Original Article

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Analysis of Quality Indicators of Preanalytical and Analytical Phases in Cervical Pap Smear Cytology: A Cross-sectional Study

Pathology Section

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ABSTRACT

Introduction: The Pap smear is a procedure used to collect cells from the cervix to screen for cancerous and dysplastic precancerous cells under a microscope. Internal quality control refers to the measures taken within a laboratory to ensure the accuracy, reliability and consistency of cytology results. It ensures that the technical quality of products in the preanalytical and analytical phases meets pre-established tolerance limits.

Aim: To assess the quality indicators of the preanalytical and analytical phases of cervical Pap smear cytology in order to evaluate Non Conformity (NC) through root cause analysis for appropriate corrective and preventive measures.

Materials and Methods: The cross-sectional study was conducted in the cytology section of the Department of Pathology at Mahatma Gandhi Medical College and Research Institute in Puducherry, India, planned and executed over a period of twelve months, starting in June 2023 and concluding in May 2024. The study cohort comprised 366 female patients who underwent Papanicolaou (Pap) smear testing as a routine screening test, systematically recruited from both the Outpatient (OP) clinics and the inpatient wards of the study Institute. Grading was performed for each parameter in the preanalytical and analytical phases using the Visual Analogue Scoring System (VASS). The grades used were: 3 - Good satisfactory smears; 2 - Extended time in fixative, average nuclear and cytoplasmic staining; 1 - Drying artefacts, air bubbles; and 0 - Inadequate smears, no clinicopathological correlation.

Results: A total of 366 cases were studied and the study demonstrated strong adherence to quality standards, with 94.2% correct slide labelling and 98% excellence in fixation and transportation processes. Findings from the analytical phase revealed that 83% of smears were of excellent quality, with a swift Turnaround Time (TAT) of 90% processed within 24 hours and a clinicopathological correlation rate of 73.49%. Interobserver variation was minimal, with a 96.17% agreement among pathologists. The study identified significant preanalytical errors, primarily due to human factors. The quality indicators met grade 3 in the majority of the samples, indicating robust diagnostic reliability. Grades 0, 1 and 2 in all parameters were categorised as NC and were evaluated for root cause analysis, followed by appropriate corrective and preventive measures.

Conclusion: Standardised protocols and continuous training can minimise variability and enhance the quality of smear preparation, fixation and staining, thereby ensuring that high standards are consistently met. The study proposes VASS as a validated benchmark system for evaluating the quality aspects of cytological smears. Additionally, focusing on value-added activities will optimise resource utilisation and enhance overall efficiency.

Keywords: Cervical cancer, Precancerous cells, Quality parameters

INTRODUCTION

Cervical cancer originates in the cervix and is the fourth most common cancer worldwide. Globally, the incidence of cervical cancer is such that one out of every 70 women develops the disease between birth and the age of 79 years [1]. Consequently, it remains a significant global health burden, particularly in regions lacking robust screening programmes and access to healthcare services. The introduction of cervical Pap smear cytology, pioneered by Dr. George Papanicolaou in the 1940s, revolutionised cervical cancer detection and prevention by enabling the identification of pre-cancerous and cancerous lesions at an early, treatable stage [2].

Quality control measures in cervical cytology tests are essential to ensure accurate and reliable results. The reliability and accuracy of Pap smears depend on three essential processes: the preanalytical, analytical and post-analytical phases.

To verify the precision of clinical findings, a Pap smear audit is conducted. A Pap smear audit involves a systematic review and evaluation of Pap smear cytology reports and related processes to assess the quality, accuracy and effectiveness of the screening programme. The audit aims to identify any deficiencies, errors, or areas for improvement in the interpretation of Pap smear results, laboratory procedures, documentation and reporting protocols [3,4]. The goal is to ensure the reliability and quality of Pap smear cytology testing for the early detection of cervical abnormalities and the prevention of cervical cancer [5].

Quality control in Pap smear cytology helps eliminate inefficiencies, minimise variation and ensure consistent quality, thus improving patient outcomes and promoting better utilisation of resources within the Pap smear cytology service.

The objective of the present study is to assess the quality indicators of the preanalytical and analytical phases of cervical Pap smear cytology in order to evaluate NC through root cause analysis for appropriate corrective and preventive measures.

