feedback during this preliminary phase. Although this homogeneous sample limits broader generalizability, it effectively supports the intended methodological purpose of refining the survey instrument for future validation with larger and more diverse populations.

2.4. Survey Instrument Development

The Adult Obesity Risk Assessment Questionnaire was a newly developed 30-item tool designed to assess key behaviors linked to obesity risk. It covered four domains: (1) demographics (e.g., age, gender, race/ethnicity, education, employment, marital status, insurance); (2) lifestyle behaviors (physical activity, fruit and vegetable intake, sleep); (3) psychosocial and behavioral factors (stress, screen time, tobacco and alcohol use); and (4) health self-monitoring and healthcare access (check-up frequency, self-weighing). (See Appendix 2). The survey items were adapted from previously validated instruments to enhance construct validity and measurement reliability. Physical activity measures were informed by the International Physical Activity Questionnaire [27], psychological stress by the Patient Health Questionnaire, and dietary intake behaviors by food frequency indices validated in obesity research [34]. The survey design emphasized clarity, brevity, and logical flow, aiming to minimize participant burden and maximize response accuracy.

2.5. Instrument Validity Check

To ensure face and content validity, the draft survey instrument underwent expert review by two faculty members specializing in public health and behavioral epidemiology. Reviewers assessed each item for relevance, clarity, and appropriateness for the target population. Feedback was incorporated to improve question wording, reduce ambiguity, and align response options with best practices for survey design. In addition, internal consistency across similar constructs was examined during the pilot phase through a preliminary reliability check using Cronbach's alpha. However, given the small sample size, these results were interpreted descriptively rather than inferentially.

2.6. Pilot Testing Process

The pilot testing phase was conducted with six adult participants recruited through convenience sampling. The sample size of six participants was selected based on established guidelines for cognitive interviewing in initial instrument development phases, where samples of 5-10 participants are standard practice to preliminarily assess item comprehension, usability, and survey flow (Willis, 2005). Participants completed the full survey online in a single sitting. The primary goals of the pilot were to evaluate the technical functionality of the survey platform (Microsoft Forms), assess participant comprehension of survey items, ensure logical flow

and skip patterns operated correctly, and measure average completion time. Participants were encouraged to report any confusion or technical difficulties encountered during survey completion, although no such issues were ultimately reported. The average completion time was five to seven minutes, consistent with the survey design expectations. Pilot feedback was used to confirm the instrument's readiness for broader field application in future studies. (See Appendix-1 for pilot data summary)

2.7. Sampling Method, Sampling Frame, and Sampling Strategy

A non-probability convenience sampling method was employed for this pilot study. The sampling frame consisted of adult individuals within the United States who had access to the survey link, primarily through academic networks and community outreach. Convenience sampling was deemed appropriate given the pilot nature of the study, which prioritized instrument testing overpopulation representativeness. Recruitment focused on adults familiar with online surveys and willing to participate voluntarily without incentives. Although this method introduced potential biases related to self-selection and digital access, it was appropriate for the methodological goals of assessing survey usability and initial data trends.

2.8. Data Collection

Data collection occurred entirely through Microsoft Forms, which allowed for secure, anonymous, and user-friendly administration of the survey instrument. Participants accessed the survey via a secure web link and were required to review and electronically agree to an informed consent preamble before proceeding. The consent document outlined the purpose of the study, the voluntary nature of participation, data anonymity, lack of personal data collection, and contact information for questions or withdrawal. No identifiable information, such as names, email addresses, device information, or IP addresses, was collected at any stage. All submitted survey responses were encrypted and stored securely within the Microsoft cloud platform, accessible only by authorized research team members. Using an online platform enabled flexible participation across geographic locations while ensuring adherence to privacy and security standards.

2.9. Data Analysis

Upon completion of data collection, responses were exported into Microsoft Excel for cleaning and initial analysis. Data cleaning procedures involved checking duplicate entries, reviewing skip logic adherence, validating categorical variable coding, and confirming the completeness of responses. No missing or invalid responses were identified, and all participants successfully completed the full survey. Descriptive statistical analyses were conducted to summarize participant demographics and behavioral patterns. Given the small sample size and developmental intent of this pilot study, the analysis was limited strictly to descriptive statistics aimed at assessing feasibility and usability. No inferential statistical tests were conducted as the study was not powered to detect statistical relationships or validate hypotheses. Frequencies and percentages were calculated for each categorical variable, including physical activity levels, dietary intake, sleep duration, screen time, stress perception, tobacco and alcohol use, and self-monitoring behaviors. Cross-tabulations were prepared to explore potential relationships between demographic variables and key behavioral factors, although no inferential testing was conducted due to the limited sample size. Trends were visually reviewed to identify areas of concern, such as the high prevalence of sedentary behavior or excessive screen time, that might warrant further investigation in larger samples [35]. The evaluation of pilot data also included an internal check of logical consistency across related items. For instance, participants reporting no physical activity were expected also to report lower levels of self-monitoring of weight, allowing the research team to assess the internal coherence of survey responses informally [36]. In addition, preliminary observations regarding the variability and spread of responses were recorded to inform necessary revisions or additions to the survey instrument before scaling for larger applications [37].

2.10. Ethical Considerations

This study was conducted in accordance with the highest ethical standards for research involving human participants. Approval was granted by the University of Louisville Institutional Review Board under protocol reference number PHEP-6273264. Participation was entirely voluntary, with participants retaining the right to withdraw at any point without penalty. All participants provided electronic informed consent prior to survey initiation. Privacy and confidentiality were rigorously protected, with no collection of personally identifiable information and full encryption of response data. In addition, the survey was designed to minimize psychological or social risk by avoiding sensitive or stigmatizing language, particularly in questions related to mental health, substance use, and weight-related behaviors. The researchers maintained a strong commitment to transparency, respect, and participant autonomy throughout the study process. (See Appendix 2)

3. Results

The results presented here strictly represent preliminary insights regarding survey feasibility and usability. Interpretations about specific behavioral patterns are exploratory and do not imply validated associations with obesity outcomes.

3.1. Participant Demographic Characteristics

A total of six adult participants completed the pilot survey in full. All participants identified as female, representing 100% of the sample. Age distribution indicated that all respondents were 18–33 years old, reflecting a relatively young adult cohort. In terms of racial and ethnic identity, half of the participants (50%) identified as White, 33% identified as Asian, and 17% identified as Black or African American. Educational attainment was uniformly high among participants, with 100% reporting possession of a graduate-level degree. Regarding employment status, the majority (67%) were employed part-time, while the remaining 33% reported full-time employment. Health insurance coverage was reported by 83% of participants, indicating that most had access to regular healthcare services. Marital and family structures varied, with 33% identifying as single with no children, 17% as married with children, 17% as married without children, and 17% as single with children. These demographic patterns suggest a highly educated, predominantly young, and professionally engaged group, though not fully representative of the broader U.S. adult population [17] **(Table 1)**.

3.2. Behavioral and Lifestyle Factors

3.2.1. Physical Activity

Physical activity data analysis revealed trends toward low engagement in regular exercise. Half of the participants (50%) reported engaging in physical activity only 1–2 days per week. An additional 33% reported 3–5 days of physical activity per week, suggesting some adherence to recommended guidelines. However, 17% of participants indicated no physical activity engagement, highlighting a critical area for behavioral intervention. These findings parallel national data indicating that insufficient physical activity remains

Table 1: Participant Demographics (N=6)

Characteristic	Category	Frequency (%)
Gender	Female	6 (100%)
Age Range	18–33 years	6 (100%)
Race/Ethnicity	White	3 (50%)
	Asian	2 (33%)
	Black or African American	1 (17%)
Education Level	Graduate degree	6 (100%)
Employment Status	Part-time	4 (67%)
	Full-time	2 (33%)
Health Insurance Coverage	Yes	5 (83%)
	No Response	1 (17%)
Marital/Family Structure	Single no children	2 (33%)
	Single with children	1 (17%)
	Married with children	1 (17%)
	Married no children	1 (17%)
	Missing/Other	1 (17%)

Survey performance metrics were strong, with no missing data, average completion time under seven minutes, and no technical issues reported. Participants' qualitative feedback indicated clear item comprehension and acceptable respondent burden.

Table 2: Behavioral and Lifestyle Factors (N=6)

Behavior	Category	Frequency (%)
Physical Activity	1–2 days/week	3 (50%)
	3–5 days/week	2 (33%)
	Never	1 (17%)
Fruit/Vegetable Intake	0–1 servings/day	2 (33%)
	2–3 servings/day	2 (33%)
	4–5 servings/day	2 (33%)
Sleep Duration	6–7 hours	5 (83%)
	4–5 hours	1 (17%)
Screen Time	2–4 hours/day	3 (50%)
	5–7 hours/day	1 (17%)
	>8 hours/day	2 (33%)
Stress Level	Occasionally	3 (50%)
	Often	3 (50%)
Alcohol Use	Yes	5 (83%)
	No	1 (17%)
Tobacco Use	Yes	1 (17%)
	No	5 (83%)

Integrated Behavioral Patterns

a major public health challenge among adults in the United States [4, 38] **(Table 2) (Figure 1)**.

3.2.2. Fruit and Vegetable Intake

Dietary behaviors related to fruit and vegetable consumption were notably inconsistent. Responses were evenly distributed across three intake categories: 33% of participants reported consuming 0–1 serving daily, another 33% reported 2–3 servings, and the remaining 33% reported 4–5 servings daily. No participants reported consuming six or more servings daily, despite public health recommendations encouraging at least five servings per day for chronic disease prevention. These findings mirror broader dietary trends in the United States, where inadequate fruit and vegetable intake remains a persistent challenge contributing to obesity risk [39].

3.2.3. Sleep Duration

Sleep patterns among participants were generally within the recommended range. Five of the six participants (83%) reported receiving 6–7 hours of sleep per night, while one participant (17%) reported only 4–5 hours of sleep per night. Although most participants achieved relatively sufficient sleep, the presence of even one individual reporting short sleep duration underscores the need to integrate sleep hygiene promotion into obesity prevention efforts [40].

3.2.4. Screen Time

Excessive screen time emerged as a significant behavioral concern. Half of the sample (50%) reported spending 2–4 hours daily on digital devices, while an additional 33% reported more than 8 hours of screen time daily. Only 17% reported moderate screen exposure of 5–7 hours daily. Given the well-documented link between prolonged screen time, physical inactivity, disrupted sleep patterns, and increased obesity risk, this finding highlights a critical intervention target for behavioral modification [35].

3.3. Psychosocial and Behavioral Factors

Stress levels were substantial among participants. Exactly half (50%) reported feeling stressed "often," while the other half reported stress "occasionally." No participants reported feeling stress "rarely" or "never." Chronic stress is known to influence hormonal pathways, dietary behaviors, and physical activity patterns, contributing indirectly but powerfully to obesity risk [13] **(Figure 2)**.

3.3.1. Alcohol and Tobacco Use

Alcohol consumption was reported by 83% of participants, with most indicating moderate intake (1–2 drinks per session). Only one participant (17%) reported no alcohol use. Tobacco use was low, with only one participant (17%) reporting use of tobacco products within the past six months. While the rates of tobacco use were encouragingly low, the high prevalence of alcohol use suggests the importance of integrating alcohol-related behavioral counseling into obesity prevention programming where relevant [41]. Substance use behaviors, particularly alcohol consumption and tobacco use, have been associated in epidemiological studies with metabolic alterations and obesity risk, highlighting the need for their inclusion in holistic obesity risk assessments.

When examining integrated behavioral patterns, a concerning synergy among multiple risk factors became evident. Participants with lower physical activity levels also reported higher screen time and inconsistent fruit and vegetable intake. Those who reported feeling stressed "often" were also more likely to report inadequate sleep or greater use of digital devices late into the night. These interconnected behavioral profiles highlight the complex clustering

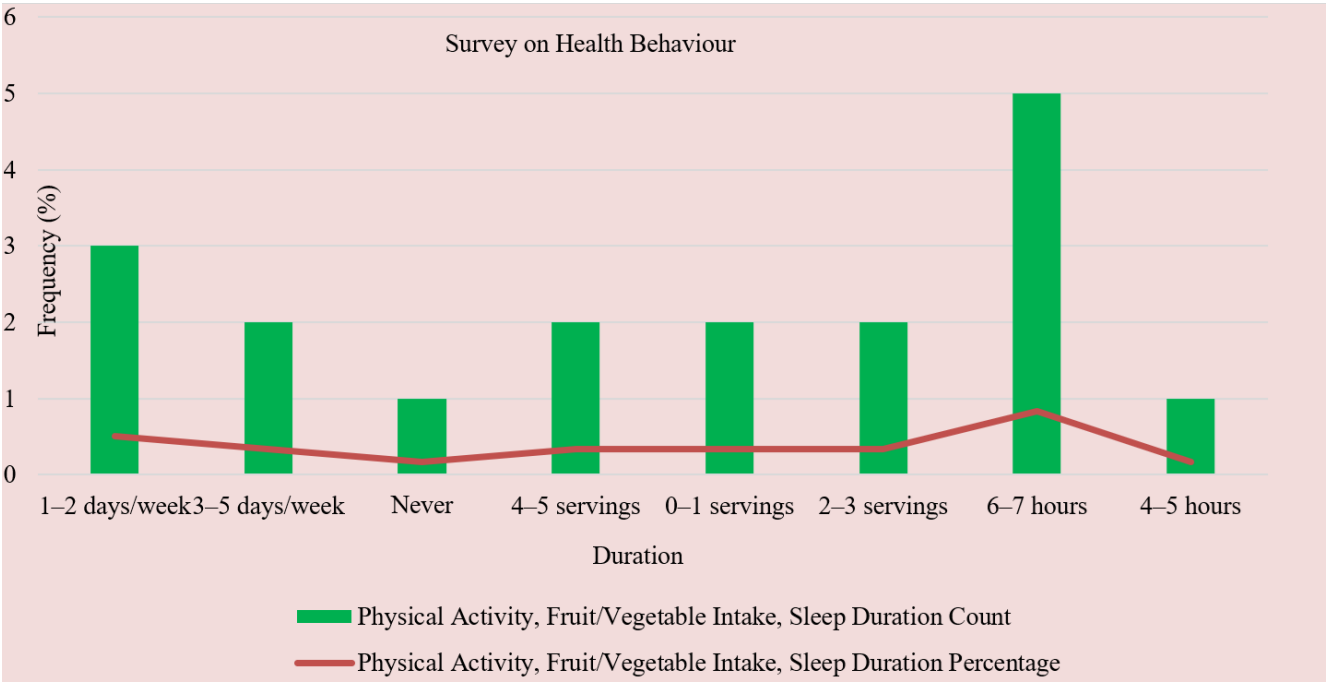

Figure 1: Patterns of Physical Activity, Dietary Intake, and Sleep Duration Among Adults (N = 6). Green bars represent the count of participants reporting each level of behavior for physical activity (days/week), fruit and vegetable intake (servings/day), and sleep duration (hours/night). The red line represents the corresponding percentages.

of risk behaviors that may compound obesity risk if not addressed holistically.

Participants who consumed 0–1 serving of fruits and vegetables daily also frequently reported higher levels of screen time and occasional or frequent stress, suggesting potential dietary coping mechanisms reinforcing obesogenic patterns. Meanwhile, individuals who were physically active 3–5 days per week tended to report higher fruit and vegetable intake and moderate screen time, indicating some alignment with healthier lifestyle patterns [42].

3.4. Data Quality and Survey Performance

From a methodological standpoint, the pilot demonstrated strong survey functionality and data quality. All six participants completed the survey without skipping any items. Logic pathways functioned correctly in Microsoft Forms, ensuring only relevant questions were displayed based on previous responses. The average completion time was between five and seven minutes, meeting the design expectation for minimal participant burden. No discrepancies or contradictions were detected across related survey items, such as consistency between self-reported health behaviors and perceived stress levels. This internal coherence further validated the survey's content structure and skipped logic programming. Overall, the pilot findings confirmed that the Adult Obesity Risk Assessment Questionnaire was both feasible and practically applicable for future larger-scale deployment.

