INTRICACIES OF ASCUS: SIL RATIO NAVIGATING ITS UNCERTAINTY AND CERVICAL CYTOLOGY COMPLEXITY IN TERTIARY CARE SETTINGS

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ABSTRACT

Introduction:

Quality indicators serve as essential tools for monitoring quality control systems and have transformed laboratory medicine. Internal quality control (QC) is essential for identifying any deviations from standards starting from the time a sample is received in the laboratory until the report is sent out. Examining the internal quality control indicators of cervical smears was the aim of this study, which aimed to determine our existing situation, pinpoint any shortcomings, and enhance the effectiveness of our laboratory services. Our objective is to improve lab services by evaluating the internal quality control indicators of cervical smears.

Materials and methods: This retrospective investigation had been done at the Department of Pathology at Saveetha Medical College and Hospital. It reviewed archived cases from March 2023 to March 2024, with clinical details extracted from the records. And focused on thin prep pap tests for diagnosing ascus conventional Pap smears classified as ASCUS, ASC-H, LSIL, HSIL, and SCC. During a span of two years, a comprehensive analysis was conducted on a total of 1,220 Pap smear cases, with a specific focus on examining 50 cases. The ASC/SIL ratio was computed, with ASC encompassing ASC-US and ASC-H, and SIL encompassing LSIL, HSIL, and SCC. The cytologic criteria for reporting adhered to the Bethesda System for Reporting Cervical Cytology 2014. Biopsies were taken to confirm epithelial cell abnormalities, routinely processed, and stained with hematoxylinand eosin.

Results: A total of 1,220 Pap smear cases were analyzed over one year, with 51 cases selected for detailed study. Among these, 34 cases were classified as ASC and 17 as SIL. The ASC/SIL ratio was determined by dividing the number of ASC instances by the number of SIL cases, yielding a ratioof 2:1, which falls below the upper threshold of 3:1. Biopsies were obtained from both cases of ASC and squamous intraepithelial lesions (SIL), revealing malignant and pre-malignant diagnoses in 62.5% of ASC cases and 88.8% of SIL cases, correspondingly.

Conclusion: The internal quality control indicators reported in the present investigation nearly conform to thestandard values specified by CAP/Bethesda. This phenomenon presents a paradoxical situation: although evaluating previous performance improves the effectiveness of screening, it also leads to an increased occurrence of incorrect identifications.

Keywords: Screening, Intraepithelial lesion, Quality control, Cervical cytology

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INTRODUCTION

In India, cervical cancer ranks second among cancer-related deaths that affect women (1). Cervical cancer incidence can be significantly reduced by putting in place effective cervical screening programs in addition to widespread HPV vaccination campaigns. Given the slow progression of cervical cancer from mild dysplasia to invasive carcinoma over several years, screening programs are vital for identifying and treating precancerous lesions before they become malignant.

Pathologists are essential for delivering high-quality and accurate cervical cytology reports. Quality control encompasses a series of operational procedures and activities that ensure the required quality of each test process. Quality control in cervical cytology is implemented to guarantee the precise analysis and documentation of Pap smears (2).

Despite being a basic and affordable procedure, the Pap test is often criticized for its high rate of false negative results, which raises concerns about its reliability. Around 20% of these errors stem from variations between different operators and within the same operator, as well as from microscopic errors (3).

A vital component of every laboratory system is QC, which is a technique for guaranteeing and preserving the appropriate standard of quality in a particular test or procedure (4).

Internal QC is essential for detecting any inconsistencies in the laboratory process, starting with the arrival of a sample until the issuance of the final report. Internal quality control indicators in cervical cytology encompass various critical factors such as the positivity rate, which refers to the proportion of ASC in both satisfactory and bad tests. Other important indicators "include the ASC/ SIL ratio, the ASCUS/SIL ratio, the percentage of low-grade SIL (LSIL) and high-grade SIL (HSIL), the rate of false-negative tests, and the percentage of unsatisfactory smears. (5,6,7,8).