MATERIALS AND METHODS

The cross-sectional study was conducted in the cytology section of the Department of Pathology at Mahatma Gandhi Medical College and Research Institute in Puducherry, India. This research was designed as a prospective study, planned and executed over a period of twelve months, starting in June 2023 and concluding in May 2024.

The study cohort comprised 366 female patients who underwent Pap smear testing as a routine screening test.

Inclusion and Exclusion criteria: All female patients who required Pap smear testing were included in the present study. The study

did not impose any exclusion criteria, allowing for the inclusion of a broad spectrum of patients with various clinical backgrounds and conditions. Pap smears were collected from patients in both the OP clinics and the OBG wards and their identities were anonymised. These samples were then sent to the cytology laboratory for analysis.

Sample size calculation: The formula used to calculate the sample size is as follows:

$$N = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 p(1-p)}{d^2}$$

$Z_{1-\frac{\alpha}{2}} = 1.96$ at 95% of confidence with $\alpha = 0.05$

$Z_{1-\beta}$=0.84 at 80% of power with β=0.20

p=$\frac{3662}{4232}$=0.8653 from record of MRD from 1st July 2022 to 30th June 2023

d=5%=0.05 Absolute precision

$$p=\frac{(1.96+0.84)^2\ 0.8653(1-0.8653)}{(0.05)^2}$$

n=366

Confidence level: 95%, Margin of error: 5%, Minimum sample size needed: 366.

Study Procedure

Alcohol-fixed Pap smears collected from the OP and wards were received in the cytology laboratory and details were obtained from the Hospital Information Management System (HIMS)- Aosta backbone system. These smears were processed in strict adherence to the laboratory's Standard Operating Protocol (SOP), ensuring consistency and reliability in the handling and analysis of specimens.

To achieve the study sample, smears were selected using a continuous sampling method until the predetermined sample size was reached.

Each smear, upon receipt, was assigned a unique Aosta number as per the SOP. Subsequently, a comprehensive log sheet was created for each sample required for the study. This log sheet included a checklist of preanalytical and analytical phase quality indicators, which were attached to the routine Aosta-generated forms. This systematic documentation was designed to maintain a detailed record of each step, from sample receipt to final analysis. The processing of the slides involved standard staining and mounting procedures, following the laboratory's established protocols. Throughout these processes, the quality of the smears was precisely monitored; however, reports were dispatched according to the routine SOP of the laboratory to ensure that the TAT was not affected because of the study. This quality control extended to the reporting phase, where the smears were evaluated for both technical quality and diagnostic accuracy.

The sequence of events from the preanalytical phase (sample collection, preparation, staining and mounting) to the analytical phase (diagnosis and clinicopathological correlation) was carefully categorised and documented using the checklist, following the SOP. In the present study, analytical indicators were evaluated from the point at which the pathologist received the slide.

After processing, the collected data were systematically tabulated and analysed to evaluate all relevant quality parameters. The VASS was utilised and grading was performed for each parameter in the preanalytical and analytical phases. The grades used were: 0, 1, 2 and 3, as detailed in [Table/Fig-1]. This comprehensive analysis aimed to identify any NCs in the process. Upon detection of NCs, further investigations were conducted to understand the root causes and corrective actions were implemented to resolve these issues. This continuous feedback loop was essential for maintaining and improving quality control within the laboratory.

Checklist for quality indicators in cervical pap smears - preanalytical and analytical indicators:

Preanalytical indicators:

- Age, clinical history, menstrual history and examination findings
- Identification
- Fixation of slides
- Transportation of slides
- Staining of slides
- Mounting of slides
- Preparation of slides

Analytical Indicators:

- Nature of smear
- Interobserver variation
- Turnaround Time (TAT)
- Clinicopathological correlation