4. Discussion

This pilot study represents an initial methodological step focused on evaluating the feasibility and clarity of a new behavioral lifestyle assessment tool. Findings are strictly preliminary and limited to feasibility insights, not comprehensive behavioral patterns or validated relationships with obesity outcomes. The findings from this pilot survey provide foundational insight into behavioral and lifestyle risk factors associated with adult obesity and demonstrate the potential value of the instrument for future field deployment. Although the sample size was small and purposively selected, the behavioral patterns observed—such as low physical activity levels, inconsistent dietary intake, high screen time, and self-reported stress—closely mirrored broader national and state-level trends, particularly those documented in Kentucky and similarly affected regions [4, 23]. These parallels suggest that, even in a limited pilot setting, the instrument was able to capture meaningful and relevant risk behaviors. Furthermore, the high rate of item completion, logical consistency of responses, and positive user experience reinforced the survey tool's usability, clarity, and interpretability. These outcomes collectively support its readiness for further refinement and scaled implementation across more diverse and representative populations. In doing so, the tool can serve as a practical, ethically grounded resource for identifying at-risk groups and informing the development of tailored community-based obesity prevention strategies.

One of the most striking patterns emerging from the pilot was the prevalence of sedentary behavior. Fifty percent of participants reported engaging in physical activity only 1–2 days per week, while another reported no physical activity at all. This aligns with long-standing evidence that inadequate physical activity is a key contributor to obesity and related chronic conditions[24]. Similarly, fruit and vegetable intake showed inconsistent trends, with one-third of respondents reporting as few as 0–1 serving per day. These findings echo concerns highlighted by the CDC and other national surveillance systems about dietary quality as a determinant of poor health outcomes [4]. Excessive consumption of sugar-sweetened beverages (SSBs) is strongly associated with increased obesity risk, as these drinks contribute significant added sugars and empty calories with minimal satiety, often leading to

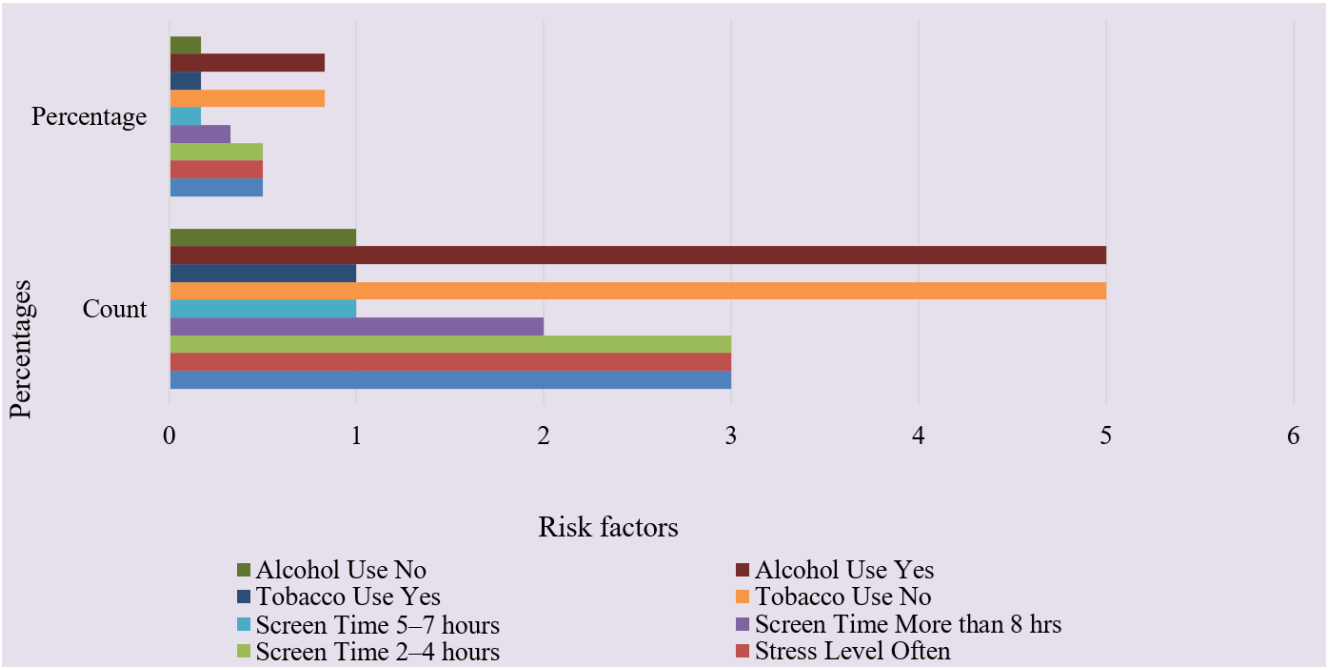

Figure 2: Behavioral Risk Factors: Screen Time, Alcohol Use, and Tobacco Use Among Adults (N = 6). Horizontal bars depict the number and percentage of participants reporting screen time (5–7 hours, >8 hours), alcohol consumption (yes/no), and tobacco use (yes/no). Each color represents a distinct behavioral category.

weight gain over time [43]. The metabolic effects of high sugar intake, including insulin resistance and elevated fat storage, further compound this risk[44]. Beyond physical health, individuals with obesity often experience psychosocial consequences such as low self-esteem, body dissatisfaction, and social stigma, which can contribute to mental health conditions like depression and anxiety [45]. This bidirectional relationship—where obesity can both lead to and be exacerbated by poor mental health—underscores the need for integrated approaches that address both nutritional behaviors and emotional well-being. Substance use, particularly alcohol and tobacco consumption, has been increasingly recognized as a contributing factor to adult obesity [46]. Alcohol introduces excess calories, alters appetite regulation, and is often associated with binge eating behaviors, while tobacco use can disrupt metabolic processes and promote weight cycling [47]. These behaviors are further complicated when combined with chronic stress or poor mental health, which may drive emotional eating and physical inactivity. Acknowledging the role of substance use in the development of obesity allows for a more comprehensive and realistic understanding of the behavioral patterns that shape health outcomes [48]. Its inclusion in this assessment tool supports a multidimensional approach to identifying modifiable risk factors within diverse populations.

High screen time was another notable outcome, with more than 80% of participants reporting over 2 hours per day and 33% exceeding 8 hours. Excessive screen time has been repeatedly linked with sedentary lifestyles, disrupted sleep, and metabolic dysfunction [49]. In this project, its measurement served as both a behavioral risk indicator and a window into how modern digital life may be shaping obesity trajectories. From a methodological perspective, the survey instrument performed reliably. Participants completed the tool within the intended timeframe (5–7 minutes), and no logic errors or technical issues were identified. The clarity of the questions, supported by the use of familiar response structures such as Likert scales and multiple-choice formats, allowed for smooth participant navigation. The high completion rate and consistency of data indicate strong face and content validity [50, 37].

This pilot study revealed several critical behavioral gaps that, if unaddressed, may contribute to the onset and persistence of obesity among young adult populations [51]. Notably, half of the participants engaged in physical activity only 1–2 days per week, and one reported no activity. Dietary behaviors were equally fragmented, with no respondents meeting the recommended daily intake of fruits and vegetables, and one-third reported as few as 0–1 serving per day. Although sleep duration was relatively adequate, elevated stress levels and screen time exceeding 8 hours per day among several respondents suggest psychosocial strain and sedentary routines that may reinforce obesogenic behaviors [52]. These patterns, especially when clustered—highlight the importance of integrative, multi-domain interventions. Policymakers and public health stakeholders can play a pivotal role by supporting access to affordable, nutritious food, investing in safe and inclusive recreational infrastructure, and promoting workplace wellness programs that reduce digital overexposure and support stress reduction [53]. Additionally, the behavioral domains captured in this tool—screen time, stress, dietary habits, and physical activity—can inform targeted messaging in community health campaigns [54]. From a research perspective, this pilot identified the need for scalable, behaviorally inclusive instruments that not only measure individual risk factors but also detect patterns across interrelated domains [55]. Future applications of this tool in larger, more diverse samples can support the development of tailored strategies to mitigate modifiable risk factors early and equitably.

This pilot study's survey development and administration process reinforced the centrality of ethical integrity in public health research. Even within an educational research context, deliberate attention was given to ensuring participant anonymity, transparency, and informed participation. Including a clear consent preamble, excluding personally identifiable data, and using neutrally worded items for sensitive topics such as substance use and stress were crucial in fostering trust and eliciting honest responses. These ethical safeguards aligned with institutional standards and enhanced the collected data's validity and reliability. This experience underscores a key principle in behavioral epidemiology: ethical rigor is inseparable from data quality, particularly in community-based health assessments where respondent trust directly influences disclosure [56]. The findings from this pilot also highlight the importance of behavioral specificity in survey instrument design. Rather than relying on general or vague assessments, the tool employed clearly defined frequency-based response options (e.g., "1–2 days of physical activity per week," "2–3 servings of fruits and vegetables per day"). This approach improved the interpretability of responses and increased the potential for translating data into practical public health recommendations. In the context of obesity research—where behavioral patterns are highly variable—precise questioning identifies concrete intervention points. Generic or overly broad survey items risk diluting actionable insights and may fail to capture the behavioral nuances needed to inform targeted programs or policies [57].

Furthermore, the pilot reinforced the value of localizing public health tools to reflect target populations' specific sociocultural and environmental conditions. In Kentucky, obesity prevalence is among the highest nationally, exacerbated by intersecting challenges such as rural geographic distribution, food insecurity, transportation barriers, and limited access to preventive care services [58]. Although the current sample consisted of graduate-level participants, future iterations of the instrument should be adapted to address diverse community contexts by incorporating items on community infrastructure, neighborhood safety, access to affordable healthy food, and transportation to health services. While descriptive statistics were suitable for the initial pilot phase, subsequent instrument deployments could benefit from inferential statistical analyses—including logistic regression and multivariable modeling—to identify predictors and correlates of obesity risk across demographic and behavioral strata. Such analyses would facilitate the development of evidence-informed, population-specific interventions that move beyond general health promotion toward precision public health.

4.1. Study Limitations

This pilot study has several important limitations that should be acknowledged when interpreting the findings. First, this study's key limitation was its notably homogeneous and small sample, consisting exclusively of graduate-level female students aged 18-33. This does not reflect the demographic diversity or socioeconomic variation present in the general adult population, particularly within high-risk regions such as rural Kentucky. The absence of male participants, the narrow educational and age ranges, and the lack of socioeconomic and geographic diversity significantly constrain the generalizability of findings. Future research must prioritize recruiting larger, diverse samples across genders, educational backgrounds, socioeconomic statuses, and geographic locations to ensure broader applicability and validity of the instrument. Additionally, the cross-sectional design and self-reported nature of the data introduce the potential for recall bias, social desirability bias, and underreporting of sensitive behaviors such as tobacco or alcohol use. Because the study relied solely on descriptive statistics, no causal inferences can be drawn regarding the relationships between behavioral patterns and obesity risk. These limitations are typical of pilot studies but underscore the need for more extensive validation in future research.

4.2. Recommendations and Implications

Despite inherent limitations, this pilot study provides valuable insights that can inform future research, public health planning, and policy development. Future applications of the behavioral risk assessment tool should prioritize larger, demographically diverse samples that capture variation in age, race/ethnicity, gender, education, income, and geographic context, particularly extending to high-risk rural areas. Employing multivariate analyses will allow researchers to identify behavioral clusters that act synergistically to elevate obesity risk, enabling more targeted and efficient intervention strategies [2]. Public health experts and policymakers should recognize the interconnectedness of risk behaviors—such as the coupling of low physical activity, high screen time, dietary inconsistency, and elevated stress—and avoid isolated or siloed programmatic responses. Integrating concise, behaviorally specific surveys into community health assessments and surveillance systems can enhance local needs mapping and support more responsive public health initiatives. Strategic policy investments should focus on improving environmental supports for healthy living, such as expanding access to affordable fresh foods, creating safe and accessible recreational spaces, addressing digital overexposure, and increasing the availability of preventive healthcare services in underserved areas [51]. By bridging behavioral data collection with structural interventions, future efforts can move toward more equitable, systemic reductions in adult obesity prevalence. Future research directions must involve larger-scale evaluations with diverse demographic groups—including varied genders, ages, educational levels, and geographic locations—to rigorously test the tool's psychometric properties, measurement invariance, and construct validity.

5. Conclusions

This pilot study successfully developed, implemented, and evaluated a 30-item behavioral risk assessment tool to identify lifestyle factors associated with adult obesity. Despite the intentionally limited sample of six graduate-level women, the instrument demonstrated strong feasibility, full response completion, and the ability to capture meaningful behavioral trends. Findings echoed national concerns around inconsistent dietary habits, low physical activity, prolonged screen exposure, and elevated stress levels among young adults. The study also highlighted the importance of ethical integrity, behavioral specificity, and practical usability in public health survey design. While preliminary, this work lays a foundation for future research involving larger, more diverse populations across gender, socioeconomic status, and geographic regions. Refinement and broader application of the instrument can support the development of targeted, community-centered interventions, especially in high-risk areas such as Kentucky. Ultimately, by translating behavioral science into practical tools, this study contributes to bridging the gap between research and real-world public health action in chronic disease prevention.

Conflicts of Interest

The authors declare no conflicts of interest related to this study.

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Institutional Review Board (IRB)

This study received ethical approval from the University of Louisville Institutional Review Board (Protocol No. PHEP-6273264). All procedures followed appropriate ethical guidelines and informed electronic consent was obtained from all participants prior to data collection.

Large Language Model

The authors used Microsoft Copilot to improve language clarity and grammar while preparing this manuscript. The authors have reviewed and edited the content accordingly and are fully responsible for the final version of the manuscript.

Authors Contribution

MRH led the development of the study, including survey design, literature review, data collection, analysis, and manuscript drafting. AH supported the methodological planning and provided critical feedback during manuscript revisions. Both authors reviewed and approved the final version of the manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The survey data was collected anonymously and has been securely stored by ethical guidelines.

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ASIDE Health Sciences

Original Article

Comparative Antimicrobial Efficacy of Cetrimide, Dettol, and Lizol Against Six Different Microbial Species on Epoxy-Coated Pharmaceutical Surfaces Using Surface Challenge Method

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ABSTRACT

Introduction: Disinfectants are vital in the pharmaceutical industry's sanitization process and contamination control programs. However, many pharmaceutical companies lack systematic policies for selecting appropriate disinfectants, often relying solely on manufacturer claims, which may not always be reliable. The complexity of existing disinfectant testing methods further complicates proper evaluation, highlighting the need for practical, efficient approaches.

Methods: This study used a simple surface challenge method to mimic real-world pharmaceutical conditions to test disinfectant efficacy. Three disinfectants, 1% Cetrimide, 2.5% Dettol, and 2% Lizol, were evaluated for antimicrobial activity. The test organisms included E. coli ATCC 8739, Salmonella typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 25619, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231, and an environmental isolate (Bacillus spp.). All testing was conducted on epoxy-coated floors within pharmaceutical industry premises.

Results: All three disinfectants demonstrated excellent antimicrobial activity against the tested organisms. After a 20-minute contact time, each disinfectant achieved a 6-log reduction in test organisms. The comparative evaluation indicated that 1% Cetrimide exhibited superior antimicrobial effectiveness compared to 2.5% Dettol and 2% Lizol.

Conclusions: The surface challenge method offers a practical approach for assessing disinfectant efficacy under pharmaceutical conditions. Among the disinfectants tested, 1% Cetrimide provided the most effective microbial reduction, suggesting its suitability for contamination control in pharmaceutical environments.

1. Introduction

Disinfectants are chemical or physical agents that destroy or remove vegetative forms of harmful microorganisms when applied to the surface. Disinfectants are classified by their types. These include aldehydes, alcohols, halogens, peroxides, quaternary ammonium, and phenolic compounds. Disinfectants vary in their spectrum of activity, mode of action, and efficacy. The first step of an efficient disinfection program is the choice of disinfectants that guarantee bactericidal, fungicidal, and sporicidal actions. The effectiveness of disinfectants can be affected by several factors, including pH, temperature, organic soiling, water hardness, and several dilutions [1, 2].

A disinfection efficacy study is part of a pharmaceutical manufacturing facility's overall contamination control program. It includes verifying proper cleaning and disinfection procedures and demonstrating that a product possesses antimicrobial activity under defined laboratory test conditions [3, 4, 5]. The disinfectants should be tested in several stages, such as preliminary suspension tests to verify whether a product deserves the qualification of a "disinfectant" and tests on surfaces that mimic practical conditions [6, 7].

Disinfectant efficacy studies demonstrate that disinfectants used on surfaces in manufacturing areas effectively inactivate or remove microorganisms, such as bacteria, fungi (yeast and molds), and bacterial spores, and validate the established disinfection procedures that provide the expected level of disinfection [8, 9, 10].