Quality indicators are crucial in enhancing the quality of cervical cytology. Promoting the updating of cytomorphological categorization among pathologists" can greatly enhance the precision of detecting early preinvasive lesions and decrease the occurrence of false-negative outcomes and unsatisfactory smears. These indicators enable laboratories to compare their performance with others and pinpoint areas needing improvement. Laboratories striving for excellence should aim to meet these quality benchmarks to deliver optimal services (6,9,10).

The objectives of our study include analyzing cases with discrepancies between cytological and histological findings as well as assessing the ratio of ASC/SIL categories. This analysis will enable us to evaluate the quality control measures in cytology smears.

MATERIALS AND METHODS:

This retrospective investigation had been performed at the "Department of Pathology, Saveetha Medical College and Hospital. Archived cases from the previous year, covering from March 2023to March 2024, were reviewed, with clinical details extracted from records. The study specifically examined the utility of conventional Pap smears for the diagnosis of ASCUS, ASC-H, LSIL, HSIL, and SCC. Out of a total of 1,220 Pap smear cases analyzed within a year, a specific examination was conducted on 50 cases. The ASC/SIL ratio was determined by considering the ASC component, which consists of ASC-US and ASC-H, and the SIL component, which includes LSIL, HSIL, and SCC. The cytological criteria were determined as per the Bethesda System for Reporting Cervical Cytology" 2014 (11). Histological findings obtained from medical records were documented for a period of one year. A cytohistological correlation was conducted for all instances exhibiting aberrant findings.

Inclusion criteria: Patients exhibiting symptoms but with a clinically normal cervix, and patients showing symptoms with suspicious cervical lesions.

Exclusion criteria: Individuals undergoing therapy, pregnant women, individuals who have had a hysterectomy, and those experiencing significant vaginal bleeding during the examination

SAMPLE COLLECTION:

A healthcare provider gathers cervical cells using a brush or spatula, swirling the collection device vigorously in a vial filled with a preservative solution tailored for the Thin Prep procedure. This action releases the cervical cells into the liquid.

In instances in which aberrant epithelial cells were found on Pap smears and in cases with extensively degraded cervix, biopsies were predominantly collected from the cervix. This was accomplished by adding three percent acetic acid, which resulted in areas that were acetowhite. In accordance with the standard procedure, these biopsies were fixed in formalin at a concentration of ten percent and stained using hematoxylin and eosin.

ASC-US: The nuclei of these cells are larger than those of normal intermediate squamous cells, ranging in size from 2.5 to 3 times the area, and their nuclear to cytoplasmic ratio (N/C) is slightly greater. They exhibit traits such as uneven nuclear morphology or chromatin distribution, aberrant parakeratosis, and nuclear hyperchromasia.

ASC-H: Cells are frequently found alone or in tiny fragments of little more than ten cells. The cells resemble metaplastic cells in size, with nuclei that are between 1.5 and 2 times larger than typical. HSIL may be roughly represented by the N/C area ratio.

LSIL: A slightly higher ratio of N/C area results from a significant growth of the nucleus, this is larger than the typical intermediate nuclei by more than threefold. Nuclear hyperchromasia in varied degrees is accompanied by variations in nuclear quantity, size, along shape. The chromatin has an appearance of being evenly dispersed, coarsely granular, and lacking or barely noticeablenucleoli. A common symptom of HPV infection is koilocytosis, which is characterized by clearly defined perinuclear halos and a highly eosinophilic cytoplasmic border around the periphery. Onthe other hand, the cytoplasm could seem dense and orange-colored, which could indicate keratinization.

HSIL: The range of nuclear enlargement is higher than it was in LSIL. A significant increase in the N/C ratio results from a decrease in the cytoplasmic area. The nuclear membrane has a non- uniform shape with many noticeable indentations. There are no nucleoli present. Immature, fragile, and lacy is the cytoplasm.

