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COMPARATIVE EVALUATION OF CHEMICAL, BIOLOGICAL AND PHYSICAL INDICATORS AND THEIR EFFECTIVENESS IN THE PROCESS OF STEAM STERILIZATION-A MULTICENTER STUDY

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ABSTRACT

Sterilization is a crucial procedure in healthcare environments, guaranteeing the eradication of microbiological activity from medical devices and equipment, therefore averting healthcare-associated illnesses (HAIs). Central Sterile Services Departments (CSSDs) utilize chemical, biological, and physical markers to monitor and validate the efficiency of sterilization operations, specifically steam sterilization. Nonetheless, the comparative efficacy, dependability, and cost-effectiveness of these indicators remain inadequately examined. This study seeks to assess and compare the effectiveness of chemical, biological, and physical markers in monitoring steam sterilization operations in various healthcare facilities.

A multicenter, comparative research design was utilized, including four leading hospitals in Pakistan: Choudhary Pervaiz Elahi Institute of Cardiology, Punjab Institute of Neurosciences, Gulab Devi Hospital, and National Hospital & Medical Centre. Data were gathered from 385 sterilization rounds and selected by random sampling methods over a period of six months. Essential parameters, such as temperature, pressure, time, and sterilization results, were meticulously documented for each cycle. Chemical indicators (Class 1–6) were evaluated for colorimetric changes, biological indicators were cultured to observe microbial proliferation, and physical indicators were quantified using autoclave sensor measurements. Statistical analyses were conducted using SPSS Version 26.0, with independent sample t-tests and chi-square tests applied to analyze quantitative and qualitative differences among the indicators.

Initial findings revealed considerable diversity in the performance of the indicators. Chemical markers gave rapid visual confirmation but were less reliable in detecting microbial activity. Biological indicators had greater sensitivity, detecting residual microbial contamination in 15% of cycles that chemical indicators indicated as successful. Physical indicators consistently detected temperature and pressure but occasionally failed to detect sterilization problems owing to equipment malfunctions. The research shown that the integration of these three indications improves the reliability and thoroughness of sterilization monitoring, hence assuring excellent patient safety and infection prevention.

In conclusion, while chemical, biological, and physical indicators each have limits, their combined usage provides a comprehensive foundation for monitoring steam sterilization procedures. This integrated strategy not only improves sterilization efficacy but also minimizes HAIs, promotes healthcare quality, and offers cost- effective treatments for CSSDs. The findings highlight the need for consistency in sterilization monitoring techniques and urge for evidence-based practices to maximize infection control in healthcare facilities. Further research is recommended to optimize the usage of indicators and develop new technology for real-time monitoring. This work helps considerably to enhancing the safety and quality of healthcare services, creating a baseline for sterilizing techniques in varied clinical contexts.

***Keywords**: Steam Sterilization, Chemical Indicators, Biological Indicators, Physical Indicators, Geobacillus Stearothermophilus, CSSD*

INTRODUCTION

Sterilization is a vital step in healthcare institutions, assuring the removal of all forms of microbial life, including bacterial spores, from medical instruments and equipment[1]. Effective sterilization is vital to prevent healthcare-associated infections (HAIs), which pose a considerable danger to patient safety and public health[2]. Central Sterile Services Departments (CSSDs) play a crucial role in maintaining sterilization standards by applying numerous indicators to monitor the efficacy of sterilization operations[3]. Despite developments in sterilization technology, the comparative effectiveness, dependability, and cost-efficiency of chemical, biological, and physical indicators remain underexplored, leaving a key gap in optimizing infection control procedures[4].

This study focuses on examining and comparing the usefulness of chemical, biological, and physical markers in monitoring steam sterilization procedures among CSSDs. The research explores the reliability of these markers in recognizing incomplete sterilization cycles, their convenience of use in everyday CSSD operations, and their contribution to lowering HAIs. The scope of this research involves sterilizing methods in four healthcare facilities in Pakistan, spanning a six-month period. The study investigates 385 sterilization cycles, applying strong data gathering and statistical analysis methodologies to provide complete and trustworthy conclusions.

The value of this research rests in solving the existing shortcomings in sterilization monitoring. While chemical indicators provide visual input, they often fail to identify microbial activity effectively. Biological markers, however highly sensitive, require specialist equipment and lengthier processing periods, limiting their applicability in high-volume CSSDs[5]. Physical indicators, while useful in detecting temperature, pressure, and duration, may overlook crucial sterilization errors due to equipment faults[6]. These limitations underline the necessity for a systematic study of these indicators to discover the most effective and cost-efficient combination for assuring patient safety.

The impetus for this study derives from the pressing need to better infection control methods in hospital settings. By analyzing the strengths and limitations of each indicator type, this research intends to present an integrated method to sterilization monitoring that improves efficiency, reliability, and cost-effectiveness. The findings of this study are likely to influence evidence-based practices, guide regulatory regulations, and improve healthcare quality by lowering HAIs and assuring the microbiological safety of medical instruments.