The research was conducted at Quest Pharmaceuticals Pvt., Ltd. from 20/05/2024 to 25/06/2024. It was designed to test the efficacy of disinfectants used in sanitizing areas in the pharmaceutical industry and the use of approved disinfectants during area sanitization procedures in the plant.

Surface challenge tests are widely considered for disinfection efficacy tests. This study is intended to provide an overview of disinfection efficacy testing and highlight its significance within the pharmaceutical industry for controlling contamination within the premise of the pharmaceutical industry. This study aims to find the efficacy of using disinfectants in plants and define the contact time of disinfectants in clean rooms for proper sanitization.

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Table 1: Materials and Equipment Used for Disinfectant Efficacy Testing

S.No	Materials and Equipment	Manufacturer
1	Pre Sterilized Petri plates	Tarsons
2	Soybean Casein Digest Agar	Hi Media
3	Sabouraud Dextrose Agar	Hi Media
4	Hot Plate	Lab Quest
5	Autoclave	Equitron
6	Biosafety Cabinet	Thermolab
7	Incubators	Allyone
8	Colony Counter	Lapiz
9	Cetrimide	Thermo Fisher
10	Iso Propyl Alcohol	Qualigens
11	Dettol	Rekitt
12	Lizol	Rekitt
13	Buffered peptone alkaline water pH 7.0	Hi Media
14	Sterile swab	Hi Media

2. Methods

2.1. Material

The materials and equipment utilized in this study are detailed in **(Table 1)**.

2.2. Media preparation

All media used in the study were prepared strictly per the manufacturer's recommendation (Hi Media).

2.2.1. Preparation and Identification of Environment Isolate

Two Petri plates of Soyabean Casein Digest Agar were exposed to air in a clean room for 30 minutes. After exposure, the plates were incubated at 35 °C for 48 hours. The plates were observed for the growth of organisms after the incubation period. A single isolated colony was selected randomly from the Soyabean casein digest agar plate, and the test organism was identified as Bacillus spp.

2.2.2. Culture Preparation

From a recently grown stock culture, the Subculture of each of the test organisms: E. coli ATCC 8739, Salmonella typhimurium ATCC14028, Pseudomonas aeruginosa ATCC 25619, Bacillus subtilis ATCC 6633, Candida albicans ATCC10231 and Environment Isolate (Bacillus spp) were performed on the surface of Soyabean casein digest agar and Sabouraud dextrose agar using Pour Plate Method. Using 0.9% Nacl, a ten-fold serial dilution of organisms was done. From test dilutions 10-5, 10-6, and 10-7, 1ml solution was pipetted in triplicate into 90 mm pre-sterilized Petri plates.15-20 ml of Soyabean casein digest agar (SCDA) were poured into plates containing bacterial culture, and 15-20 ml of Sabouraud dextrose agar (SDA) was poured into plates containing fungal culture. The SCDA plates were incubated at 30 °C to 35 °C for 48 hours and the SDA plates at 25 °C for 5 days. After incubation, the colonies in plates were counted, and the concentration of organisms in initial dilution was determined.

2.2.3. Disinfectant Preparation

1% cetrimide, 2.5% Dettol, and 2% Lizol solution were prepared using purified water.

2.3. Test Method

The test areas of 10×10 cm^2 were prepared duplicated on the epoxy-coated floor for the individual organisms and labeled as contact times 10 minutes and 20 minutes, along with the organism's name. Altogether, 12 areas of 10×10 cm^2 were prepared, and 1 ml culture of organisms was applied on the test area from known culture dilution with the help of Sterile Micro Pipette tips. The culture was spread on the test area uniformly with the help of a sterile inoculating loop and leaves for air drying. After air drying the test area, 2 ml of 1% Cetrimide, 2.5% Dettol, and 2% Lizol were applied to the 100 cm^2 test area. Disinfectants were spread uniformly with a sterile micropipette, and complete surface coverage of disinfectant on the test surface was visually confirmed before initiating contact time.

2.4. Swab Collection

Swabs containing the test organism were collected from the floor surface with sterile swabs, covering an area of 10x 10 cm^2, in unidirectional movements, first with 10 horizontal strokes followed by 10 vertical strokes.

Swab samples were taken from the test area labeled as contact time 10 minutes and 20 minutes for each organism at 10 minutes and 20 minutes time intervals individually. The swabs were dipped in a test tube containing 10 ml of Buffered peptone alkaline water with pH 7.0.

2.5. Sample Analysis

Each tube containing the swabs was shaken for 1-2 minutes, and 1 ml of the solution was pipetted individually for each organism in triplicate into 90 mm pre-sterilized Petri plates. 15-20 ml of Soyabean casein digest agar (SCDA) was poured into plates containing bacterial culture, and 15-20 ml of Sabouraud dextrose agar (SDA) was poured into plates containing fungal culture. The SCDA plates were incubated at 30 °C to 35 °C for 48 hours, and the SDA plates at 20 °C to 25 °C for 5 days. After the incubation period, the colonies in plates were counted, and the concentration of organisms in the test solution was determined.

2.6. Calculation of test Result

2.6.1. TAMC (cfu/ml)

$$\text{TAMC (cfu/ml)} = \frac{\text{No. of colonies per ml} \times \text{Dilution factor}}{\text{Sample in ml}}$$

2.6.2. Logarithmic Reduction Factor (RF)

$$\text{RF} = \log N_c - \log N_t$$

Where,

N_c: Number of colonies used during the test

N_t: Number of colonies observed after the test

3. Results

1% Cetrimide shows excellent antimicrobial activity and reduces the tested organisms by 6 logs at an exposure time of 10 minutes, except for E. coli **(Table 2)**.

2% Lizol shows excellent antimicrobial activity against Pseudomonas aeruginosa and Candida albicans and gives a 6-log

Table 2: Efficacy Result of 1% Cetrimide

S.no.	Organisms	Concentration of organisms used	Contact time	Colony observed (cfu/ml) after test	Kill colony %	Log reduction
1	Bacillus subtilis	4×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
2	E. coli	29×10^6 cfu/ml	10 min	30	99.9998%	5 log reduction
			20 min	0	100%	>6 log reduction
3	Salmonella typhi	16×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
4	Pseudomonas aeruginosa	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
5	Candida albicans	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
6	Environment Isolate (Bacillus spp)	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction

CFU, Colony Forming Unit

Table 3: Efficacy Result of 2% Lizol

S.no.	Organisms	Concentration of Organisms Used	Contact Time	Colony Observed (Cfu/ml) After Test	Kill Colony %	Log Reduction
1	Bacillus subtilis	4×10^6 cfu/ml	10 min	23	99.9994%	5 log reduction
			20 min	3	99.9999%	6 log reduction
2	E. coli	23×10^6 cfu/ml	10 min	63	99.9997%	5 log reduction
			20 min	3	99.9999%	6 log reduction
3	Salmonella typhi	11×10^6 cfu/ml	10 min	17	99.9998%	5 log reduction
			20 min	3	99.9999%	6 log reduction
4	Pseudomonas aeruginosa	5×10^6 cfu/ml	10 min	27	99.9994%	5 log reduction
			20 min	3	99.9999%	6 log reduction
5	Candida albicans	2×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
6	Environment Isolate (Bacillus spp)	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction

CFU, Colony Forming Unit

reduction at an exposure time of 10 minutes. Still, Bacillus subtilis, E. coli, Salmonella typhi, and Environment Isolate (Bacillus spp) fail to give a 6-log reduction at an exposure time of 10 minutes **(Table 3)**.

2.5% Dettol shows excellent antimicrobial activity against Bacillus subtilis, E. coli, Salmonella typhi, and Candida albicans and gives a 6-log reduction at an exposure time of 10 minutes. Still, Pseudomonas aeruginosa and Environment Isolate (Bacillus spp) fail to give a 6-log reduction **(Table 4)**.

4. Discussion

Disinfectants kill the bacteria by damaging their cell wall or cell membrane at specified Concentration and contact time. The study's observations were expressed in log10 reductions against different contact times (10 minutes and 20 Minutes). All disinfectants showed good antimicrobial activity and had 5 log reductions or more at a contact time of 10 minutes and 20 Minutes, respectively. The study was conducted on epoxy-coated floors as most of the classified areas in the pharmaceutical industry are epoxy-coated where production activities are done.

Table 4: Efficacy Result of 2.5% Dettol

S.no.	Organisms	Concentration of Organisms Used	Contact Time	Colony Observed (Cfu/ml) After Test	Kill Colony %	Log Reduction
1	Bacillus subtilis	4×10^6 cfu/ml	10 min	10	100%	>6 log reduction
			20 min	20	100%	>6 log reduction
2	E. coli	29×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
3	Salmonella typhi	16×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
4	Pseudomonas aeruginosa	3×10^6 cfu/ml	10 min	30	99.999%	5 log reduction
			20 min	0	100%	>6 log reduction
5	Candida albicans	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
6	Environment Isolate (Bacillus spp)	9×10^6 cfu/ml	10 min	100	99.9998%	5 log reduction
			20 min	3	99.9999%	6 log reduction

CFU, Colony Forming Unit

Antimicrobial efficacy of 1% Cetrimide showed excellent antimicrobial activity and a 6-log reduction of tested organisms. E coli fails to give a 6log reduction at a contact time of 10 minutes. This might be due to the impact of environmental factors on the organisms under test conditions. 2.5% Dettol also showed excellent antimicrobial efficacy against most tested organisms, but Pseudomonas aeruginosa failed to give a 6log reduction at a contact time of 10 minutes; this might be due to more resistance mechanism of the organism in the tested 2.5% Dettol solution. 2% Lizol showed poor activity against Bacillus subtilis, E. coli, Salmonella typhi, and Environment Isolate (Bacillus spp) and failed to give a 6-log reduction at an exposure time of 10 minutes. This might be due to the organisms' high resistance to Lizol on low contact time.

The results showed that 1% Cetrimide had excellent antimicrobial activity compared to 2.5% Dettol and 2 % Lizol. This result agrees with Joshi et al. 's test. [6], in which the test disinfectants gave more than a 4-log reduction for the tested organisms. In similar studies by Bhosale et al. [11], the disinfectant activity of tested disinfectants has more than a 5 log reduction. Our findings also agree with a similar study by Kumar et al. [12], who stated more than 5 log reduction of test organisms under study. However, the result of Olasehinde et al. [13] disagrees with our study, which stated 4 logs or less reduction of the organisms in the study. This could be possibly due to improper dilation or incorrect concentration details mentioned by the manufacturer.

Therefore, this indicates that all the test disinfectants have excellent antimicrobial efficacy at the recommended concentration and contact time of 10 minutes and 20 minutes on test surfaces. Using all the mentioned disinfectants may reduce the contamination caused by the test microorganisms and is an important means of controlling contamination in the disinfectant control program in the pharmaceutical industry.

Several limitations should be acknowledged in this study. First, negative, positive, and sterility controls were not incorporated into the experimental design. This decision was made because the study aimed to evaluate antimicrobial efficacy under practical field conditions with naturally occurring microbial loads on surfaces rather than standardized laboratory conditions with defined inoculation. Second, the statistical analysis is limited due to the study's primary objective of determining whether the tested disinfectants achieve a 6-log reduction in microbial populations. While log reduction values are reported, the experimental design was not optimized for comprehensive statistical evaluation of treatment differences. However, this approach aligns with industry standards for disinfectant efficacy testing. Third, the differential susceptibility observed between E. coli and Pseudomonas aeruginosa to 1% Cetrimide warrants consideration. Under the tested conditions, E. coli demonstrated reduced susceptibility compared to P. aeruginosa, which may be attributed to species-specific differences in cell wall composition, efflux mechanisms, or environmental stress responses. This finding is consistent with known variations in antimicrobial resistance patterns among gram-negative bacteria and does not compromise the overall validity of the results.

5. Conclusions

The study shows that the In-use disinfectants are effective at contact times of 10 and 20 minutes, respectively. If the desired log reduction is 6 log reduction during a contamination control program in the Pharmaceutical Industry, then a contact time of 20 minutes should be determined for each disinfectant.1% Cetrimide has excellent antimicrobial activity compared to 2.5% Dettol and 2% Lizol.

Our study concluded that all three disinfectants had broad activity against the organism. Proper concentration and contact period are crucial for any disinfectant to give an excellent result. So, proper concentration and contact time should be determined under practical conditions to get better results when selecting disinfectants for contamination control programs in the pharmaceutical industry.

Conflicts of Interest

The authors declare no competing interests that could have influenced the objectivity or outcome of this research.

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Institutional Review Board (IRB)

This study did not involve human participants, human data, or human tissue. Therefore, approval from an IRB or ethics committee was not required. All procedures were carried out in accordance with relevant guidelines and regulations for laboratory-based research.

Large Language Model

No large language model was used in the preparation of this manuscript.

Authors Contribution

SKS: Conceptualization, methodology, investigation, data collection, analysis, and writing—original draft and final manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Original Article

Dual versus Single Ovulation Triggers in In Vitro Fertilization and Intracytoplasmic Sperm Injection: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Infertility remains a significant global health concern. Optimizing hormonal triggers, such as human chorionic gonadotropin (HCG) with or without gonadotropin-releasing hormone (GnRH) agonists, is crucial to enhance reproductive outcomes. We aim to evaluate and compare the reproductive success rates of dual trigger protocols (HCG + GnRH agonist) versus HCG alone in women undergoing assisted reproductive technologies (IVF/ICSI).

Methods: A systematic search was conducted in PubMed, Scopus, and Web of Science for studies published up to January 2025. Studies comparing reproductive outcomes in women undergoing IVF/ICSI who received either dual trigger (HCG + GnRH agonist) or HCG alone were included. Data were analyzed using RevMan version 5.4 and R Studio version 4.4.1. The primary outcome was the clinical pregnancy rate. Secondary outcomes included live birth rate, fertilization rate, and embryo quality metrics.

Results: Seventeen studies with a total of 2,239 women were included: 1,118 in the dual trigger group and1,121in the HCG onlygroup.Thedualtriggergroupshowedsignificantlybetteroutcomesinterms of total oocytes retrieved, fertilized oocytes, follicles >15mm on trigger day, viable embryos, two pronuclei (2PN) formation, clinical pregnancy, biochemical pregnancy, live birth rate, good quality embryos, and fertilization rate.

Conclusions Dual triggering with HCG and GnRH agonist appears to significantly enhance reproductive outcomes compared to HCG alone in women undergoing IVF or ICSI. These findings support the broader adoption of dual trigger protocols in assisted reproductive practice.

1. Introduction

Infertility is defined as a failure to achieve conception after 1 year of regular unprotected sexual intercourse, according to the World Health Organization (WHO). Around 16-17.5% of couples suffer from infertility. [1]. To address these growing health issues, many drugs and interventions have emerged. The most commonly used intervention worldwide was assisted reproductive technology (ART), which is a tool that reshapes human reproduction to help millions of couples suffering from infertility to conceive. It involved In Vitro Fertilization (IVF) and Intracytoplasmic sperm injection (ICSI) [1, 2] In the 1990s, ICSI technology emerged as an intervention aid, especially in male infertility, to achieve a higher pregnancy rate [3, 4]. To improve the success rate and improve pregnancy outcomes in ART technology, many protocols have emerged, such as Controlled ovulation hyper stimulation (COH); these treatment protocols are used during IVF intervention to retrieve high-quality numbers of eggs and embryos and thus improve the success rate of pregnancy [5]. The basis of using COH depends on mimicking physiological luteinizing hormone (LH) surge through administration of a single dose of human chorionic gonadotropin (HCG) after 18 hours of LH surge to stimulate division of meiosis and final follicular maturation [6, 7].

However, hCG injections can trigger final oocyte maturation even without a concomitant FSH surge, leading to a prolonged luteotropic effect that is associated with an increased risk of ovarian hyperstimulation syndrome (OHSS).[8, 9] In response to reduce this risk, several studies searched for ovulation triggers other than HCG, for example, GnRH agonist (GnRH) was first introduced thirty years ago [10]. GnRH has a pharmacological action that mimics the physiological mid-cycle hormonal profile that occurs during the natural ovulation through inducing simultaneous surges of both LH and FSH to stimulate oocyte maturation [11]. By adding benefits for reducing the prevalence of OHSS[12]. This reduces risk through endogenous release of physiological LH, which is more physiological than HCG [13]. Although other studies found that using GnRH trigger leads to luteal phase defect resulting in decreased implantation, pregnancy rate, and abortion, notably in fresh embryo transfer cycles than HCG triggers IVF cycles [8].