Indicators	Parameters	Grade 0- Poor	Grade 1- Average	Grade 2- Good	Grade 3- Excellent
Preanalytical indicators	Age, clinical and menstrual history with examination findings	No history and examination findings	Wrong entry of age, sex and wrong history	Inadequate history and not providing complete examination findings	Complete history with examination findings with correct age and sex of the patient
	Identification of slides	Mix up of slides and mismatch in register entry	Mismatched labelling of pap slides	Receiving and proper entries without delay	Proper labelling and numbering of slides with the form
	Fixation of slides	Unfixed slides	Inadequate fixative	Extended time in fixative	Slides sent with adequate fixatives
	Transportation of slides	Delay in sending samples after procedure	Transportation errors (Container without proper capping)	Partially immersed slides in fixation	Containers and fixative were appropriate
	Staining of slides	Lack of expertise in staining procedure and using unfiltered and expired stains	Inadequate timing for nuclear and cytoplasmic staining	Inadequate washing and not checking the integrity of stain before usage	Excellent nuclear and cytoplasmic staining
	Mounting of slides	Reverse mounting	Inadequate mountant and presence of air bubbles	Excessive mountant	Well mounted providing good resolution
	Put up of slides	Breakage of slides before handover to pathologist	No proper handing over of slides to the pathologist	Delayed handing of slides to the pathologist	Slides were brought to the pathologists immediately
Analytical indicators	Nature of smear (satisfactory/ unsatisfactory)	Unsatisfactory due to inadequate smear and inflammation obscuring the cells	Background staining deposits, air bubbles and artefacts obscuring the cells	Average nuclear and cytoplasmic staining	Satisfactory smear
	Clinicopathological correlation	Typographical errors in the diagnosis of reports	Reports not correlating with clinical diagnosis	Consensus obtained in hierarchical reporting after discussion	Clinicopathological correlation with second opinion

[Table/Fig-1]: Preanalytical and analytical indicators as well as their grades.

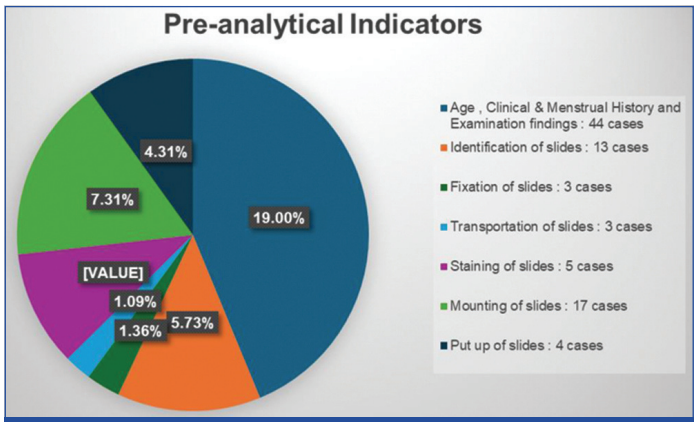
Grades 0, 1 and 2 in all parameters were categorised as NC and were evaluated for root cause analysis, followed by appropriate corrective and preventive measures. The quality indicators measured included TAT and interobserver variation. TAT was calculated from the time of receiving the specimen to the finalisation of a report by the faculty in Aosta and concurrence/non concurrence with TAT was noted. The clinicopathological correlation of the final report was verified.

STATISTICAL ANALYSIS

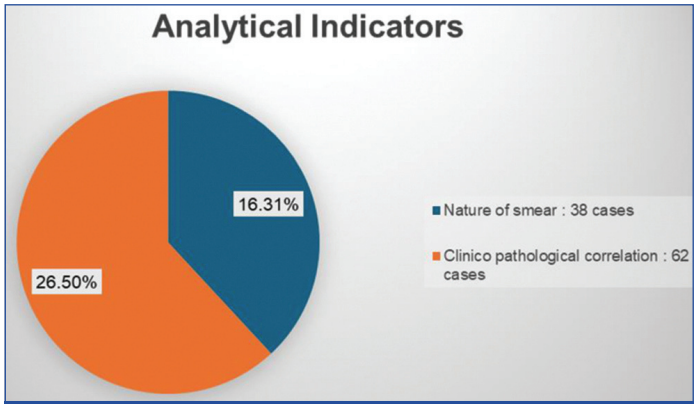
Statistics were represented as frequency and percentage.

RESULTS

The preanalytical and analytical parameters of the 366 samples of Pap smears were graded using the VASS, with grades 0, 1 and 2 in all the parameters categorised as NC. [Table/Fig-2,3] represent the NC in preanalytical and analytical parameters plotted in pie charts, respectively.



[Table/Fig-2]: NC in preanalytical parameters.



[Table/Fig-3]: NC in analytical parameters.

In the assessment of TAT for reports in cervical cytology, the majority of cases (331 out of 366, 90.43%) achieved a TAT of 24 hours, reflecting a prompt and efficient diagnostic process. However, 13 cases (3.55%) were completed within 36 hours and 22 cases (6.01%) were completed within 48 hours.