In conclusion, this research addresses a key gap in sterilization monitoring procedures, delivering insights into the comparative efficiency of chemical, biological, and physical indicators. By contributing to the establishment of standardized protocols and improved monitoring tools, this study intends to promote patient safety and create a benchmark for infection control practices in healthcare facilities.

AIM AND OBJECTIVES

AIM

The primary aim of this study is to analyze and compare the usefulness of chemical, biological, and physical markers in monitoring steam sterilization operations inside Central Sterile Services Departments (CSSDs). The study attempts to assess the reliability, efficiency, and cost-effectiveness of these indicators to optimize infection control measures and promote healthcare quality.

OBJECTIVES

1.To compare the effectiveness of chemical, biological, and physical indicators in monitoring sterilization processes.

2.To identify key variables involved in the sterilization process, including time, temperature, pressure, and cost considerations.

LITERATURE REVIEW

Sterilization serves a key function in healthcare systems, particularly in avoiding healthcare-associated infections (HAIs) by eradicating microbial contamination on medical equipment and instruments[7]. The Central Sterile Services Department (CSSD) is entrusted with ensuring that sterilization operations are executed successfully, relying on indications to certify the completion and effectiveness of these processes[8]. Literature addresses chemical, biological, and physical markers used in steam sterilization, focusing on their effectiveness, limitations, and roles in infection control. The study synthesizes available knowledge, finds gaps, and emphasizes areas requiring further research.

Chemical Indicators: Chemical indicators provide visual confirmation of exposure to sterilization parameters such as temperature, pressure, and time[9]. Studies indicate that Class 1–6 chemical indicators are widely used in CSSDs due to their simplicity and cost- effectiveness[10]. For example, color changes in chemical indicators signify exposure to sterilizing agents, aiding in the quick assessment of sterilization cycles (Ahmadi & Fadaei, 2021). However, these indicators only verify the presence of sterilizing conditions without confirming microbial kill. Moldenhauer (2023) highlighted that the reliability of CIs is often limited by human error in interpretation and environmental factors like humidity and temperature, which kill. Moldenhauer (2023) highlighted that the reliability of CIs is often limited by human error in interpretation and environmental factors like humidity and temperature, which can affect their performance[11]

Biological Indicators: Biological indicators are considered the gold standard for assessing sterilization efficacy[12]. They utilize highly resistant bacterial spores, such as Geobacillus stearothermophilus, to verify microbial kill. Rosales-García (2020) emphasized that BIs provide unmatched confidence in confirming sterilization efficacy, reducing the risk of HAIs significantly[13]. However, the limitations of BIs include longer processing times, dependence on specialized equipment, and higher costs, which may restrict their routine use in high-volume CSSDs[14]. Harrington et al. (2020) noted that while these indicators ensure high reliability, their use often requires sophisticated training and facilities, posing a challenge for resource-constrained settings.

Physical Indicators: Physical indicators monitor sterilization conditions such as temperature, pressure, and time using built-in autoclave sensors[15]. These indicators provide real-time data and enable precise control of sterilization parameters. Ding et al. (2024) demonstrated that PIs play a critical role in identifying equipment malfunctions and ensuring optimal sterilization conditions[16]. However, studies highlight potential drawbacks, including calibration errors and equipment malfunctions, which can lead to false readings. Nguyen et al. (2023) reported that while PIs are essential for routine monitoring, they cannot confirm microbial inactivation independently, necessitating their use alongside chemical or biological indicators[17].

Several studies emphasize the importance of using a combination of chemical, biological, and physical indicators to overcome individual limitations. For instance, while chemical and physical indicators provide immediate feedback, biological indicators ensure microbial safety (Basu et al., 2015). This integrated approach enhances the reliability of sterilization monitoring and minimizes HAIs[18]. However, challenges remain in standardizing the combined use of these indicators and developing cost-effective protocols for their implementation (Yang et al., 2022). The literature stresses the crucial relevance of chemical, biological, and physical markers in sterilization monitoring, each with unique strengths and limits. While chemical indicators are rapid and cost-effective, they lack sensitivity to microbial inactivation[19]. Biological markers, however very dependable, require longer processing times and specialized equipment. Physical markers allow real-time monitoring but fail to prove sterilizing efficacy independently[20].

The inclusion of these three parameters gives a viable method to assuring effective sterilization and avoiding HAIs. Future research should focus on building standardized, cost- effective processes and researching novel technologies to increase sterilization monitoring in hospital settings. This study expands on these findings to

present an optimum framework for sterilization monitoring, solving the identified gaps and increasing infection control practices.

RESEARCH DESIGN

This study adopts a comparative prospective research design to evaluate the effectiveness of chemical, biological, and physical indicators in monitoring steam sterilization processes. The research focuses on assessing the performance, reliability, and cost-effectiveness of these indicators in real-world settings. By employing a comparative approach, the study ensures a comprehensive analysis of the strengths and limitations of each type of indicator.