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In recent years, many studies have used both GnRH and a low dose of HCG. They called "Dual Triggers," which has been very effective for final oocyte maturation [14], increasing pregnancy rate, and decreasing risk of OHSS compared with single HCG triggers [15]. We aimed from this systematic review and meta-analysis to investigate Dual triggers versus single HCG triggers in women undergoing IVF/ICSI cycles in different reproductive outcomes.

2. Methods

2.1. Study Registration

This study is a systematic review and meta-analysis design, outlined in the Cochrane Handbook for Systematic Reviews of Interventions and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [16]. Each procedural step was precisely executed according to the methodologies outlined in the Cochrane Handbook for Systematic Reviews of Interventions [17]. This review was registered in the PROSPERO database under registration number (CRD420251036833) on 13 March 2024.

2.2. Literature Search Strategy and Information Sources

We searched for a comprehensive search of many electronic databases, including MEDLINE via PubMed, Scopus, Web of Science, and previously published meta-analyses. The search strategy incorporated a combination of Medical Subject Headings (MeSH) and free-text terms, integrated with Boolean operators ("AND" and "OR") to ensure a balance between sensitivity and specificity. The key search terms included Articles were identified using the following strategy: ("reproductive outcome" OR "pregnancy outcome" OR "birth outcome") AND "in vitro fertilization" OR "IVF" OR "ICSI" AND "HCG))" OR "human chorionic gonadotropin" AND "GnRH" OR "gonadotropin-releasing hormone". The detailed search strategy is included in the Online Resource. No restrictions by language or publication period were employed.

2.3. Eligibility Criteria

Studies were eligible if they included women of childbearing age undergo IVF/ICSI, treated with dual trigger treatment combination between (HCG + GnRH agonist) as a primary intervention compared to (HCG alone) and reported outcomes such as mature oocyte, total oocyte, clinical pregnancy, biochemical pregnancy, implantation, abortion, fertilization, ongoing pregnancy, live birth, good quality embryo, embryo transferred, duration of stimulation, follicle size, oocytes retrieval, and duration of stimulation. Eligible study designs included randomized controlled trials (RCTs). On the other side, excluded Study designs involving observational, retrospective, non-RCTs, case series, case reports, reviews, and expert opinions. Additionally, studies that were only available as conference abstracts or protocols, those lacking complete full texts, and non-English studies were excluded.

2.4. Study Selection

Four authors [M.W, A.E, E.M, and A.M] independently screened the studies according to the previously mentioned eligibility criteria. Where Eligibility screening was performed in two steps by Rayyan software [18], the first step was to screen the titles and abstracts for eligibility. In the second step, full-text articles of eligible abstracts were retrieved and screened for inclusion eligibility. With discrepancies resolved by consensus or consultation with another reviewer [E.E].

2.5. Primary and secondary outcomes

The primary outcomes of interest were pregnancy outcomes (oocyte, clinical pregnancy, biochemical pregnancy, implantation, and abortion). Whereas, the secondary outcomes were as 2PN, cleavage rate(oocyte &embryo), fertilization, ongoing pregnancy, live birth, good quality embryo, embryo transferred, Viable embryo, cancellation rate, multiple pregnancy, duration of stimulation, follicles >10 mm at trigger day, follicles >15 mm at trigger day, cryopreserved, oocyte /follicle aspirate, oocytes retrieval, duration of stimulation, endometrial thickness on trigger, total dose gonadotropin, estradiol level (E2) on trigger day, and progesterone level on trigger day.

2.6. Data Extraction

Three authors shared independently extracted data using a standardized electronic form, recording key information. The extracted data encompassed several comprehensive categories of information from the included studies. First, they recorded summary information including authors, year, study design, intervention, and measured outcomes from each study. Additionally, they documented baseline characteristics such as authors, year, age, BMI, infertility duration, primary and secondary infertility classifications, causes of infertility including tubal and male factors, and basal hormonal levels including FSH/IU, LH, IU/l, E2 pmol/l, and AMH ng/ml. The extraction process also involved assessing risk of bias domains for each study, along with capturing the study outcomes that were previously identified as primary and secondary endpoints. Discrepancies during data extraction were resolved by re-verification and discussions through the senior investigator. The extracted data were organized into tables to ensure consistency and facilitate subsequent analysis.

2.7. Quality Assessment

We assessed the risk of bias for randomized controlled trials (RCTs) using the Risk of Bias 2.0 (RoB 2) tool [17]. Bias was evaluated by an intention-to-treat perspective across seven key domains. These domains included random sequence generation, allocation concealment, deviations from intended interventions, measurement of the outcome, including blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. Each domain and the overall study were assigned a risk of bias rating of 'low', 'some concerns', or 'high'. An overall 'low' risk of bias was given only if all domains were rated 'low'; an overall 'some concerns' rating was assigned if one or more domains were rated 'some concerns'; and an overall 'high' risk of bias was assigned if one or more domains were rated 'high' **Figure S2**. Evaluating domains such as randomization, deviations from intended interventions, missing outcome data, outcome measurement, and selective reporting. One reviewer performed the assessments, and discrepancies were resolved through discussion with the senior author.

2.8. Statistical Analysis

All statistical analyses were conducted using Review Manager (RevMan) version 5.4. Continuous outcomes were evaluated using the mean difference (MD) with corresponding 95% confidence intervals (CI), while dichotomous outcomes were assessed using odds ratios (OR) and the Mantel-Haenszel method. Heterogeneity among the included studies was evaluated using both the Chi-square test and the I^2 statistic. And I^2 value exceeding 50% was interpreted as indicative of significant heterogeneity, warranting the use of a random-effects model. Heterogeneity was interpreted by the Cochrane Handbook (Chapter 9), with I^2 values categorized as follows: 0–40% (low), 30–60% (moderate), 50–90% (substantial), and 75–100% (considerable). A Chi-square p-value of less than 0.1

was considered statistically significant for heterogeneity, while a p-value < 0.05 was deemed statistically significant for all other analyses. To assess potential publication bias, funnel plots were visually inspected, and Egger's regression test was performed using the standard error of the observed outcomes as predictors to detect asymmetry.

Heterogeneity was assessed through visual inspection of the forest plots and measured using the I^2 and Chi-square tests. Heterogeneity was considered significant when the chi-square test p-value is less than 0.1 and the I^2 test is greater than 50%, following the recommendations of the Cochrane Handbook for Systematic Reviews and Meta-Analysis. Using a random effect model for the outcome reveals significant heterogeneity (chi-square p-value < 0.1 and I^2> 50%), and the meta-analysis excludes studies with missing outcomes.

We performed sensitivity analyses to ensure that none of the included studies affected the results and to examine whether the overall effect size is statistically significant among them. In each scenario, we excluded one study to ensure the overall effect size was not dependent on any single study. Additionally, we focused only on RCTS and cohort studies and excluded case-control studies and cross-sectional studies. In cases of significant heterogeneity (Chi-Square P<0.1& I^2 > 50%), sensitivity analyses were conducted to address the heterogeneity. **Figure S1** PRISMA flow diagram for new systematic reviews that included searches of databases and registers only.

3. Results

3.1. Search and Screening:

The initial search yielded 7897 records from three electronic databases, trial registries, and previous meta-analyses. After removing 1022 duplicates, 6875 records remained for title and abstract screening. Of these, 6784 records were excluded based on irrelevance to the research question, inappropriate study design, or population mismatch. The full texts of 91 articles were reviewed for eligibility, and 17 studies met the inclusion criteria. Ultimately, 17 studies were included in qualitative and quantitative synthesis (meta-analysis) (Supplementary file).

3.2. Study Characteristics

A total of 17 studies were included, comprising randomized controlled trials. Intervention groups received human chorionic gonadotropin (HCG) hormones, combined with gonadotropin-releasing hormone (GnRH). At the same time, control groups received human chorionic gonadotropin hormones (HCG) only. Women were predominantly childbearing age, with mean ages ranging from 20 to 40 years **Figure S1**.

3.3. Risk of bias assessment

We assessed the risk of bias in the included studies using The Cochrane risk of bias 2 (RoB 2.") tool used for randomized controlled trial studies (RCTs), where most often of the included RCTs (14 studies) had low risk of bias for overall judgement other than three study was some concerns, where all studies had a low risk of bias judgment for 3 domains (randomization, missing outcome, and selection of reported results), a moderate risk of bias judgment for 2 domains (blinded outcome assessment, and other bias) With only one study high in risk due to not mentioned data about randomization. Careful revision of the data presented in the published articles (**Figure s2**).

3.4. Outcomes

Seventeen studies involving a total of 2239 women were included in this quantitative analysis that compares reproductive outcomes among patients who received human chorionic gonadotropin hormones (HCG) and who received (human chorionic gonadotropin hormones (HCG) combined with gonadotropin-releasing hormone (GnRH)).

3.4.1. Total Oocyte

Four studies [19, 20, 21, 22] involving a total of 380 women measured the total oocyte count. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled MD: 2.05, 95% CI [0.44; 3.67], P= 0.0125). The Pooled studies were homogenous (Chi-square P= 0.19, I^2= 36.8%) with mild heterogeneity **Figure S3**.

3.4.2. Mature Oocyte (MII)

Twelve studies [23, 8, 24, 25, 2, 22, 26, 27, 28, 29, 30, 11] involving a total of 1714 women measured the MII. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger did not favor either of the two groups (pooled MD: 2.91, 95% CI [-1.03; 6.85], P= 0.148). Pooled studies were not homogenous (Chi-square P< 0.0001, I^2 = 88.3%) **Figure S4**. To resolve the heterogeneity, we conducted a sensitivity analysis in multiple scenarios, excluding one study in each scenario. Heterogeneity was best resolved by omitting Meng-Han Yan, 2023 MD: 0.74, 95% CI [0.50; 0.98], (P = 0.47, I^2 = 0%) **Figure S35**.

3.4.3. Oocyte Retrieval

Eleven studies [23, 8, 25, 2, 26, 27, 28, 29, 31, 30, 11] involving a total of 1528 women measured the oocyte retrieval. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled MD: 0.71, 95% CI [0.38; 1.03], P< 0.0001). Pooled studies were homogenous (Chi-square P= 0.09, I^2= 37.9%) with mild heterogeneity **Figure S5**.

3.4.4. Fertilized Oocytes

Four studies [19, 2, 26, 31] involving a total of 393 women measured the fertilized oocytes, the overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled MD: 0.50, 95% CI [0.12; 0.87], P= 0.0098). Pooled studies were homogenous (Chi-square P= 0.55, I^2= 0.0%) **Figure S6**.

3.4.5. Cryopreserved Oocyte

Four studies [8, 24, 29, 31], involving a total of 393 women, measured the cryopreserved oocyte. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled MD: 0.87, 95% CI [- 0.04; 1.79], P=0.06). Pooled studies were not homogenous (Chi-square P=0.02, I^2 = 68.9%). **Figure S7**. To resolve the heterogeneity, we conducted a sensitivity analysis in multiple scenarios, excluding one study in each scenario. Heterogeneity was best resolved by excluding the study of Svenstrup (2024 (I^2 = 24.3%) **Figure S28**.

3.4.6. Follicles > 10mm on trigger day

Four studies [8, 20, 27, 31], involving a total of 488 women, measured the Follicles > 10mm on the trigger day. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled MD: 0.37, 95% CI [- 0.24; 0.97], P= 0.23). Pooled studies were homogenous (Chi-square P= 0.41, I^2= 0.0%) **Figure S8**.

Table 1: Baseline characteristics and hormonal parameters across included studies*

Study ID	Group	Tubal	Male	FSH (IU/l)	LH (IU/l)	E2 (pmol/l)	AMH (ng/ml)
Abed, 2020 [19]	Intervention	14 (35%)	NA	6.81 (2.51)	5.13 (2.63)	36.25 (16.43)	NA
	Control	8 (20%)	NA	6.04 (2.38)	4.98 (2.63)	34.05 (13.33)	NA
Ali, 2020 [23]	Intervention	10 (12.5%)	29 (36%)	5.65 (2.23)	3.74 (2.03)	NA	2.38 (1.59)
	Control	16 (32%)	36 (45%)	5.95 (2.22)	3.75 (2.71)	NA	2.05 (1.34)
Decleer, 2014 [24]	Intervention	NA	NA	6.9 (3.4)	NA	2164.57 (262.13)	NA
	Control	NA	NA	7.5 (2.3)	NA	2207.78 (173.71)	NA
Eftekhar, 2017 [25]	Intervention	NA	NA	6.59 (2.76)	NA	2164.57 (262.13)	NA
	Control	NA	NA	6.14 (2.59)	NA	2207.78 (173.71)	NA
Farouk, 2024 [2]	Intervention	NA	NA	9.27 (0.97)	5.9 (1.3)	46.38 (6.93)	0.674 (0.15)
	Control	NA	NA	9.5 (0.89)	5.8 (0.75)	45.73 (6.46)	0.719 (0.12)
Haas, 2020 [20]	Intervention	NA	17 (22%)	NA	NA	8120 (4273.65)	22.41 (14.4)
	Control	NA	17 (21%)	NA	NA	6818 (3614.75)	20 (18.18)
Keskin, 2023 [22]	Intervention	NA	NA	NA	NA	NA	2.45 (0.71)
	Control	NA	NA	NA	NA	NA	2.98 (1.44)
Kim, 2014 [26]	Intervention	32 (53.3%)	28 (46.7%)	6.2 (1.9)	5.5 (1.8)	48.9 (18.5)	NA
	Control	35 (58.3%)	25 (41.7%)	6 (2)	5.7 (1.9)	46.7 (14.8)	NA
Maged, 2020 [27]	Intervention	24 (30%)	21 (26.3%)	12.3 (1.8)	6.1 (1.6)	NA	0.9 (0.1)
	Control	26 (32.5%)	20 (25%)	12.2 (1.6)	5.8 (1.2)	NA	0.9 (0.1)
Meng-Han Yan, 2023 [30]	Intervention	14 (35.9%)	24 (61.5%)	6.46 (2.16)	4.95 (3.65)	40 (23.3)	2.96 (1.46)
	Control	18 (52.9%)	16 (47.1%)	6.96 (1.83)	4.33 (1.98)	38.2 (16.1)	3.58 (1.41)
Schachter, 2008 [32]	Intervention	17 (16%)	52 (49.5%)	7.2 (4.1)	NA	NA	NA
	Control	14 (13%)	50 (47%)	6.9 (2.8)	NA	NA	NA
Singh, 2023 [29]	Intervention	NA	NA	5.82 (1.7)	4.38 (1.89)	2539.86 (252.8)	2.75 (0.87)
	Control	NA	NA	5.54 (1.77)	4.17 (1.93)	2765.86 (271.29)	2.79 (0.84)
Svenstrup, 2024 [31]	Intervention	2 (8%)	10 (40%)	6.2 (1.5)	7.8 (4.3)	NA	NA
	Control	1 (5%)	6 (27%)	5.7 (1.8)	5.9 (3.0)	NA	NA
Zhou, 2022 [15]	Intervention	93 (56.7%)	20 (12.2%)	9.83 (3.58)	4.86 (2.53)	164.13 (74.69)	1.77 (1.84)
	Control	89 (54.3%)	29 (17.7%)	9.63 (3.07)	4.9 (2.58)	163.69 (71.71)	1.84 (1.51)
Mahajan, 2016 [28]	Intervention	NA	NA	7.7 (3.0)	5.3 (3.1)	NA	2.3 (1.3)
	Control	NA	NA	7.2 (2.5)	4.8 (2.8)	NA	2.0 (1.0)
Alleyassin, 2018 [8]	Intervention	NA	NA	4.91 (2.30)	8.31 (4.32)	NA	4.29 (3.5)
	Control	NA	NA	5.54 (2.45)	10.22 (7.29)	NA	3.72 (2.39)
Humaidan, 2006 [21]	Intervention	NA	NA	NA	NA	NA	NA
	Control	NA	NA	NA	NA	NA	NA

AMH, anti-Müllerian hormone; E2, estradiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; LH, luteinizing hormone; NA, not available. *Note: Continuous variables are described as Mean (SD), and the categorical variables are described as N (%).

3.4.7. Follicles >15mm on trigger day

Five studies [25, 27, 29, 30, 11], involving a total of 816 women, measured the Follicles > 15mm on the trigger day. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled MD: 0.51, 95% CI [0.16; 0.86], P= 0.004). Pooled studies were homogenous (Chi-square P= 0.40, I^2 = 0.0%) **Figure S9**.