Squamous Cell Carcinoma (SCC): These cells display noticeable differences in size and shape, with some appearing individually while others form loose clusters. There is considerable variability in cellular size and shape, including elongated and spindle-shaped cells containing dense, orangeophilic cytoplasm. The nuclear membranes may have unusual shapes, nuclei vary significantly in size, and there are often multiple dense, opaque nuclei present. Atypical cells areassociated with keratotic changes such as

"hyperkeratosis" or "pleomorphic parakeratosis.

RESULTS:

Over the course of one year, a total of 1,220 cases were examined. The age range of women screened varied from 26 to 72 years, each presenting with diverse clinical backgrounds. Among these, 210 cases (17.2%) were deemed unsatisfactory. These unsatisfactory smears were categorized into qualitative inadequacy (50 cases, 23.8%), which included issues like inadequatefixation, poor staining quality, drying artifacts, and broken slides, and quantitative inadequacy (160 cases, 76.2%), which included factors such as the absence of the junctional component, thepresence of inflammation, and the presence of blood.

Unsatisfactory Smears			
Qualitative	**Number (Percentage)**	**Quantitative**	**Number (Percentage)**
Poor Fixation& Staining Quality	15(30%)	Absence of endocervical cells	90(56.3%)
Artefacts	25(50%)	Existence of Blood	56(35%)
Damaged slides	10(20%)	Existence of Inflammation	14(8.7%)
Total	50(23.8%)	Total	160(76.2%)
Total = 210 (17.2%)			

Table 1. Distribution of Unsatisfactory Smears

Of the satisfactory cases, 1,010 (82.7%) were found, with 959 (94.9%) showing no signs of intraepithelial lesions and 51 (5.04%) displaying neoplastic abnormalities. Among these 51 abnormal cases, there were 34 with ASC components and 17 with SIL components. The ASC/SIL ratio was determined by dividing the number of ASC cases by the number of SIL cases, resulting in a 2:1 ratio, which is below the maximum benchmark of 3:1. Biopsies were performed on 16 ASC component cases and 9 SIL component cases. The positivity rate for malignant and pre- malignant diagnoses in biopsy samples was 62.5% for the ASC component and 88.8% for the SILcomponent. Despite the higher positivity rate in the SIL component, a significant number of positive biopsies were also found in the ASC component. Only 50% of the cases had appropriate histological follow-up, with some patients unable to complete the follow-up process, and discrepancies were noted in the cytohistological correlation for some cases.

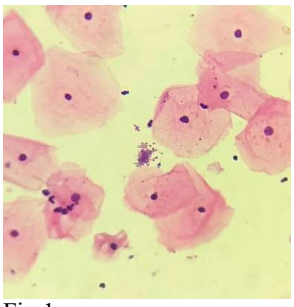


Fig 1

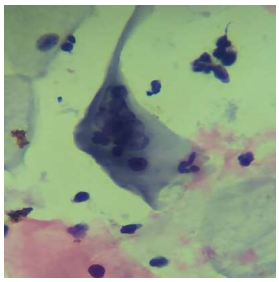


Fig 2

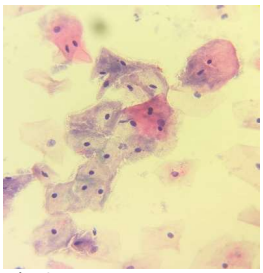


Fig 3

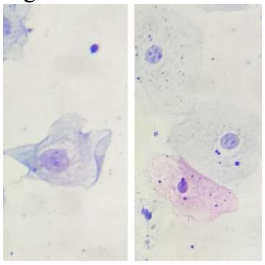
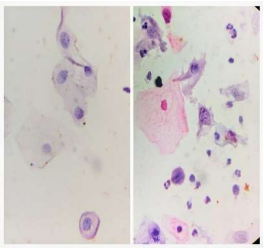