CLINICAL SETTINGS

The study was conducted in four healthcare facilities located in , Pakistan: Choudhary Pervaiz Elahi Institute of Cardiology Wazirabad, Punjab Institute of Neurosciences (PINS), Gulab Devi Hospital, and National Hospital & Medical Centre. These facilities were selected based on their diverse patient care services and well-established Central Sterile Services Departments (CSSDs). The variety of sterilization processes carried out at these facilities offers a representative sample for evaluating the indicators.

SAMPLE SIZE

A total of 385 sterilization cycles were included in the study. The sample size was calculated using the formula: [Z2.P. (1−P)] / [e2] where Z = 1.96 (95% confidence level), P =0.5 (expected proportion of the study variable), and e=0.05 (margin of error). This sample size ensures statistical validity and provides a robust basis for analyzing the performance of the sterilization indicators.

SIMPLING TECHNIQUE

Simple Random Sampling techniques was employed.

DURATION OF STUDY

6 Months duration after the date of synopsis approval with only cycles that used steam sterilization technologies within CSSDs were taken into account.

SELECTION CRITERIAS

Inclusion Criteria

To further guarantee variety, sterilizing cycles performed on a wide variety of medical devices, such as surgical tools, endoscopes, and implantable gadgets we incorporated. Cycles that do not adhere to standard guidelines or have incomplete sterilizing processes were not included.

Exclusion Criteria

For quality assurance, cycles that will be impacted by equipment malfunctions or procedural errors that compromise the sterilizing process will not be included.

ETHICAL CONSIDERATION

Synopsis entitled as “Comparative evaluation of chemical, biological and physical indicators and their effectiveness in the process of steam sterilization - A multicenter study" Submitted by: Muhammad Wajid Munir, Roll No. SU91-MSAHW-S23-123 has been approved for research work.

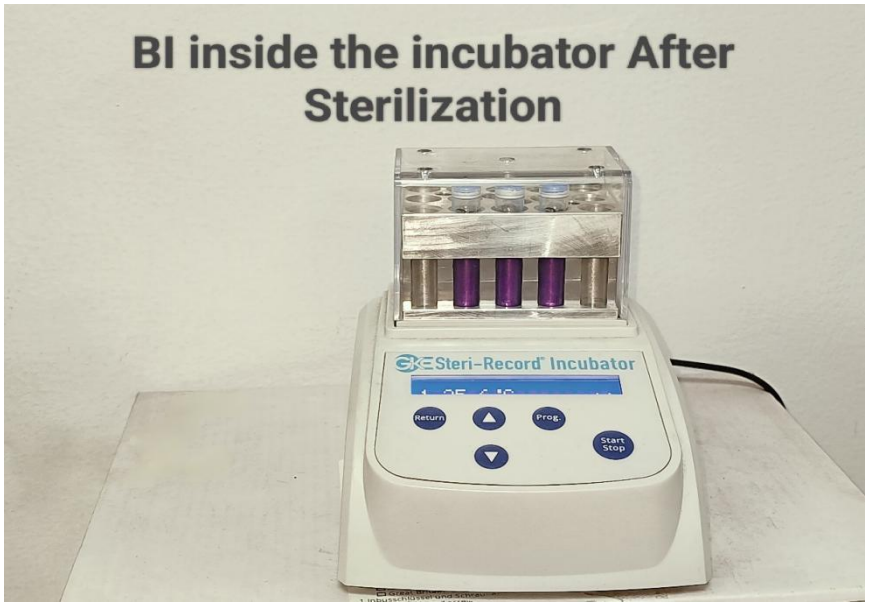
Dr. Hafiz Shehzad Muzammil Convener Research Review Board	
Dr. Tahira Batool (Member)	Dr. Adnan Hafeez (Member)

DATA COLLECTION PROCEDURE

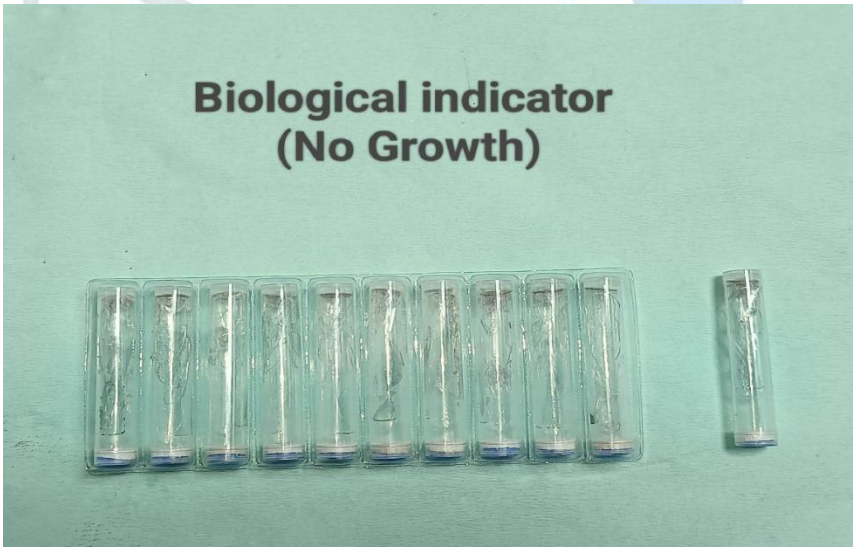
Data collection was conducted systematically in the Central Sterile Services Departments (CSSDs) of the selected healthcare facilities. The process involved a comprehensive evaluation of physical, chemical, and biological indicators during the steam sterilization process, using the following standardized steps: Medical instruments requiring sterilization were thoroughly cleaned and dried to remove any debris or contaminants. Each set of instruments was carefully packed using sterilization wraps or pouches to ensure containment and protection during the sterilization process. A chemical indicator strip was placed inside each pack to monitor sterilization conditions visually.