3.4.8. Cleavage Rate Oocyte/ Embryo

Four studies [25, 27, 31, 11], involving a total of 741 women, measured the cleavage rate of oocyte/embryo. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger did not favor either of the two groups (pooled MD: 0.88, 95% CI [- 0.06; 1.82], P=0.06). Pooled studies were not homogenous (Chi-square P= 0.09, I^2= 53.2%). **Figure S10**, to resolve the heterogeneity, we

Table 2: Baseline demographic and infertility characteristics across included studies

Study ID	Group	Age (Mean ± SD)	BMI	Infertility duration (years)	Primary infertility	Secondary infertility
Abed, 2020 [19]	Intervention	28.63 (4.71)	26.82 (3.21)	6.1 (3.46)	29 (72.5%)	11 (27.5%)
	Control	28.18 (5.88)	26.11 (3.40)	6.7 (4.4)	31 (77.5%)	9 (22.5%)
Ali, 2020 [23]	Intervention	29.88 (4.45)	27.25 (3.52)	7.3 (4.16)	NA	NA
	Control	30.45 (4.55)	27.96 (4.08)	7.39 (4.23)	NA	NA
Decleer, 2014 [24]	Intervention	30 (3.6)	23.8 (4.6)	NA	NA	NA
	Control	30.5 (4.1)	23.5 (5.1)	NA	NA	NA
Eftekhar, 2017 [25]	Intervention	30.06 (5.3)	24.13 (2.87)	6.34 (3.85)	71 (71.3%)	27 (28.7%)
	Control	30.49 (4.79)	24.07 (2.98)	6.23 (4.09)	78 (78.4%)	20 (21.6%)
Farouk, 2024 [2]	Intervention	41 (1.12)	29.6 (1.77)	NA	NA	NA
	Control	41 (0.99)	29.9 (1.86)	NA	NA	NA
Haas, 2020 [20]	Intervention	35.4 (3.52)	23.6 (3.52)	NA	NA	NA
	Control	36 (3.55)	24.1 (4.85)	NA	NA	NA
Keskin, 2023 [22]	Intervention	34.23 (4.62)	NA	NA	NA	NA
	Control	32.75 (4.65)	NA	NA	NA	NA
Kim, 2014 [26]	Intervention	36.2 (3.7)	21.7 (2.0)	46.6 (24.2)	NA	NA
	Control	35.8 (3.8)	21.4 (2.2)	49 (29.1)	NA	NA
Maged, 2020 [27]	Intervention	39.1 (2.5)	27.3 (1.8)	5.7 (3.1)	NA	NA
	Control	38.9 (2.2)	26.9 (1.4)	5.2 (2.9)	NA	NA
Meng-Han Yan, 2023 [30]	Intervention	31.26 (4.05)	22.5 (4.65)	3 (2.5)	19 (48.7%)	20 (51.3%)
	Control	30.97 (3.65)	22.1 (4.0)	3 (2.75)	24 (70.6%)	10 (29.4%)
Schachter, 2008 [32]	Intervention	33.7 (5.6)	NA	NA	NA	NA
	Control	34.7 (4.7)	NA	NA	NA	NA
Singh, 2023 [29]	Intervention	30.98 (4.34)	24.37 (3.59)	NA	NA	NA
	Control	30.88 (3.70)	24.6 (2.64)	NA	NA	NA
Svenstrup, 2024 [31]	Intervention	30.1 (3.9)	23.93 (3.88)	2 (1.58)	NA	NA
	Control	30.9 (3.6)	24.7 (5.66)	2.3 (0.79)	NA	NA
Zhou, 2022 [15]	Intervention	38.49 (3.19)	22.49 (2.62)	4.56 (3.54)	68 (41.5%)	96 (58.5%)
	Control	38.88 (2.95)	22.6 (2.53)	4.59 (3.6)	66 (40.2%)	98 (59.8%)
Mahajan, 2016 [28]	Intervention	32.4 (4.5)	25.8 (3.9)	NA	NA	NA
	Control	33.1 (4.1)	24.2 (3.2)	NA	NA	NA
Alleyassin, 2018 [8]	Intervention	32.09 (5.52)	NA	NA	NA	NA
	Control	31.57 (6.02)	NA	NA	NA	NA
Humaidan, 2006 [21]	Intervention	NA	NA	NA	NA	NA
	Control	NA	NA	NA	NA	NA

BMI, body mass index; NA, not available; SD, standard deviation.

conducted a sensitivity analysis in multiple scenarios, excluding one study in each scenario that did not solve the heterogeneity

3.4.9. Viable embryo

Three studies [27, 29, 11] involving a total of 588 women, measured the viability of the embryo. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled MD: 0.98, 95% CI [0.33; 1.62], P= 0.002). Pooled studies were not homogenous (Chi-square P= 0.06, I^2= 63.8%). **Figure S11** shows the results of a sensitivity analysis conducted in multiple scenarios, excluding one study in each scenario. Heterogeneity was best resolved by excluding the study of Singh (2023 (P = 0.22, I^2 = 0%), (MD: 0.73, 95% CI [0.46; 1.00]) **Figure S33**.

3.4.10. Duration of Stimulation

Sixteen studies, [23, 8, 24, 25, 2, 20, 21, 22, 26, 33, 27, 28, 32, 29, 31, 30, 11], A total of 2159 women were involved, and the duration of stimulation was measured. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the HCG group (pooled MD: 0.16, 95% CI [0.05; 0.28], P= 0.004). Pooled studies were homogenous (Chi-square P= 0.135, I^2= 28.7%) **Figure S12**.

3.4.11. Endometrial thickness on trigger day

Seven studies [23, 8, 2, 26, 27, 32, 31], involving a total of 970 women, measured the endometrial thickness on trigger day. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the HCG group (pooled MD: -0.15, 95% CI

[-0.28; -0.02], P= 0.026). Pooled studies were homogenous (Chi-square P= 0.26, I² = 21.3%). With mild heterogeneity, **Figure S13**.

3.4.12. Estradiol level (E2) on trigger day

Eleven studies [19, 23, 24, 25, 20, 27, 28, 32, 29, 30, 11], involving a total of 1655 women, measured the estradiol level (E2) on trigger day. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled MD: 86.64, 95% CI [-38.78; 212.06], P=0.175). Pooled studies were not homogenous (Chi-square P< 0.0001, I²= 78.3%) **Figure S14**. We conducted a sensitivity analysis across multiple scenarios, excluding one study in each scenario, to avoid leading to a solution **Figure S29**.

3.4.13. Progesterone level on trigger day

Six studies [19, 28, 32, 29, 31, 30], involving a total of 587 women, measured the Progesterone level on trigger day. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger did not favor either of the two groups (pooled MD: - 1.87, 95% CI [- 5.31; 1.57], P= 0.28). Pooled studies were not homogenous (Chi-square P< 0.0001, I²= 82.8%). In **Figure S15**, we conducted a sensitivity analysis in multiple scenarios, excluding one study in each scenario. Heterogeneity was best resolved by excluding the study of Svenstrup 2024 MD: -0.01, 95% CI [-0.16; 0.14], (P = 0.19, I² = 35.2%) **Figure S32**.

3.4.14. X2 Pronucleate (2PN)

Five studies [24, 28, 29, 30, 11], Involving a total of 697 women, the X2 Pronucleate (2PN) was measured. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled MD: 1.89, 95% CI [- 0.07; 3.85], P= 0.058). Pooled studies were not homogenous (Chi-square P < 0.0001, I²= 88.6%). In **Figure S16**, we conducted a sensitivity analysis in multiple scenarios, excluding one study in each scenario. Heterogeneity was best resolved by excluding the study of Meng Han, 2023, with (MD: 0.91, 95% CI [0.0, 1.82]) I²= 63.0%) **Figure S31** .

3.4.15. Biochemical Pregnancy Rate

Fifteen studies [19, 23, 8, 25, 2, 20, 21, 22, 33, 27, 32, 29, 31, 30, 11], involving a total of 1818 women, measured the Clinical Pregnancy. The overall Risk Ratio (RR) between the Dual trigger group and the HCG trigger group favored the dual trigger group (pooled RR: 1.30, 95% CI [1.15; 1.46], P< 0.0001). Pooled studies were homogenous (Chi-square P= 0.21, I²= 21%) **Figure S34**.

3.4.16. Clinical pregnancy

Six studies, [23, 8, 25, 2, 27, 11], involving a total of 925 women, measured the biochemical Pregnancy. The overall Risk Ratio (RR) between the Dual trigger group and the HCG trigger group favored the dual trigger group (pooled RR: 1.26, 95% CI [1.02; 1.55], P= 0.031). Pooled studies were homogenous (Chi-square P= 0.52, I²= 0.0%) **figure S36**.

3.4.17. Ongoing Pregnancy Rate

Five studies [8, 24, 25, 32, 15], involving a total of 790 women, measured the ongoing pregnancy rate. The overall Risk Ratio (RR) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled RR: 1.14, 95% CI [0.92; 1.4], P= 0.23). Pooled studies were homogenous (Chi-square P = 0.21, I²= 30.4%) **Figure S26**.

3.4.18. Implantation Rate

Nine studies [23, 24, 25, 20, 21, 26, 27, 32, 11], Involving a total of 1900 women, the overall Risk Ratio (RR) between the Dual trigger and the HCG trigger did not favour either of the two groups (pooled RR: 1.24, 95% CI [1.04; 1.48], P= 0.0176). Pooled studies were not homogenous (Chi-square P = 0.0169, I-square 57.1%). **Figure S25** with best case scenario by omitting Decleer, 2014 with (RR: 1.37, 95%CL [1.11, 1.68], I² =35.5%)

3.4.19. Live Birth Rate

Six studies [23, 20, 22, 33, 30, 11], involving a total of 775 women, reported the live birth rate. The pooled analysis showed that the Dual trigger group had a significantly higher live birth rate compared to the hCG trigger group (pooled RR: 1.38, 95% CI [1.12; 1.68], P=0.0019). Pooled studies were homogenous (Chi-square P= 0.705, I²= 0%). The absolute live birth rates were 42.5% in the Dual trigger group and 30.8% in the hCG trigger group **Figure S24**.

3.4.20. Abortion Rate

Seven studies [23, 8, 25, 21, 22, 26, 11], involving a total of 552 women, measured the abortion rate. The overall Risk Ratio (RR) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled RR: 0.95, 95% CI [0.58; 1.56], P= 0.83). Pooled studies were homogenous (Chi-square P = 0.455, I²= 0.0%) **Figure S22**.

3.4.21. Cancellation rate

Three studies [8, 2, 27], involving a total of 432 women, measured the cancellation rate. The overall odds Ratio (OR) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled OR: 0.56, 95% CI [0.29; 1.07], P= 0.08). Pooled studies were homogenous (Chi-square P = 0.31, I²= 14.1%) with mild heterogeneity **Figure S23** .

3.4.22. Embryo transfer

Five studies [25, 2, 26, 27, 32], involving a total of 664 women, measured the embryo transfer. The overall Risk Ratio (RR) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled RR: 1.28, 95% CI [1.03; 1.59], P= 0.02). Pooled studies were homogenous (Chi-square P = 0.62, I²= 0.0%) **Figure S17**.

3.4.23. Multiple pregnancy

Three studies [23, 22, 26], involving a total of 325 women, measured the multiple pregnancy. The overall odds Ratio (OR) between the Dual trigger and the HCG trigger did not favor either of the groups (pooled OR: 1.49, 95% CI [0.75; 2.95], P= 0.25). Pooled studies were homogenous (Chi-square P = 0.52, I²= 0.0%). **Figure S18** .

3.4.24. Good quality embryo odds ratio (OR)

Three studies [24, 22, 11], involving a total of 565 women, measured the good quality of embryos. The overall odds Ratio (OR) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled OR: 2.20, 95% CI [1.29; 3.76], P= 0.0038). Pooled studies were homogenous (Chi-square P = 0.149, I²= 47.4%) **Figure S19**.

3.4.25. Good quality embryo Mean Difference (MD)

Five studies [23, 8, 29, 30, 11], involving a total of 787 women, measured the good quality embryo. The overall Mean Difference (MD) between the Dual trigger and the HCG trigger favored the dual trigger group (pooled MD: 1.23, 95% CI [0.54; 1.92], P=

0.0005). Pooled studies were not homogenous (Chi-square P= 0.0009, I^2= 78.5%). We conducted a sensitivity analysis in multiple scenarios, excluding one study in each scenario. Heterogeneity was best resolved by excluding the study of Singh, 2023 (P = 0.48, I^2 = 0.0%) **Figure S20**.

3.4.26. Total dose gonadotropin

There was no statistically significant difference in the total gonadotropin dose between the dual trigger and HCG trigger groups (MD: 16.02 IU/m, 95% CI [-47.60, 79.65], P = 0.6216). This confirms non-significance, with I^2 = 0.0%, indicating that the studies were highly consistent **Figure S21**.

4. Discussion

This study has been the most comprehensive systematic review and meta-analysis, including seventeen RCTs for reproductive outcomes and different types of ovulation triggers, which included women during IVF/ICSI. Ovulation trigger is the most essential step that dramatically contributes to the success of IVF. Therefore, the optimal timing of ovulation trigger and the pharmacokinetics and pharmacodynamics of the triggering agents were crucial in fertility treatment. This determination was essential for ending the follicular phase, selecting triggering agents, determining doses, timing oocyte retrieval, and mitigating potential consequences.

For decades, human chorionic gonadotropin (HCG) has been utilized as controlled ovarian stimulation (COS) during ovulation trigger to stimulate the development of multiple follicles and induce final oocyte maturation as a substitute for the natural endogenous LH surge in IVF, as HCG has similar structures and biological functions to luteinizing hormone (LH). There is only one receptor for them. It's inducing ovulation, resumption of meiosis in the oocyte, and formation of the corpus luteum. Moreover, HCG had a pivotal role in facilitating implementation through improving endometrial receptivity. [34, 35] However, HCG can lead to complications such as ovarian hyperstimulation syndrome (OHSS), which occurs in approximately 20-30% of cycles and potentially leads to severe consequences [36]. Consequently, scientists investigated various strategies to decrease the prevalence of OHSS, including a combination of gonadotropin-releasing hormone (GnRH) agonists and human chorionic gonadotropin (HCG) triggers (dual trigger). Where GnRH stimulates the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are the most critical hormones for the maturation of the follicles. Therefore, dual trigger has been an effective way to maintain the optimal luteal phase function and decrease the time of using HCG alone, thus significantly reducing the prevalence of OHSS [37, 36]. This discussion summarizes the results of available studies in our systematic review and meta-analysis regarding the use of two different triggering methods (HCG only compared with Dual trigger). Firstly, these comparisons between both groups represented a statistically significant favor of dual trigger groups for (total oocyte, oocytes retrieval, fertilized oocytes, follicles >15mm at trigger day, viable embryo, X2 Pronucleate (2PN), clinical pregnancy, biochemical pregnancy rate, live birth rate, good quality embryo, and fertilization rate) more than HCG group. Secondly, these results illustrated that there were no statistically significant favor between both groups for (mature oocyste (MII), cryopreserved oocyte, follicles > 10mm at trigger day, cleavage rate oocyte/embryo, estradiol level (E2) on trigger day, progesterone level on trigger day, ongoing pregnancy rate, implantation rate, abortion rate, cancellation rate, embryo transfer, and multiple pregnancy). Moreover, there was a statistically significant favor for the HCG group for the duration of stimulation and endometrial thickness on trigger day, more than the dual trigger group.

A comparison of the current results with previous studies of systematic review and meta-analysis, as Chen et al. conducted research titled Dual triggering with GnRH agonist plus hCG versus triggering with hCG alone for IVF/ICSI outcome in GnRH antagonist cycles and reported that dual trigger favored more than HCG regarding (total oocytes, retrieval oocytes, mature oocytes, and good quality embryos) in several studies that agreed with the current results [38]. Moreover, Bourdon et al., who performed a systematic review and meta-analysis of randomized trials for Dual trigger improves the pregnancy rate in fresh in vitro fertilization (IVF) cycles compared with the human chorionic gonadotropin (hCG) trigger and matched with these results where, revealed that dual trigger had significant higher number of retrieved oocytes, number of mature oocytes, pregnancy rate, and live birth rate than HCG only trigger [39].