In evaluating interobserver variation among four pathologists in cervical cytology, 352 cases (96.17%) showed agreement in their interpretations, indicating no interobserver variation. However, in 14 cases (3.80%), there was discordance in observations between at least two pathologists. The calculation of the kappa statistic reveals a moderate level of agreement (κ=0.511) between the pathologists, indicating a moderate level of consistency beyond chance.

DISCUSSION

The preanalytical phase holds significant importance as it directly involves specimen collection procedures and often lies beyond the laboratory's immediate control. Additionally, most preanalytical errors stem from human factors [6]. This aligns with the findings of the current study, where the majority of errors are linked to the preanalytical phase. The age of the studied patients ranged between 13 and 70 years, with a mean (±standard error) age of 42.4098±0.5288 years. Mistakes in age, clinical history, menstrual history and examination findings on the request form were primarily attributed to human error. This reflects a strong adherence to quality control measures in the collection of patient information and examination findings during the preanalytical phase. However, there is a significant number of patients with incomplete or unsatisfactory data.

Similarly, during the identification of slides, instances of slide mix-up, mismatched register entries and mismatched labelling of Pap slides were observed. Nonetheless, 94.2% of the slides were correctly labelled and numbered according to the form, with only 5% deviating from this standard. This suggests a strong framework for slide identification and numbering, which enhances the reliability and effectiveness of follow-up analysis procedures.

Alternatively, during fixation and transportation, no specimens were recorded in the grade 0 and grade 1 categories. This high percentile underscores the effectiveness of the fixation process in preserving specimen integrity and facilitating accurate analysis. Attention should be given to addressing the minor issue of extended fixative exposure observed in a small subset of slides to further enhance overall quality. Unfixed slides, inadequate fixative during fixation and delays in sending samples after the procedure, as well as transportation errors such as containers lacking proper capping, did not occur. However, occurrences of extended fixative exposure during fixation and partially immersed slides during transportation were observed at a frequency of approximately 1%. This indicates that 98% of the samples met excellent criteria.

This highlights the efficiency of the transportation system, which significantly contributes to timely processing and the effectiveness of the fixation process in preserving specimen integrity and facilitating accurate analysis. In staining procedures, the errors accounted for a collective frequency of 5% within the sample set. As a small subset showed minor deficiencies, areas for improvement in washing procedures and stain integrity checks are suggested.

The vast majority, comprising 95% of samples, exhibited excellence in both nuclear and cytoplasmic staining processes. In the subsequent preanalytical stage of mounting, there was a slight increase in errors, with approximately 6% of cases exhibiting issues such as inadequate mountant resulting in air bubbles and excessive mountant application. On the other hand, 94% of the specimens were well-mounted, providing good resolution. Additionally, only a minimal 0.5% of cases demonstrated reverse mounting, indicating meticulous attention to detail and expertise in slide preparation. The implementation of standardised staining protocols will achieve clear nuclear and cytoplasmic details. Furthermore, providing continuous training for clinicians and lab technicians on best practices in smear preparation and handling will enhance perfection in the analytical phase [7].

In the final preanalytical phase, the occurrence of slide breakage before handover to the consultant and inadequate handing over of slides accounted for 4%, while 96% demonstrated excellent grading. Notably, there was a complete absence of delayed handover of slides to the pathologists, emphasising the commitment to the process. Our study stands out significantly amidst broader global trends in preanalytical error rates.

Overall, it is crucial to provide proper staff education, standardise procedures, implement quality control measures, utilise automation technologies (such as Hologic's ThinPrep Imaging System and Becton Dickinson's Focal Point GS Imaging System) and employ Computer-Aided Diagnosis (CAD) Systems (such as Cytyc's AutoPap and Visioneer's Pathfinder) to reduce or avoid preanalytical errors. In addition, ensuring proper sample handling by rejecting

unlabelled samples and documenting any relabelling with physician approval is also essential [8-11].