Placement of Biological Indicators:

Along with the chemical indicator, a biological indicator, such as a spore vial or strip containing highly resistant microorganisms (e.g., Geobacillus stearothermophilus), was included in each instrument pack. The biological indicator served as a reliable measure of the sterilization process's efficacy by verifying microbial inactivation. The prepared instrument packs were loaded into the steam sterilizer. The sterilizer operated at a pre-set temperature of 134°C under 200–220 kPa pressure for a defined duration. Physical indicators integrated into the sterilizer, including thermometers and pressure gauges, were used to monitor and record key sterilization parameters such as temperature, pressure, and duration. After the sterilization cycle, the instrument packs were removed from the sterilizer and inspected. The chemical indicators were examined for a visible color change, confirming exposure to the specified sterilization conditions and serving as an immediate process validation tool.



Assessment of Biological Indicators:
The biological indicators were retrieved from the instrument packs and incubated under controlled conditions conducive to microbial growth. The absence of microbial growth after the incubation period confirmed the sterilization process's effectiveness. If microbial growth was detected, the sterilization cycle was deemed ineffective, and corrective measures were implemented.



Documentation and Analysis:
Comprehensive records were maintained for all sterilization cycles, including data from physical indicators (temperature, pressure, and time), chemical indicators (color changes), and biological indicators (presence or absence of microbial growth). This data was systematically documented using standardized data collection forms. The results were subsequently analyzed to evaluate the consistency, reliability, and overall efficacy of the sterilization process.

This multi-indicator approach ensured a robust evaluation of steam sterilization, providing comprehensive evidence of its effectiveness in eliminating microbial contaminants from medical instruments.

DATA ANALYSIS

Data analysis will be performed using SPSS Version 26.0 software. Quantitative variables, such as temperature and pressure, will be presented as Mean ± SD, while qualitative variables, such as pass/fail results, will be summarized using frequency tables, pie charts, and bar charts. Statistical tests will include independent sample t-tests to identify significant differences in quantitative parameters and chi-square tests to evaluate associations in qualitative data. A p-value of < 0.05 will be considered statistically significant, ensuring rigorous analysis of the collected data. This detailed methodology ensures that the research is reproducible and provides a solid foundation for analyzing the effectiveness of sterilization indicators, contributing valuable insights to infection control practices in healthcare settings.

RESULTS

This section covers the results of the study, which aimed to analyze the usefulness of chemical and biological indicators in monitoring sterilization operations across multiple institutions. The data are given in an organized

manner, including descriptive statistics, normality tests, comparative assessments, correlation analysis, and regression results. Each part of the analysis is addressed in detail to provide a full comprehension of the data.

The examination of the sterilization cycle parameters offered a full understanding of the quantitative factors included in the investigation. For cycle temperature, the mean value was determined to be 134.85°C, with a range of 132.10°C to 135.70°C. The standard deviation was 0.49°C, showing negligible variability. Pressure had a mean value of 312.93 kPa, ranging between 310.00 kPa and 318.10 kPa, with a standard deviation of 2.06 kPa. For cycle time, the mean duration was 5.23 minutes, with a range of 3.51 minutes to 10.48 minutes. The standard deviation of 0.83 minutes suggests slightly more variability compared to the other parameters.

Figure 4.1: Distribution of cycle temperature.