In addition, Zhang et al. study was carried out to assess the outcomes comparison of IVF/ICSI among different trigger methods for final oocyte maturation: A systematic review and meta-analysis, which illustrated that dual trigger had a significantly higher number of MII oocytes retrieved and fertilized oocytes, supporting the results of this study [40]. At the same line, Bourdon et al. evaluated whether gonadotropin-releasing hormone agonist (GnRH) triggering improves oocyte maturation, pregnancy outcomes, and safety compared with human chorionic gonadotropin (hCG) triggering during controlled ovarian stimulation, and indicated that there was a statistically significantly higher number of oocytes retrieved and mature oocytes after utilizing dual triggering compared with HCG alone [39]. In addition to the results of other systematic reviews Hu et al. reported that dual trigger was associated with a significantly higher live birth rate (LBR) per started cycle, as well as higher rates of ongoing pregnancy, implantation, clinical pregnancy, oocytes, mature oocytes, fertilized oocytes, and a higher number of usable embryos compared to HCG trigger [41]. Furthermore, Sloth et al. demonstrated an increase in both clinical pregnancy and live birth rates in the dual trigger group compared to the HCG trigger [42]. It was reported that there was no significant difference between the two groups regarding implantation rate.

In several studies focusing on using different ovulation triggers Yuan et al. [36] reported that it was slightly lower than the MII oocyte rate in the dual-trigger group. However, there was a significantly higher ICSI oocyte fertilization rate. Both groups were approximately equal in the number of 2PN embryos and the high-quality embryo rate. Zhou et al. showed that there was a high statistical oocyte retrieval rate in the dual trigger group, which may indicate that dual trigger had a positive effect on oocyte maturation, in addition to a higher number of good-quality embryos, viable embryos [11]. Moreover Lin et al. revealed that There was no statistically significant difference between both groups regarding total r-FSH dose, duration of stimulation, endometrial thickness, hCG day serum hormone profiles, total retrieved oocytes and mature metaphase II (MII) oocytes but illustrated that dual trigger were a significantly higher for fertilization rate, clinical pregnancy rate and live birth rate more than HCG trigger group [43]. In addition, dual trigger had positive effects on the cycle cancellation rate and abortion rate, but there was no incidence of OHSS in either group. On the other hand, Guner et al. reported that there were no differences between the two groups regarding implantation rate, clinical pregnancy, miscarriage, and live births [44].

A study comparing dual triggers for final follicular maturation with HCG trigger in ovarian stimulation for freeze-all in vitro fertilization/intracytoplasmic sperm injection cycles found that dual trigger significantly improved cumulative live-birth rates. Specifically, it revealed a statistically significantly higher biochemical pregnancy rate, clinical pregnancy rate, and live birth rate compared to HCG trigger [15].

Furthermore, Dong et al, who carried out a retrospective cohort study with propensity score matching for Reproductive outcomes of dual trigger with combination GnRH agonist and hCG versus trigger with hCG alone in women undergoing IVF/ICSI cycles and reported that there was no significant difference between both groups for the number of oocytes retrieved, embryos available, top-quality embryos, or the rate of normal fertilization, the incidence of ovarian hyperstimulation syndrome, implantation rate, biochemical pregnancy rate, clinical pregnancy rate, ectopic pregnancy rate, early miscarriage rate, and live birth rate, while the miscarriage rate higher in dual trigger [45].

Two studies [26, 33] revealed that there was a higher statistically significant difference for the number of mature oocytes retrieved and the oocyte maturation rate for the dual trigger compared with HCG trigger, while there was no difference between both groups regarding the duration of stimulation, total dose of follicle-stimulating hormone, and total number of oocytes retrieved. [46] who reported that the number of oocytes, the number of M2 oocytes, and the number of 2PN embryos were higher in group HCG than in the dual trigger group. At the same time, there were no significant differences between the two groups in terms of fertilization rate, the number of embryos, chemical pregnancy, clinical pregnancy, ongoing pregnancy, and implantation rate. Addition. Tu et al. [47] showed that dual trigger cycles yielded a significantly higher number of 2PN cleavage embryos, top quality embryos (TQEs), number of cleavage stage embryos, 2PN cleavage stage embryos, and number of oocytes retrieved, clinical pregnancy rate, persistent pregnancy rate, and live birth rate compared to HCG trigger?

This discrepancy may be attributed to many factors such as embryo quality, endometrial receptivity, sample size, and baseline patient characteristics (age and BMI, highlighting the complexity of reproductive outcomes beyond fertilization success.

5. Limitations:

While the superiority of dual trigger in specific patient subgroups is evident, we need studies to clarify its impact on OHSS risk and long-term reproductive success. Despite these constraints, the current evidence supports dual trigger as a promising strategy for improving key IVF/ICSI outcomes.

6. Conclusion

Dual trigger group can improve the quantity and quality of embryos in normal responders where it associated with higher of statistically significant for (total oocyte, oocytes retrieval, fertilized oocytes, follicles >15mm at trigger day, viable embryo, X2 Pronucleate (2PN), clinical pregnancy, biochemical pregnancy rate, live birth rate, good quality embryo, and fertilization rate) more than HCG group. Moreover, there were no statistically significant favor between both groups for (mature oocyste (MII), cryopreserved oocyte, follicles > 10mm at trigger day, cleavage rate oocyte/embryo, estradiol level (E2) on trigger day, progesterone level on trigger day, ongoing pregnancy rate, implantation rate, abortion rate, cancellation rate, embryo transfer, and multiple pregnancy). On the other side, there was a statistically significant favor for the HCG group for the duration of stimulation and endometrial thickness on trigger day, more than the dual trigger group.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Authors Contribution

EE supervised the project, provided a valid idea, contributed to the screening and data extraction, conducted quality assessments, wrote the discussion section and abstract, and was the first reviewer for the final manuscript. MW contributed to screening and data extraction, performed the meta-analysis, and was the second reviewer for the final manuscript. AE contributed to data extraction and wrote the introduction and methods. ENM contributed to screening, data extraction, and results. ASM contributed to screening and data extraction. All authors participated in the review and editing of the manuscript. Each author has read and approved the final manuscript.

Data Availability

All data supporting the findings are derived from previously published studies included in the systematic review and meta-analysis, which are fully cited in the References section.

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Original Article

Aqueous *Basella alba* Mitigates Cyclosporine-Induced Nephrotoxicity in Wistar Rats: Relevance in Adjuvant Therapy

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ABSTRACT

Introduction: Cyclosporine A (CsA) is an immunosuppressant agent that is usually considered as a first-line therapy against organ rejection after a transplant procedure. However, its administration is often associated with nephrotoxicity and a compromise of kidney function. There is a paucity of literature on the effects of aqueous *Basella alba* leaf extract (ABALE) in this condition. This study aimed to bridge the knowledge gap.
Methods: Thirty male Wistar rats were divided into 6 groups of 5 rats each, such that the experimental groups received graded doses of ABALE at 100mg/kg, 200mg/kg, and 400mg/kg for 21 consecutive days, after inducing nephrotocity with CsA at 20mg/kg/day (i.p).
Results: Treatment with ABALE resulted in a dose-dependent reduction of oxidative stress, inflammation, and elevated plasma markers of kidney dysfunction, with the highest dose showing the greatest protective effect ($p < 0.05$). Histological analysis of the kidneys also revealed near-normal architecture following ABALE treatment, while CsA administration was associated with marked vacuolation of the kidney interstitium and glomerular atrophy. However, no significant difference was observed between the untreated recovery group and the nephrotoxicity model group.
Conclusion: ABALE mitigated cyclosporine-induced nephrotoxicity by suppressing plasma pro-inflammatory cytokines and restoring antioxidant balance. These findings suggest that the extract may serve as a promising adjuvant therapy in CsA-induced nephrotoxicity.

1. Introduction

The kidney, recognized as the principal organ of homeostasis, serves both excretory and regulatory functions, primarily through its capacity to produce urine [1, 2, 3]. Impairment of normal kidney function can lead to the accumulation of metabolic waste products, which may have harmful effects on other organs [1, 2]. Progressively, such dysfunction manifests as chronic kidney disease (CKD), a condition with rising global prevalence that, if left unaddressed, can significantly reduce life expectancy [4].

CKD affects approximately 10% of the global population, predominantly adults, and contributes to millions of premature deaths due to its associated health risks [1, 4]. Current treatment strategies focus on slowing disease progression and preventing related complications, with the primary approaches being pharmacological intervention and adherence to a regulated dietary plan.

Cyclosporine is an immunosuppressive agent commonly used as a first-line treatment to prevent organ rejection following transplantation and to manage various immunologically mediated disorders [5, 6]. However, its use is frequently associated with adverse side effects, most notably nephrotoxicity, which can impair the kidneys' essential excretory and regulatory functions. The treatment of such complications carries a significant financial burden worldwide, particularly in low- and middle-income countries. Given these challenges, there is growing interest in exploring plant-based or natural products as potential adjuvant therapies. In this context, the present study investigates the medicinal potential of *Basella alba* in mitigating cyclosporine-induced nephrotoxicity.

Basella alba is an annual and perennial leafy vegetable that is native to Africa and tropical Asia [7, 8, 9]. Commonly known as Malabar spinach, its pharmacological properties have been widely documented, demonstrating notable health benefits, including anti-diabetic, antioxidant, anti-inflammatory, antimicrobial, digestive, and wound-healing effects [8, 10, 9]. These therapeutic effects are largely attributed to its rich phytochemical composition, including tannins, flavonoids, polyphenols, phytosterols, alkaloids, and triterpenoids [11, 12]. Despite advances in medical research and these documented benefits, there remains a scarcity of studies investigating the effects of aqueous *Basella alba* extract on cyclosporine-induced nephrotoxicity. Most science indexing databases, including PubMed, scopus, and web of science, have journals with publications that seem to focus on the anti-diabetic,

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Table 1: Dose Regimen and Experimental Protocol

N = 30	Description	Duration
Group 1 (n = 5)	Normal rat chow and water	28 days*
Group 2 (n = 5)	Cyclosporine A (CsA) at 20 mg/kg/day (i.p.), in olive oil as vehicle	7 days*
Group 3 (n = 5)	CsA (7 days) + recovery period (21 days)	28 days*
Group 4 (n = 5)	CsA (7 days) + oral 100 mg/kg ABALE (21 days)	28 days*
Group 5 (n = 5)	CsA (7 days) + oral 200 mg/kg ABALE (21 days)	28 days*
Group 6 (n = 5)	CsA (7 days) + oral 400 mg/kg ABALE (21 days)	28 days*

CsA, Cyclosporine A; i.p., Intraperitoneal; ABALE, Aqueous *Basella alba* leaf extract; * point at which rats were sacrificed; N, total number of rats recruited for the study; n, number of rats in a group.

antimicrobial, anti-stress, cardio-protective, and anti-inflammatory potential of different alcohol fractions of the extract of *Basella alba* [13, 14, 8, 15, 10, 9], other than the effects of its aqueous extract in an experimental model of CsA-induced nephrotoxicity. To mimic the natural mode of ingestion in humans, this study aims to address the existing literature gap by investigating the potential nephroprotective effects of its aqueous extract in a Wistar rat model of cyclosporine-induced kidney injury.

2. Methods

2.1. Animal Management and Experimental Protocol

Thirty (30) male Wistar rats of about 6 to 8 weeks old and weighing 130 to 145g were recruited for this study. The rats were housed in standard, conventional plastic cages under a natural light-dark cycle, as well as natural temperature and humidity conditions during the harmattan season. The study was conducted in a hygienic laboratory with a natural ecosystem at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The rats were divided into six (6) groups of five (5) rats each as follows: Groups 1 were allowed ad libitum feeding on normal rat chow throughout the study period, after which they were euthanized. Group 2 served as the model for CsA-induced nephrotoxicity. They were administered intraperitoneal CsA administration at 20 mg/kg/day for 7 consecutive days using olive oil as a vehicle, after which they were euthanized. This model was as delineated by Murray et. al [16] and validated in our pilot study. Group 3 was pre-treated as group 2 and, thereafter, left for a recovery period of 21 days before they were euthanized. Groups 4, 5, and 6 were also pre-treated as group 2, after which they received graded doses of aqueous *Basella alba* leaf extract (ABALE) at 100mg/kg, 200mg/kg, and 400mg/kg, respectively, for 21 consecutive days before they were euthanized **(Table 1)**. The rats were euthanized under ketamine anesthesia (60mg/kg i.m.) and blood samples were collected into separate lithium heparinized tubes. These samples were centrifuged at 4000 rpm for 15 minutes using a cold centrifuge (Centrium Scientific, model 8881) at -4 °C. The obtained plasma was decanted into separate plain bottles for biochemical assays. Thereafter, the kidney of each rat was carefully excised and fixed in 10% formal saline solution for histological examinations.

2.2. Dose Regimen and Stock Solution of *Basella alba*

According to Krishna [17], *Basella alba* has an oral LD50 of > 2,000 mg/kg. This indicates that the plant is of very low toxicity. The adopted graded doses for this study were 100mg/kg, 200mg/kg, and 400mg/kg.

A stock solution for 100mg/kg of ABALE was prepared by dissolving 1g of the extract in 20 mL of normal saline. This was done to prevent overloading, such that each 100g rat received 0.2 mL of ABALE. On the other hand, both 200 and 400 mg/kg of ABALE were prepared by dissolving 2g and 4g of the extract in separate 20ml of normal saline, respectively.

Each stock solution was refrigerated after use at 5°C, while fresh samples were prepared every 48 hours throughout the study period.

2.3. Assessment of Plasma Concentrations of Kidney Function Biomarkers

Plasma concentrations of creatinine and urea were assayed using the Randox standard laboratory test kit (Randox Lab. Ltd., County Antrim, United Kingdom). At the same time, the level of kidney injury molecule-1 (KIM-1) was determined using the ELK Biotechnology (Rat) KIM-1 ELISA kit (China), catalog number ELK11099, with a species sensitivity range of 0.32 to 20 ng/mL.

2.4. Assessment of Plasma Concentrations of Pro-inflammatory Cytokines and Oxidative Stress Biomarkers

Standard biochemical kits for systemic inflammatory profiling were procured from Elabscience (China); hs-CRP (rat) ELISA kit with catalog number E-EL-R3002 and species sensitivity of 4.69 to 500 pg/mL, IL-6 (rat) ELISA kit with catalog number E-EL-R0015 and species sensitivity of 7.5 to 800 pg/mL, and TNF-α (rat) ELISA kit with catalog number E-HSEL-R0006 and species sensitivity of 0.94 to 100 pg/mL. The protocols were performed as delineated by the manufacturer.

The plasma GSH concentration was determined according to the method of Beutler and Kelly [18], as described as follows: To 1 mL of the sample, 0.5 mL of Ellman's reagent (10 mM) and 2 mL of phosphate buffer (0.2 M, pH 8.0) were added. The yellow precipitate developed was read at 412 nm against a blank containing 3.5 mL of phosphate buffer. A series of standards was treated similarly, and the amount of GSH was expressed in μg/mg protein. Meanwhile, SOD activity was determined by the method of McCord and Fridovich [19]. In contrast, the TBARS level was determined by the method of Ohkawa and co-workers [20], as described as follows: To 0.5 mL of the sample, 0.5 mL of phosphate buffer (0.1 M, pH 8.0) and 0.5 mL of 24% TCA were added. The resulting mixture was incubated at room temperature for 10 min, followed by centrifugation at 2000 rpm for 20 min. To 1 mL of the supernatant, 0.25 mL of 0.33% TBA in 20% acetic acid was added, and the resulting mixture was boiled at 95 °C for 1 h. The resulting pink-coloured product was cooled, and the absorbance was read at 532 nm.

2.5. Histological Examination

The excised kidneys, fixed in a 10% formalin-saline solution, were subjected to histological examination using conventional hematoxylin and eosin (H&E) staining techniques. Tissue preparation

Table 2: Effects of ABALE on Plasma Concentration and Kidney Function Indices of Wistar Rats with CsA-induced Nephrotoxicity

Group	Creatinine (mg/dl)	Urea (mg/dl)	KIM-1 (ng/ml)
Group 1	0.62 ± 0.04	35.46 ± 0.35	7.36 ± 2.35
Group 2	0.87 ± 0.03^{a}	50.22 ± 0.37^{a}	17.62 ± 2.14^{a}
Group 3	0.84 ± 0.04^{a}	49.84 ± 0.51^{a}	16.37 ± 2.01^{ab}
Group 4	0.69 ± 0.02^{abc}	39.75 ± 0.40^{abc}	9.45 ± 1.87^{bc}
Group 5	0.65 ± 0.03^{bc}	36.61 ± 0.50^{abcd}	8.58 ± 2.17^{bc}
Group 6	0.64 ± 0.02^{bcd}	36.00 ± 0.44^{bcde}	7.69 ± 2.46^{bc}

KIM-1, Kidney injury molecule 1; a, significant difference compared with Group 1 (Negative control); b, significant difference compared with Group 2 (CsA); c, significant difference compared with Group 3 (CsA + Recovery); d, significant difference compared with Group 4 (CsA + 100 mg/kg ABALE); e, significant difference compared with Group 5 (CsA + 200 mg/kg ABALE); Values represent mean ± SEM, $p < 0.05$.