In the analytical phase of the present study, several parameters were evaluated, including the nature of the smear (satisfactory/unsatisfactory), TAT in hours, clinicopathological correlation and interobserver variation. Our results from the observations made while analysing the nature of the smear indicated that 83.60% of the samples met the excellent criteria. These findings emphasise the critical role of precise smear preparation techniques in ensuring clear visualisation of cellular morphology, thereby facilitating accurate cytological diagnosis and optimal patient management. However, 16.31% of the samples were not fully satisfactory, which was attributed to errors such as background staining deposits, air bubbles and artefacts obscuring the cells. Additionally, some samples exhibited average nuclear and cytoplasmic staining quality. The unsatisfactory samples were often due to inadequate smear preparation and inflammation that obscured the cellular details. This high percentage suggests that the majority of samples had adequate cellularity, proper fixation and clear staining, facilitating accurate diagnosis.

The TAT analysis showed that approximately 90% of samples were processed within 24 hours. This distribution highlights the importance of timely reporting, with the vast majority of cases being processed and reported within the recommended timeframe. A quick TAT not only facilitates expedited patient management and treatment decisions but also enhances overall healthcare efficiency. A small fraction of cases required 36 hours or 48 hours, indicating occasional delays that should be addressed to ensure consistent rapid processing. In most cases, TAT was exceeded for carcinoma and High-Grade Squamous Intraepithelial Lesions (HSIL) where clinical data were not provided, resulting in additional time spent gathering the necessary details.

Additionally, advanced Electronic Medical Record (EMR) systems facilitate the electronic transmission of cytology results, eliminating the need for manual result entry and reducing the risk of transcription errors. Electronic reporting speeds up result delivery to healthcare providers and patients, thereby reducing TAT [12,13].

The Clinicopathological Correlation (CPC) results reveal that approximately 73.49% of cases achieved a Grade 3, demonstrating excellent correlation between clinical findings and pathological results. Notably, there were no cases in Grade 0 or Grade 2, which reflects a high level of diagnostic accuracy and consistency. Conversely, around 27% were rated as Grade 1, indicating a need for further alignment between clinical and pathological assessments. As CPC plays a pivotal role in guiding clinical decision-making and treatment planning, ongoing monitoring and quality improvement efforts are essential to maintain CPC rates above the recommended threshold of 80%, thereby enhancing the reliability and effectiveness of cervical cytology reporting practices.

However, achieving a CPC rate above the 80% threshold is crucial for reliable diagnostic outcomes. To increase CPC rates in cervical cytology, it is essential to enhance multidisciplinary collaboration and invest in continuous education and training. Establishing regular Multidisciplinary Team (MDT) meetings involving pathologists, gynaecologists, oncologists and other specialists can significantly improve CPC rates by facilitating comprehensive case discussions and integrating clinical, radiological and pathological data for more accurate diagnoses.

The evaluation of interobserver variation among four pathologists in cervical cytology revealed that 352 out of 366 cases (96.17%) showed complete agreement, indicating a high level of consistency and reliability in diagnostic interpretations. This substantial concordance underscores the efficacy of current training, standardisation and protocols in ensuring uniformity in cytological assessments. However, the presence of discordance in 14 cases (3.80%) highlights areas where further improvement is necessary. The kappa statistic (κ=0.511) suggests moderate agreement beyond chance, signifying that while there is a solid foundation of consistency, there is room for enhancement [14].

This moderate level of agreement points to the importance of ongoing quality assurance measures, such as regular calibration meetings, continued education and the implementation of more refined diagnostic criteria. These efforts are crucial for reducing variability and ensuring that all pathologists are aligned in their diagnostic approach. Collaborative discussions and second opinions should be encouraged to mitigate discrepancies and enhance diagnostic accuracy. Ensuring high interobserver agreement is vital for the reliability of cervical cytology, as it significantly impacts clinical decision-making and patient outcomes.

Limitation(s)

The limitations of the study include the fixed time period to identify NCs to implement appropriate corrective and preventive measures. This may not accurately reflect the potential for ongoing improvements.

CONCLUSION(S)

Standardised protocols and continuous training can minimise variability and enhance the quality of smear preparation, fixation and staining, ensuring that high standards are consistently met. The study proposes the VASS as a validated benchmark for evaluating the quality aspects of cytological smears. Additionally, focusing on value-added activities will optimise resource utilisation and enhance overall efficiency. Integrating these principles will create a more efficient, reliable and high-quality cervical cytology audit process, leading to improved diagnostic accuracy and patient outcomes, as demonstrated by the high percentages of excellence achieved in various quality parameters. In conclusion, incorporating a larger sample size and a longer duration in a multicentre study can significantly enhance the statistical power and generalisability of the findings.

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