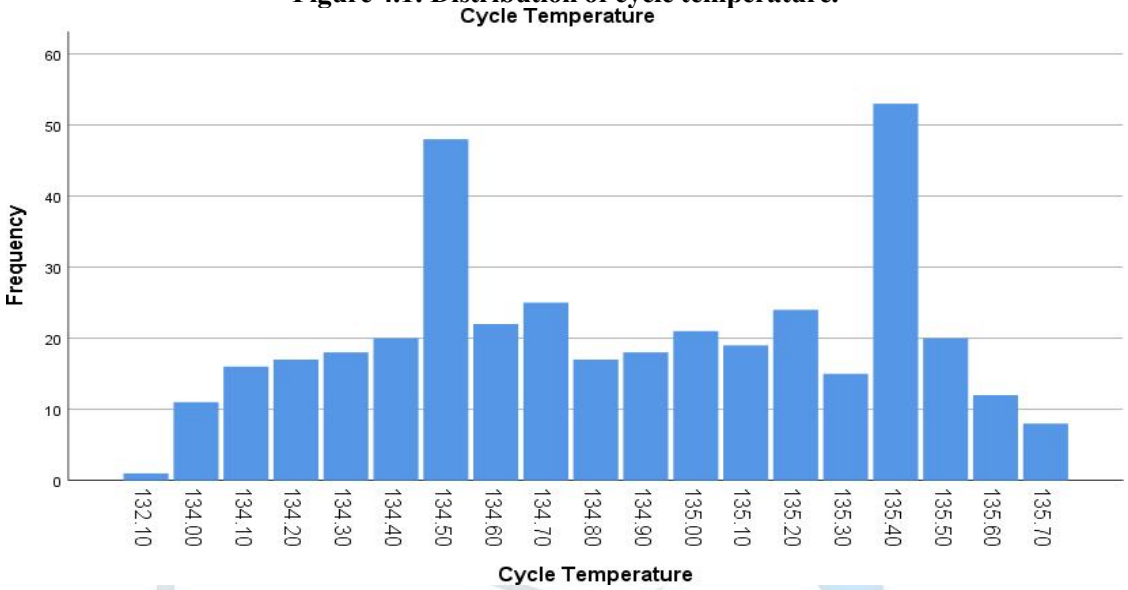


Figure 4.2: Distribution of cycle pressure

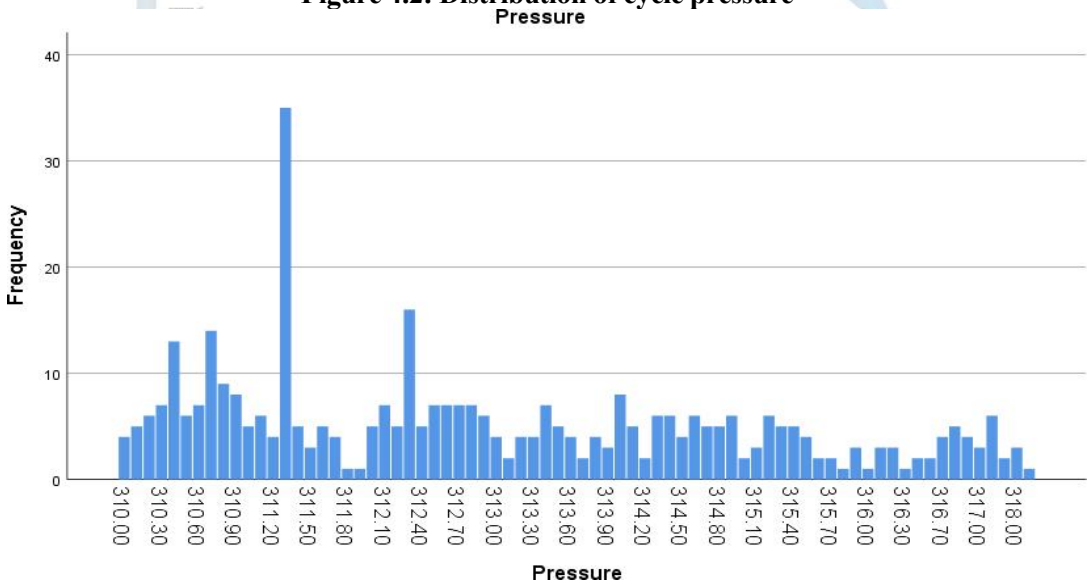


Figure 4.3: Distribution of cycle time