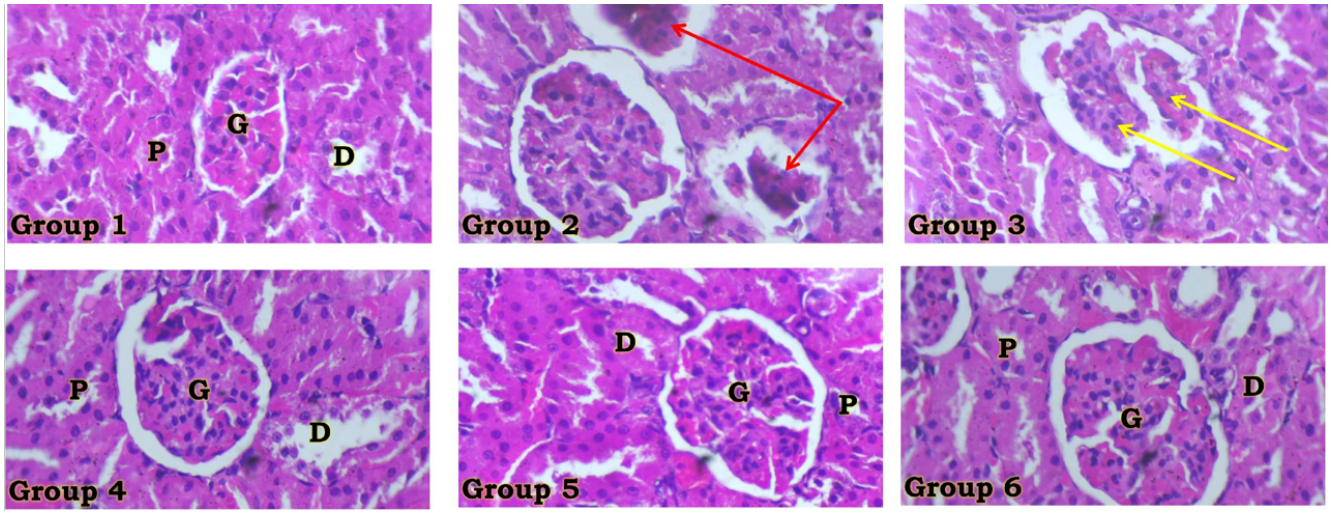

Figure 1: Effects of ABALE on Kidney Histology of Wistar Rats with CsA-induced Nephrotoxicity (magnification = x400) G = Glomerulus; P = Proximal tubule; D = Distal tubule; Red Arrow = Atrophic glomerulus; Yellow Arrow = Shrunken glomerulus.

on the slides was captured for photomicrograph assessment using a Leica DM750 microscope with a camera at a magnification of ×400.

2.6. Statistical Analysis

Data were expressed as mean ± standard error of mean at $p < 0.05$. These were subjected to the Newman-Keuls post hoc test using GraphPad Prism 5.03 (GraphPad Software Inc., CA, USA).

3. Results

3.1. Effects of ABALE on Plasma Concentrations of Kidney Function Indices of Wistar Rats with Cyclosporin-induced Nephrotoxicity

CsA administration induced significantly elevated levels of plasma creatinine (mg/dL), urea (mg/dL), and Kim-1 (ng/mL) ($p<0.0001$). While there was no significant difference between the recovery and toxic group 2, ABALE administration was associated with a dose-dependent improvement in these kidney function indices, compared with the toxic CsA group 2 ($p < 0.0001$) **(Table 2)**.

3.2. Effects of ABALE on Plasma Concentrations of Oxidative Stress Biomarkers of Wistar Rats with Cyclosporin-induced Nephrotoxicity

While plasma concentrations of GSH (μg/mg protein) and SOD (mM) were significantly reduced following CsA administration, these were dose-dependently restored by ABALE administration, with the highest dose having the most beneficial effect **(Table 3)**. These biomarkers were not significantly reversed in the recovery group compared with control group 1 ($p < 0.0001$). However, the plasma TBARS level (nmol/mg protein) was significantly elevated in the toxic group 2 compared with the ABALE-treated groups, with the highest dose (400 mg/kg) also exhibiting the most risk-averse profile ($p < 0.0001$) **(Table 3)**.

3.3. Effects of ABALE on Plasma Concentrations of Pro-inflammatory Cytokines of Wistar Rats with Cyclosporin-induced Nephrotoxicity

The pro-inflammatory cytokines (IL-6, CRP, and TNF-α) were significantly elevated in the plasma compared to the control group ($p < 0.001$). However, following administration of ABALE, there was a dose-dependent amelioration of inflammation, with the highest dose also expressing the highest risk-averse potential **(Table 4)**.

Table 3: Effects of ABALE on Plasma Concentrations of Oxidative Stress Biomarkers of Wistar Rats with CsA-induced Nephrotoxicity

Group	GSH (μg/mg protein)	SOD (U/mL)	TBARS (nmol/mg protein)
Group 1	3.62 ± 0.28	186.00 ± 14.50	29.31 ± 0.52
Group 2	2.04 ± 0.30^{a}	57.84 ± 12.35^{a}	78.75 ± 0.30^{a}
Group 3	2.15 ± 0.25^{a}	72.37 ± 12.40^{a}	77.15 ± 0.44^{ab}
Group 4	3.21 ± 0.32^{bc}	173.40 ± 14.32^{bc}	34.55 ± 0.37^{abc}
Group 5	3.38 ± 0.32^{bc}	181.10 ± 13.72^{bc}	29.82 ± 0.41^{bcd}
Group 6	3.44 ± 0.21^{bc}	182.50 ± 14.24^{bc}	29.02 ± 0.35^{bcde}

GSH, Reduced glutathione; SOD, Superoxide dismutase; TBARS, Thiobarbituric acid reactive substances; a, significant difference compared with Group 1 (Negative control); b, significant difference compared with Group 2 (CsA); c, significant difference compared with Group 3 (CsA + Recovery); d, significant difference compared with Group 4 (CsA + 100 mg/kg ABALE); e, significant difference compared with Group 5 (CsA + 200 mg/kg ABALE); Values represent mean ± SEM, $p < 0.05$.

Table 4: Effects of ABALE on Plasma Concentrations of Pro-inflammatory Cytokines of Wistar Rats with CsA-induced Nephrotoxicity

Group	IL-6 (pg/ml)	CRP (pg/ml)	TNF-α (pg/ml)
Group 1	69.21 ± 2.54	54.00 ± 2.00	33.54 ± 2.67
Group 2	102.54 ± 3.55^{a}	86.00 ± 2.00^{a}	57.44 ± 2.18^{a}
Group 3	100.63 ± 2.30^{a}	84.00 ± 3.00^{a}	55.25 ± 2.59^{a}
Group 4	76.66 ± 3.21^{abc}	61.00 ± 2.00^{abc}	40.21 ± 2.36^{abc}
Group 5	72.54 ± 3.00^{bcd}	57.00 ± 1.00^{bcd}	36.45 ± 2.61^{bcd}
Group 6	70.89 ± 3.73^{bcd}	56.00 ± 2.00^{bcd}	35.07 ± 2.42^{bcd}

TNF-α, Tumor necrosis factor-alpha; IL-6, Interleukin-6; CRP, C-Reactive protein; a, significant difference compared with Group 1 (Negative control); b, significant difference compared with Group 2 (CsA); c, significant difference compared with Group 3 (CsA + Recovery); d, significant difference compared with Group 4 (CsA + 100 mg/kg ABALE); Values represent mean ± SEM, $p < 0.05$.

3.4. Effects of ABALE on Kidney Histology of Wistar Rats with Cyclosporin-induced Nephrotoxicity

CsA administration was associated with distortion of the glomerulus, expressed as both atrophic and shrunken histo-architecture. However, the ameliorative effects of graded doses of ABALE were associated with an improved histoarchitecture of the kidney compared to that of the toxic CsA group (**Figure 1**).

4. Discussion

This study demonstrated that CsA-induced nephrotoxicity is implicated by oxidative stress, as well as an imbalance of plasma pro-inflammatory cytokines, in its pathogenesis. This is also associated with histo-architectural distortion of the glomerulus / glomerular filtration barrier (with a downstream effect on glomerular filtration), which culminated in significantly elevated plasma levels of kidney function biomarkers. If this condition is left unchecked, the resultant bio-accumulation of metabolic wastes may spiral into a compromise of body homeostasis and consequent expression of health risks. However, administration of graded doses of ABALE was associated with the amelioration of this nephrotoxicity, presenting a potential therapeutic choice in adjuvant therapy against CsA-induced kidney injury or, possibly, immunologically mediated clinical conditions. Generally, for the indices assessed in this study, the highest dose of ABALE (400mg/kg) showed the most risk-averse potential against CsA-induced nephrotoxicity, even though the extract demonstrated a dose-dependent therapeutic effect.

The use of both enzymatic (SOD) and non-enzymatic (GSH) antioxidant biomarkers [21], along with their significantly reduced plasma levels observed in this study, supports the role of oxidative stress in the development of CsA-induced nephrotoxicity. This indicates an overwhelming of the systemic antioxidant defenses, exceeding the body's capacity to neutralize free radicals. The marked increase in lipid peroxidation, measured by TBARS, provides clear evidence of heightened systemic cellular and organ damage. This oxidative damage is further corroborated by pronounced histological and architectural disruption of the glomerulus, resulting in impairment of the glomerular filtration barrier and elevated plasma kidney function biomarkers.

Both classical (creatinine and urea) and novel (KIM-1) kidney function biomarkers were significantly elevated in the plasma of experimental groups following CsA administration. These harmful effects were notably improved by ABALE treatment in a dose-dependent manner. While the recovery group failed to restore kidney excretory and regulatory functions fully (as depicted by the assayed plasma levels of kidney function biomarkers), the highest dose of ABALE demonstrated the most significant protective effect. This underscores the extract's potential as a valuable adjuvant therapeutic agent for CsA-induced nephrotoxicity. A limitation of this study, deserving further exploration, is the absence of KIM-1 measurement in urine samples, which could have been facilitated through the use of metabolic cages for clean urine collection within a Wistar rat experimental model. Additionally, circulatory CsA levels were not assessed; determining these would help clarify the relationship between ABALE administration and CsA half-life, as per its clearance by the kidney. Both aspects warrant further scientific investigation.

Administration of ABALE was linked to a significant decrease in plasma pro-inflammatory cytokines in the experimental groups. The extract's ability to reduce systemic inflammation, evidenced by notable declines in plasma CRP, IL-6, and TNF-α levels, highlights

its potent anti-inflammatory properties. Nevertheless, the underlying pro-inflammatory pathways merit further detailed investigation.

5. Conclusion

Aqueous *Basella alba* leaf extract (ABALE) mitigated cyclosporine-induced nephrotoxicity by suppressing plasma pro-inflammatory cytokines and restoring antioxidant balance. The highest dose (400mg/kg) demonstrated the most risk-averse potential, while a recovery period (without medication) is associated with significant features of nephrotoxicity. This presents ABALE as a potential therapeutic choice in adjuvant therapy for cyclosporine-induced nephrotoxicity.

Conflicts of Interest

The authors declare that they have no competing interests that could have influenced the objectives or outcome of this research.

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Ethical Approval Statement

The experimental protocols were in strict compliance with the guidelines for animal research, as contained in the NIH guidelines for the care and use of laboratory animals and approved by the local institutional research committee (Institute of Public Health of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria) with ethics reference IPH/OAU/12/110A.

Large Language Model

None

Authors Contribution

Conceptualization of the study was performed by ICE. Methodology was developed by ICE and OOG, and data analysis was conducted by ICE, OOG, and OAEO. Investigation was carried out by ICE, OOG, OAEO, and AAK, while manuscript writing was undertaken by ICE, OOG, and OAEO. Funding acquisition was managed by ICE, OOG, and OAEO, and supervision was provided by ICE and OOG. All authors contributed to data collection, interpretation of results, enhancement of the intellectual content, and approval of the final manuscript.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available, as the work was based solely on animal experiments and no additional data were created. Further details are available from the corresponding author on reasonable request.

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Case Report

Near-miss Osteomyelitis in an Immunosuppressed Crohn's Disease Patient: Diagnostic Vigilance Sparked by a Medical Student

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ABSTRACT

Crohn's disease (CD) patients on immunosuppressive therapy are at increased risk of infections, including osteomyelitis, which could be diagnostically challenging. This case highlights the importance of medical student vigilance in making a near-miss diagnosis of osteomyelitis in a CD patient.

A 56-year-old CD patient developed left shoulder pain following localized muscle injection and was initially managed as a soft-tissue abscess. The condition worsened despite 21 days of antibiotics. Investigation: Cultures were positive for methicillin-sensitive Staphylococcus aureus (MSSA). Persistent pain and new neurologic complaints prompted an MRI (T1 hypointensity, T2-STIR hyperintensity, post-contrast enhancement) at 60 days, in support of osteomyelitis without bone biopsy.Management/Outcome: The patient received 6 weeks of intravenous vancomycin (1 g every 12 hours), followed by oral antibiotics, resulting in partial relief of pain (pain score: 8/10 to 4/10) and improvement in shoulder function (Constant-Murley score: 30 to 65 at 3 months). Immunotherapy (adalimumab) was restarted after infection control.

This case highlights three practical lessons: maintaining a low threshold for advanced imaging in immunosuppressed patients with persistent pain; incorporating diagnostic time-outs to invite trainee perspectives; and seeking early infectious-disease consultation when osteomyelitis is suspected.

1. Introduction

Making a precise diagnosis is crucial in the challenging field of medicine, particularly for individuals with complex illnesses such as Crohn's disease (CD). CD is a type of chronic inflammatory bowel disease (IBD) that is most commonly associated with musculoskeletal symptoms, though it can cause several other disorders as well [1, 2]. The shoulder may also be affected; however, this is less common [3, 4, 5]. These might range from joint soreness to arthritis affecting big joints. A wide range of possibilities must be carefully considered during the diagnostic process in such circumstances. This case underscores the diagnostic pitfalls in immunosuppressed CD patients, where musculoskeletal extraintestinal manifestations (prevalence: 20–50% in IBD) can mask severe infections like osteomyelitis, particularly following injection-site complications [1, 2]. The novelty lies in the role of a medical student in averting diagnostic delay through timely suspicion of osteomyelitis. This is where the new insight of trainees can be quite helpful. Because they have access to the most up-to-date academic information and frequently have more time to spend with patients, medical students can play a crucial role [6, 7]. A student's perspective can help the medical team arrive at the correct diagnosis by noticing changes in symptoms and challenging presumptions. This helps to reinforce the idea that good patient care is a team effort, in which all opinions, regardless of position, contribute to the outcome.

2. Case Presentation

A 56-year-old man with Crohn's disease being treated with immunotherapy (adalimumab) presented to the hospital in early September 2024 (symptoms having started in July and having worsened for the last two months) with intense left shoulder pain that progressively worsened and severely limited the range of motion. An injection was done into the peri-articular area (local muscle, deltoid) for pain relief, testing, and therapeutic reasons. An abscess developed in the injection site, as a consequence of suspected (though never formally adjudicated) poor aseptic technique and contamination, which probably made pain worse. Wound cultures taken during abscess drainage on 11.08.2024 (Day 7 from injection and abscess identification) before antibiotics yielded methicillin-sensitive Staphylococcus aureus (MSSA, MIC <1 μg/mL). The patient was started on an initial antibiotic course of vancomycin 1 g IV every 12 hours (discharge summary, approximately 21 days from 08.11.2024 to 29.11.2024) and had his Crohn's immunotherapy drug stopped to reduce the risk of infection in the meantime.