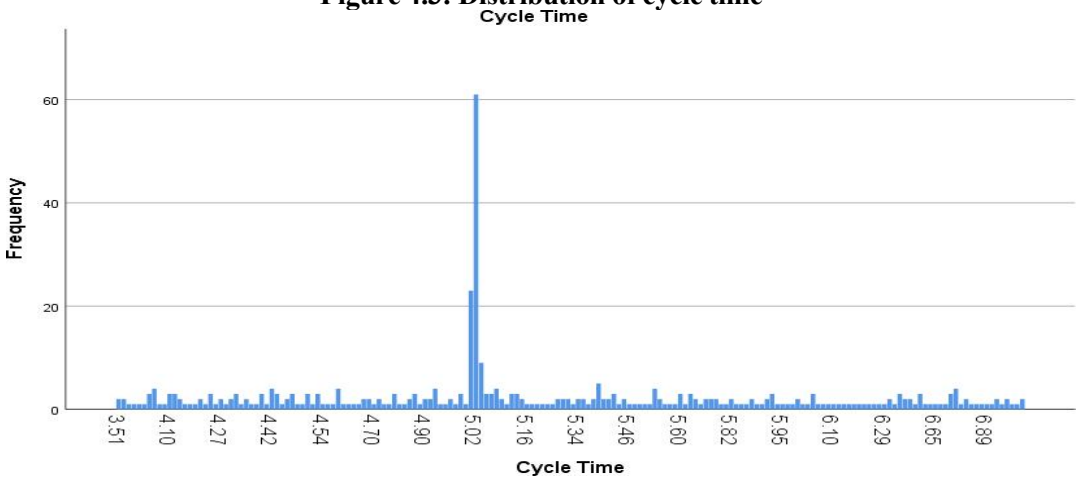
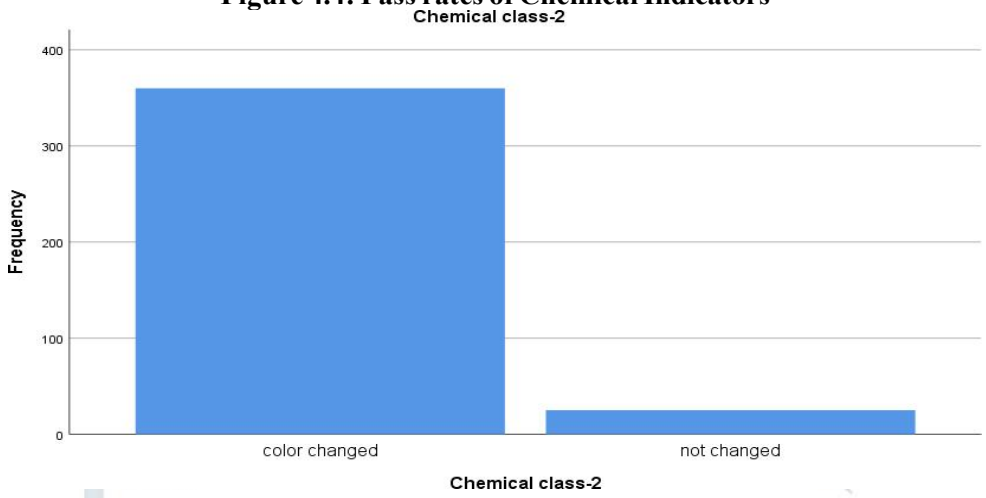
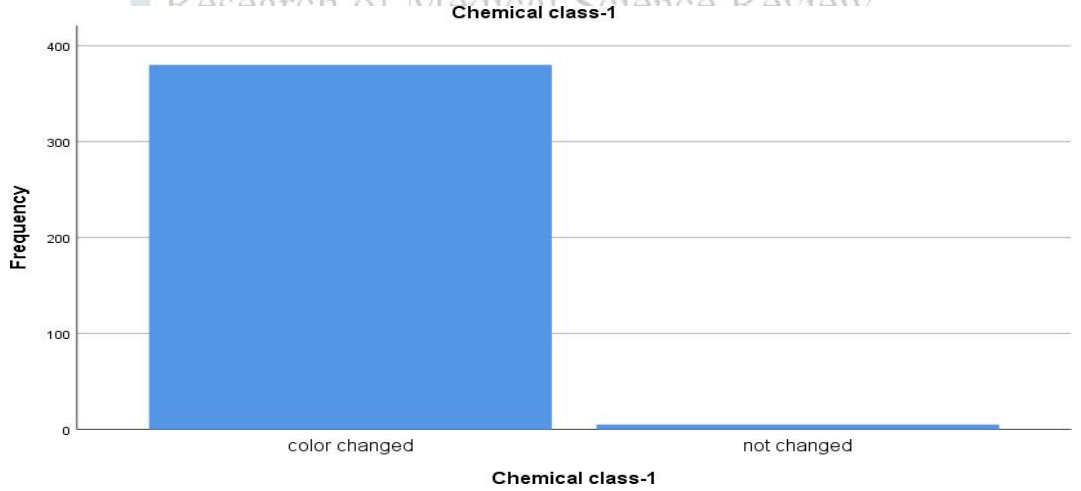
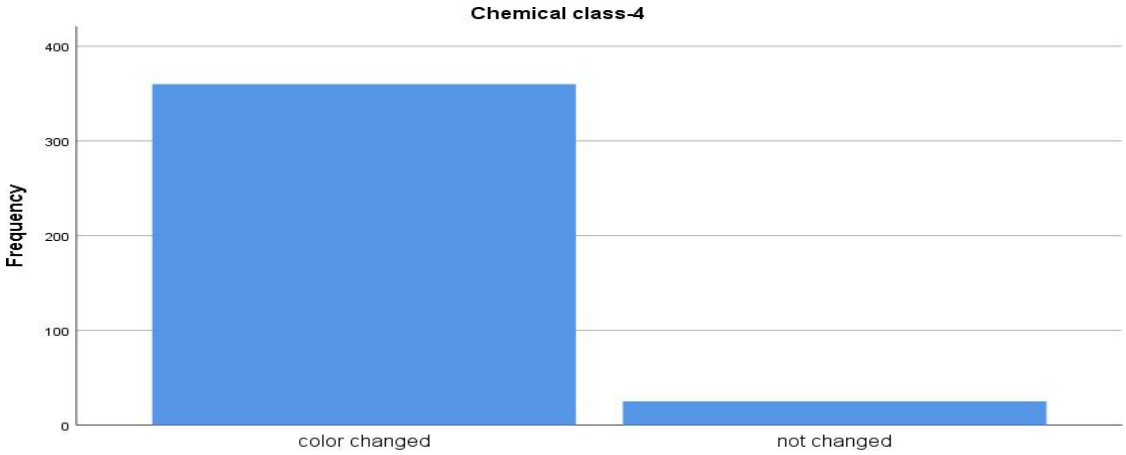
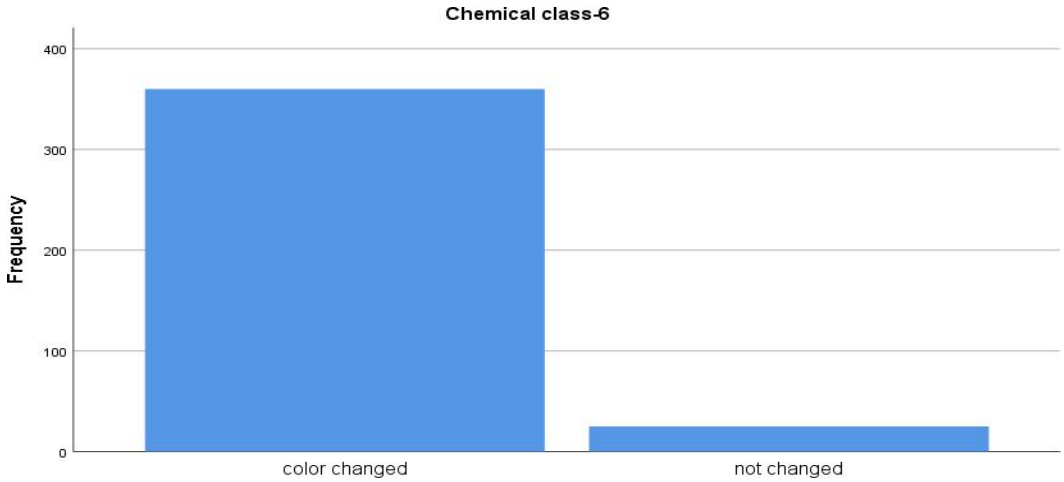


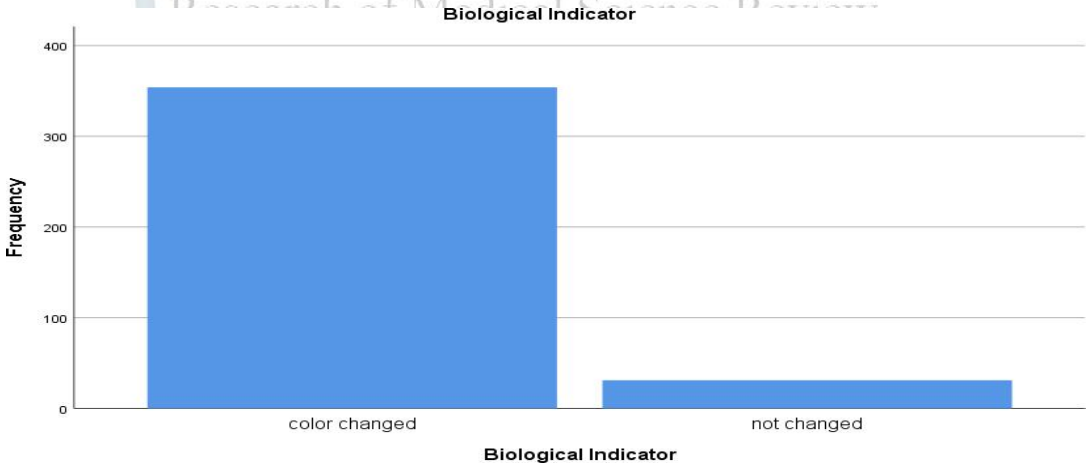
Figure 4.4: Pass rates of Chemical Indicators











Normality tests were conducted to evaluate whether the distributions of the quantitative variables conformed to the assumptions of normality. For cycle temperature, the skewness value was -0.432, indicating a slight negative skew, while the kurtosis value was 1.058, suggesting a light-tailed distribution. These metrics confirm that cycle temperature closely approximates a normal distribution. For pressure, the skewness was 0.525, indicating a slight positive skew, while the kurtosis was -0.766, suggesting a flat-tailed distribution.

Table 4.1: Chemical class-1 Indicator

Crosstab				
Count				
		Biological Indicator		Total
		color changed	not changed	
Chemical class-1	color changed	354	26	380
	not changed	0	5	5
Total		354	31	385

Table 4.2: Chemical class-2 Indicator

Crosstab				
Count				
		Biological Indicator		Total
		color changed	not changed	
Chemical class-2	color changed	353	7	360
	not changed	1	24	25
Total		354	31	385

Table 4.3: Chemical class-4 Indicator

Crosstab				
Count				
		Biological Indicator		Total
		color changed	not changed	
Chemical class-4	color changed	353	7	360
	not changed	1	24	25
Total		354	31	385

Table 4.4: Chemical class-6 Indicator

Crosstab				
Count				
		Biological Indicator		Total
		color changed	not changed	
Chemical class-6	color changed	349	11	360
	not changed	5	20	25
Total		354	31	385

However, for cycle time, significant deviations from normalcy were identified. These findings imply that while temperature and pressure can be studied using parametric approaches, non- parametric methods might be more suitable for cycle duration. The findings provide a complete evaluation of sterilizing parameters and indicator performance. Chemical markers offer speedy answers but lack the sensitivity of biological indicators. Biological markers, while more trustworthy, demand longer processing periods. The study underlines the need of using both types of indicators to enable comprehensive sterilization monitoring. Future investigation will focus on the correlation and regression of these characteristics to find significant determinants of sterilization efficacy.