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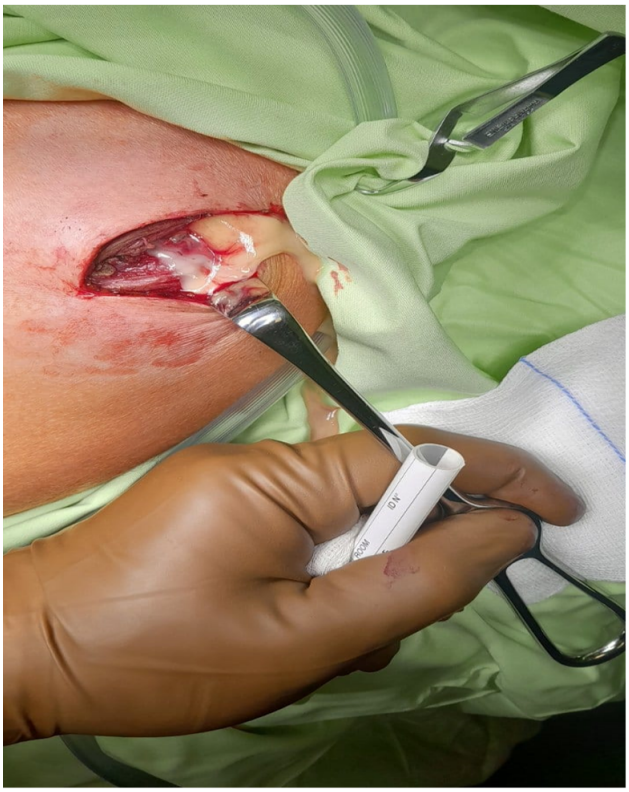

Figure 1: Presents pus leakage from abscess in the peri articular area of left shoulder.

Patient showed some improvement at 60 days after drainage (around 07.01.2025; however, pain levels increased drastically (pain score 8/10), and he developed numbness in all fingers of the left hand. Despite the worsening condition and the red flag symptoms (persistent pain greater than 72 hours duration, CRP level risen to 80 mg/L, and onset of developing neurological symptoms), the attending doctor blamed the persistence of pain on post-operative complications and reassured the patient that it would improve with time and that he was progressing well. A student doctor, observing the persistent and worsening symptoms, suggested the possibility of osteomyelitis (bone infection). Still, this opinion was initially dismissed by the attending doctor, who, in light of the initial diagnosis of a soft-tissue infection, showed confirmation bias.

Patient's condition worsened further, necessitating an emergency room (ER) admission on 10.01.2025 with severe pain and numbness. An MRI of the left shoulder on 10.01.2025 established the diagnosis of osteomyelitis with features of increased signal intensity (T1 hypointensity, T2-STIR hyperintensity, post-contrast enhancement), tendon rupture, and fluid collection. The diagnosis relied on MRI, microbiology, and the clinical course, as a bone biopsy was postponed due to surgical risk in the immunocompromised patient. No early imaging had been done to add to the diagnostic delay.

Following diagnosis, the patient received 6 weeks of intravenous vancomycin (1 g q12h, 10.01.2025 through approximately 21.02.2025), then 4 weeks of levofloxacin orally (21.02.2025 through 21.03.2025), contingent on MSSA susceptibility and antimicrobial stewardship (local MRSA <10%; toxicity is tracked with normal CBC and BMP). MRI on 17.02.2025 showed persistent changes with rotator cuff tear and likely Hill-Sachs fracture, indicating remaining postoperative complications. Partial pain relief (pain score reduced from 8/10 to 4/10) and shoulder function (Constant-Murley score from 30 to 65) gain were attained at 3 months post-diagnosis (approximately 10.04.2025). Resumption of immunotherapy (adalimumab) was recommended by infectious disease and gastroenterology experts, in accordance with the established resolution of infection (CRP <10 mg/L, absence of fever), and occurred.

The evolution of an Injection-site abscess to osteomyelitis went through contiguous spread to the humerus and was precipitated by immunosuppression, obscuring characteristic signs. This case again brings into sharp focus the diagnostic pitfalls in immunosuppressed CD patients, in which musculoskeletal extraintestinal manifestations may masquerade or disguise infections. It reminds us of the importance of trainee contributions to preventing near-miss errors through diagnostic time-out and escalation processes.

2.1. Medical Background: Crohn's Disease and Musculoskeletal Manifestations

Crohn's disease (CD) can involve any portion of the gastrointestinal tract, from the mouth to the anus. CD is also linked with extraintestinal manifestations (EIMs) beyond its mere gastrointestinal symptoms, which can involve the musculoskeletal system and other organ systems [1]. Musculoskeletal symptoms are seen in more than half of the IBD patients and stand as one of the most frequent EIMs [2]. Arthralgia, i.e., pain in the joints, and peripheral arthritis, which more frequently affect large joints such as the knees and ankles, can be among them, as can axial involvement in the form of spondyloarthritis [4, 5]. The shoulder can also be affected less frequently [3]. Although the exact pathophysiology of the majority of musculoskeletal EIMs is unknown, it is thought to include immunological dysregulation, genetics, and the interaction of systemic inflammation with gut microbiota [7]. In this case, the

Table 1: Shows the chronological order of the events

Date	Event
Around 09.07.2024	Initial onset of gradual left shoulder pain, progressively worsening over two months.
Around 09.09.2024	Presentation to the hospital with intense pain limiting the range of motion; peri-articular (deltoid) injection administered for pain management, testing, and treatment
Shortly after injection (within days, around mid-September 2024)	An abscess developed at the injection site due to infection.
08.11.2024	Abscess identified and drained; wound cultures revealed MSSA (pre-antibiotic treatment); initial vancomycin course initiated.
08.11.2024–29.11.2024	Initial IV vancomycin course; adalimumab paused.
Approximately 07.01.2025	60 days post-drainage; reported some improvement, but pain increased (8/10) with numbness in all left-hand fingers; medical student suggests osteomyelitis, dismissed.
10.01.2025	ER admission for severe pain and numbness; initial MRI confirms osteomyelitis (T1 hypointensity, T2-STIR hyperintensity, post-contrast enhancement; tendon rupture; fluid collection).
10.01.2025–21.02.2025	6-week IV vancomycin course post-diagnosis.
21.02.2025–21.03.2025	4-week oral levofloxacin course.
17.02.2025	Follow-up MRI shows persistent changes (rotator cuff tear, suspected Hill-Sachs fracture).
Approximately 10.04.2025	3 months post-diagnosis; pain improved to 4/10, Constant-Murley score 65; adalimumab restarted

MSSA, Methicillin-Sensitive Staphylococcus aureus; IV, Intravenous; ER, Emergency Room; MRI, Magnetic Resonance Imaging; T1, T1-weighted MRI sequence; T2-STIR, T2-weighted Short Tau Inversion Recovery MRI sequence

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patient's shoulder pain was initially misattributed to CD-related arthralgia, highlighting how immunosuppression can mask typical signs of infection, thereby delaying suspicion of osteomyelitis. Musculoskeletal manifestations in CD patients can have a significant negative impact on their quality of life and typically require a multidisciplinary management strategy.

2.2. Osteomyelitis: Diagnosis and Treatment

Osteomyelitis is an infectious disease that results in an inflammatory bone and bone marrow condition induced by bacterial infection, leading to progressive bone destruction [6]. It can be either acute or chronic, and it is typically diagnosed based on a constellation of clinical symptoms, imaging studies (such as X-rays, MRIs, and CT scans), and laboratory results (including elevated inflammatory markers and positive blood cultures) [8]. In this case, diagnosis relied on MRI (T1 hypointensity, T2-STIR hyperintensity, post-contrast enhancement) and clinical course, as bone biopsy was deferred due to surgical risk in an immunocompromised patient [8, 9]. A definitive diagnosis is the gold standard and typically includes a bone biopsy, histological study, and microbiological culture [9]. Osteomyelitis treatment is typically long-term and involves surgical debridement to remove infected or necrotic bone, along with long-term antibiotic treatment [10]. The antibiotics are chosen based on culture findings, as well as the patient's specific characteristics. Here, vancomycin (1 g IV every 12 hours) was administered for 6 weeks based on MSSA susceptibility (MIC < 1 μg/mL), followed by oral levofloxacin for 4 weeks. In some instances, particularly in chronic osteomyelitis, surgery is necessary for the definitive removal of the infection [11]. The duration of antibiotic treatment may vary, but it typically lasts weeks to months, depending on the seriousness and type of infection [12].

2.3. Staphylococcus aureus and Osteomyelitis

Staphylococcus aureus (S. aureus) is a common skin and nasal bacterium that is the leading cause of osteomyelitis [13]. S. aureus can produce infection of the bone by a variety of mechanisms, including hematogenous spread, direct inoculation (like trauma, surgery, or, in our patient, an unsterile injection), and contiguous spread from an infective overlying soft tissue infection [14]. Here, a contiguous spread from an injection-site abscess (MSSA, as indicated by wound culture collected prior to antibiotics) was likely, given the low local MRSA prevalence per hospital epidemiology. The ability of S. aureus to form biofilms on bone and medical devices, as well as its capacity to survive intracellularly within host cells, contributes to the recurrence and chronicity of osteomyelitis, making treatment challenging [15, 16]. Initial antibiotic failure was likely due to inadequate duration and bone penetration, underscoring the need for early imaging. The presence of S. aureus in an abscess, especially in close proximity to bone, should raise a high suspicion of underlying osteomyelitis, particularly if this does not respond to standard treatment or if it is recurrent.

2.4. Immunotherapy and Infection Risk in Crohn's Disease

Patients with Crohn's disease frequently require immunosuppressive medications, such as biologics and immunomodulators, to control their chronic inflammation [17]. While these medications are quite efficient at controlling disease activity, they do increase the patient's vulnerability to infections from bacterial, viral, and fungal pathogens [18]. The nature and severity of immunosuppression vary depending on the drug and combination therapy used. For example, anti-tumor necrosis factor (TNF) medications, which are routinely used in CD, can impede the immune system's ability to generate an effective response to infections, including those caused by S. aureus [19]. In this case, adalimumab was paused after the diagnosis of an abscess and restarted 3 months after antibiotic completion, following consultations with infectious disease and gastroenterology specialists who confirmed resolution of the infection (CRP <10 mg/L, no fever). This increased risk of infection necessitates careful monitoring and a heightened awareness of potential infectious complications, as infections in immunocompromised patients can present atypically and progress rapidly. The interruption of immunotherapy, as occurred in this case, is a common practice when an infection is suspected or confirmed.

Table 2: Presents the differential diagnosis for osteomyelitis

Condition	Symptoms and features
Osteomyelitis	Focal pain, numbness
Septic Arthritis	Joint effusion, warmth
Bursitis	Localized tenderness
Rotator Cuff Tear	Weakness, limited ROM
SIRVA	Post-injection pain
Neuropathic Pain	Burning, no focal signs

SIRVA, Shoulder Injury Related to Vaccine Administration; ROM, Range of Motion.

Table 3: Presents the safety net checklist for immunocompromised patients

Checklist Item
Persistent focused pain after more than 72 hours of antibiotics
Fever or increasing CRP (more than 20 mg/L)
New neurological symptoms (e.g., numbness)
Unresolved soft-tissue infection near the bone

CRP, C-reactive protein.

Still, it also highlights the delicate balance between controlling the underlying disease and mitigating the risk of infection.

2.5. Progression from Abscess to Osteomyelitis

The spread from soft tissue infection, i.e., abscess, to osteomyelitis is a well-documented process when infection is not managed or is managed improperly, or if it occurs in very close proximity to bone [20]. An abscess at the deltoid injection site likely seeded the humerus via contiguous spread, exacerbated by immunosuppression. In immunocompromised individuals, this can be even quicker and insidious due to a weakened immune response. An abscess, which is a localized collection of pus, can exert pressure on adjacent bone, leading to local ischemia and creating an environment conducive to bacterial invasion of the bone tissue. Furthermore, bacteria from the abscess can directly spread into the bone through microtrauma or through pre-existing vascular channels. The acute management of the abscess, although mandatory, may not always eradicate all of the bacteria, especially if the infection has already begun to involve the bone. Red flags (persistent pain for more than 72 hours, CRP 80 mg/L, and new numbness) were ignored, delaying the MRI; a safety-net checklist should have prompted faster imaging [21] (Table 3).

Persistent pain and new neurological symptoms of numbness are critical red flag signs that should be further investigated with further workup for deeper, more serious infections such as osteomyelitis, even if imaging was initially negative or inconclusive. This case exemplifies how a seemingly localized infection can escalate to a severe bone infection, especially in a vulnerable patient population.

2.6. Clinical Reasoning and Medical Education

This case exemplifies the complexity of clinical reasoning, as well as the crucial role of medical education in teaching critical thinking and a willingness to question assumptions. Clinical reasoning is an interactive process of data collection, hypothesis generation, and hypothesis testing, which is typically conducted under conditions of uncertainty [22]. Although experience is beneficial, it can also lead to cognitive biases, such as anchoring bias (overreliance on initial information) and confirmation bias (seeking information that confirms preconceived views) [23]. In this case, the attending physician, perhaps fixated on the initial diagnosis of a soft tissue infection and subsequent abscess drainage, may have overlooked the possibility of a deeper, more severe infection, blaming the patient's persistent symptoms on expected post-surgical complications. Anchoring on post-operative complications and confirmation bias delayed MRI; diagnostic time-outs and trainee escalation pathways could mitigate such errors [23]. This demonstrates a typical weakness in medical practice: the tendency to prematurely stop a diagnostic investigation when a plausible explanation is available, even if it does not fully account for all of the patient's symptoms. The medical student with a fresh perspective was able to synthesize the evolving clinical picture—the patient's immunocompromised state, the S. aureus infection, the persistent and worsening pain despite antibiotic treatment, and the new neurological symptoms—to propose an alternative, more severe diagnosis. This highlights the importance of creating an environment in medical school where students feel empowered to express their findings and hypotheses, even if they disagree with those of their superiors. Effective medical education entails more than just imparting knowledge; it also includes cultivating diagnostic curiosity, supporting a systematic approach to problem solving, and developing the courage to challenge. Although the student's suggestion was initially rejected, it ultimately proved accurate, highlighting the possibility that insightful information can come from all levels of the medical hierarchy. This instance supports the ideas that responsiveness to different viewpoints is crucial for the best possible patient outcomes and that learning in medicine is an ongoing, team-based process.

3. Conclusions

This case highlights the crucial importance of a comprehensive differential diagnosis, especially in challenging cases involving immunocompromised patients. While experience is necessary for clinical intuition, it must be combined with a continual openness to new information and a willingness to alter preliminary diagnostic impressions. Despite initial dismissal, the medical student's correct anticipation of the possibility of osteomyelitis demonstrates the absolute necessity of multiple perspectives in clinical decision-making. This incident serves as a forceful reminder to all healthcare professionals, regardless of their level of experience, to attentively listen to and take seriously the opinions of every member of the healthcare team. The generation of an atmosphere of intellectual humility and shared inquiry is not an academic goal, but a practical imperative for the delivery of optimum patient outcomes. Learning Points: 1) In immunosuppressed patients with persistent focal pain after soft-tissue infection, maintain a low threshold for advanced imaging (MRI/CT). 2) MRI findings (marrow edema, enhancement) should prompt early infectious disease/orthopedics consultation. 3) Formal trainee-voice mechanisms (e.g., diagnostic time-outs, escalation pathways) mitigate anchoring bias. 4) Persistent pain (>72 hours) or new neurological symptoms warrant urgent re-imaging. 5) Patient outcomes (pain score 8/10 to 4/10, Constant-Murley score 30 to 65) highlight the value of timely diagnosis. The patient's journey from a seemingly simple infection to a severe bone infection, and the subsequent delay in diagnosis, highlights the potential consequences of ignoring warning signs and dismissing valid clinical hypotheses. Ultimately, this case reinforces the timeless lesson that in medicine, continuous learning, critical thinking, and a commitment to thoroughness are paramount.

Conflicts of Interest

The authors declare no competing interests that could have influenced the objectivity or outcome of this research

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Large Language Model

The authors declare that generative AI was used solely for language editing and grammar correction during the preparation of the manuscript. No part of the scientific content, data interpretation, analysis, conclusions, or author responses to peer review was generated by AI. The authors take full responsibility for the integrity, originality, and accuracy of all content presented.

Authors Contribution

AB conceptualization and writing of the original manuscript, AK data collection and writing the original manuscript, AA writing the original manuscript, DK writing the original manuscript, MM writing the original manuscript, and HH drafted the manuscript, provided critical revisions, and clinical expertise.

Data Availability

Patient data related to this study are not publicly available but can be obtained upon request from the corresponding author.

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