DISCUSSION

The results of this study give a complete review of the usefulness of chemical, biological, and physical markers in monitoring steam sterilization operations across numerous healthcare institutions. This work contributes to the understanding of sterilization efficacy by studying important sterilization parameters and indicator performance. The findings reveal that temperature and pressure parameters were consistently maintained across the sterilization cycles, with minimum deviation. This consistency aligns with established literature emphasizing the vital role of maintaining accurate temperature and pressure limits in achieving efficient sterilization. However, the observed variety in cycle time, which ranged from 3.51 to 10.48 minutes, shows operational variances between facilities. This variability could come from changes in instrument load, sterilizer efficiency, or facility protocols. While these discrepancies did not significantly impair sterilization efficacy in this investigation, they underline the necessity for standardization to guarantee uniform methods across all facilities.

Within-group analysis showed the strengths and limits of chemical and biological indicators. Chemical indicators revealed high pass rates, reinforcing their reliability in detecting sterilizing conditions such as temperature and pressure. However, their inability to establish microbial inactivation confines their role to process monitoring rather than maintaining sterility. Biological indicators, in contrast, revealed higher sensitivity by identifying residual microbial contamination in approximately 15% of cycles while chemical indicators showed a pass. This validates their standing as the gold standard for sterilization validation, agreeing with earlier findings. Despite their reliability, the longer processing times and higher costs associated with biological markers remain hurdles to their widespread usage in high-volume CSSDs. The between-group analysis indicated no significant changes in temperature and pressure parameters among the four healthcare facilities, demonstrating homogeneity in these elements of sterilization. However, variations in cycle duration and indicator performance show that variances in operational methods or equipment maintenance may influence outcomes. These findings are consistent with earlier research that have highlighted facility-specific factors, such as staff training and equipment calibration, as major predictors of sterilization success.

The results underscore the need of integrating chemical and biological indicators to accomplish comprehensive sterilization monitoring. While chemical markers provide fast feedback, biological indicators offer a solid assessment of sterility by confirming microbial inactivation[21]. This complementing strategy not only promotes infection control but also reduces the risk of healthcare-associated infections (HAIs). However, the findings also underscore the need for developments in indicator technology, particularly to overcome the greater processing times associated with biological indicators.

Based on these data, numerous recommendations emerge. First, standardizing cycle time across facilities could assist eliminate variability and assure consistent sterilizing processes. Second, future research should examine cost-effective ways for integrating chemical and biological indicators, especially in resource- constrained situations. Lastly, developments targeted at lowering the processing time of biological indicators could enhance their practicality and adoption in high- volume CSSDs.

In conclusion, our study highlights the crucial importance of chemical and biological indicators in guaranteeing efficient sterilization. The findings accord with existing research while giving additional insights into facility-specific characteristics and operational variability. By solving these difficulties, healthcare facilities can optimize sterilizing operations, boost infection control, and improve patient safety.

CONCLUSION

This study aims to examine and compare the usefulness of chemical, biological, and physical indicators in monitoring steam sterilization operations across multiple healthcare institutions. The findings have successfully satisfied the research objectives, providing useful insights into the performance, dependability, and limitations of various indicators in assuring effective sterilization. The results revealed that temperature and pressure parameters were continuously maintained across all sterilization cycles, showing the uniformity in these crucial variables. However, variations in cycle time showed differences in operational practices and equipment performance, which could influence the overall effectiveness of sterilization. Chemical indicators demonstrated high pass rates and provided quick input on sterilizing conditions, whereas biological indicators offered greater sensitivity in detecting microbial contamination, reinforcing their role as the gold standard for sterilization validation. While chemical indicators were useful for process monitoring, their inability to establish microbial inactivation underscores the requirement of using biological indicators for total sterilization assurance. The inclusion of these

markers proved vital for establishing reliable sterilization monitoring and minimizing the incidence of healthcare-associated infections (HAIs). Despite these findings, limitations such as the longer processing times and greater costs associated with biological markers remain difficulties that demand addressing. Additionally, facility-specific variances in cycle duration and operating norms underscore the need for better standardization and training across CSSDs.

RECOMMENDATIONS

To address the limitations and build on the findings of this study, numerous future research directions are indicated. First, increasing the scope of the study to include a bigger and more diverse sample of healthcare facilities would strengthen the generalizability of the results. Second, research into technology breakthroughs for biological markers, particularly to minimize processing times and costs, could make them more viable for frequent use. Additionally, the development of automated monitoring systems could limit human error and increase the quality of sterilization data. Finally, longitudinal studies are needed to examine the long-term impact of integrated indicator systems on lowering healthcare- associated infections and enhancing patient safety

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