Fig 4



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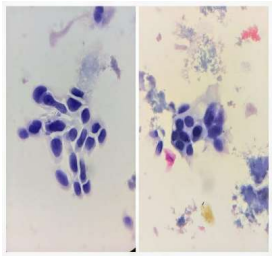

Fig 6

Figure. Representative cytomorphological features in liquid based cytology of Pap smears. (1) *Candida* infection showing budding yeast forms **(2)** Herpes simplex virus infection showing multinucleation, nuclear moulding, and ground-glass nuclei. **(3)** Bacterial vaginosis showing clue cells. **(4)** ASC-US showing mild nuclear atypia. **(5)** LSIL showing koilocytic atypia. **(6)** HSIL showing hyperchromatic cells with a high nuclear-to-cytoplasmic ratio.

Satisfactory Smears	
Negative of Intraepithelial lesion or malignancy	**959(94.95%)**
organisms	
1. Bacterial Vaginosis	10
2.trichomonas vaginalis	9
3.candida	20
4. herpes simplex	1
5. Leptothrix	6
6.aspergillus	1
Non-Neoplastic lesion	
1. Atrophy	8
2.Granulomatous lesion	1
3. Post chemotherapy Changes	1
4. cervicitis	1
Epithelial Cell Abnormalities	**51(5.04%)**
Squamous Cells	
ASC Component	
ASC-US	27
ASC-H	7
Total	34
SIL Component	
L-SIL	4
H-SIL	10
SCC	2
Total	17
ASCUS: SIL Ratio	**2:1**
Total	**1010 (82.8%)**

Table 2. Cytological Findings in Satisfactory Pap Smears

DISCUSSION:

In recent times, a growing number of women have actively engaged in pap smear screening, driven by heightened awareness. Delivering cytology results with high accuracy requires ensuring the standard of cervical cytopathology reporting. Maintaining cytopathology quality involves aiming to meet suggested benchmarks. However, achieving the recommended benchmark for detecting precancerous lesions poses challenges for numerous laboratories, often due to various factors such as sampling errors during pap smear collection.

In our present investigation, the ASC/SIL ratio of 2:1 achieved the benchmark recommended by Bethesda. A research investigation by Renshaw AA et al. found that a ratio of less than 1.5 forASC/SIL is indicative of insufficient screening. This is due to the fact that, in comparison to higher ASC/SIL ratios, lower ASC/SIL ratios indicate a higher risk of a certain diagnosis. As a result, there's a lower chance of mistaking a normal cell for an aberrant one—but at the price of less sensitivity. Sensitivity is crucial in a screening program since it reduces the chance of missing genuine positive cases compared to some false positive cases. In our study, we propose that sustaining an ASC/SIL ratio above 1.5 indicates adequate sensitivity, while achieving an ASC/SIL ratio exceeding 3 suggests the need for future revisions to detect positive cases with minimal impact on specificity (12).

The frequent use of the "ASCUS" category can significantly impact patient care. An inaccurate interpretation may lead to a significant over- or undertreatment of patients (13).

Investigating the causes of this striking discrepancy, it was found that fifty-five percent of patients had a normal cervix after a per speculum examination, on the other hand, eighteen percent of patients had visited for standard check-ups without exhibiting any specific symptoms. Cervical lesions often show clinical indicators, which is further supported by the finding that all of the study's patients with abnormal smears had symptoms. Patient histories revealed complaints of vaginal bleeding and discharge, while per

speculum examination identified cervical erosion, discharge, bleeding upon touch, and the presence of growth.

Challenges such as limited access to healthcare, educational barriers, and potential anxieties related to the invasive nature of the procedure may influence these differences. Some cases show significant disparities between tissue analysis and cytological abnormalities, which could result from limitations in sample collection or interpretation errors. To identify these disparities, two independent pathologists conducted a blinded review to establish a consensus diagnosis.

Previous research and studies have established that the ASC/SIL ratio holds greater significance as an indicator of screening quality compared to the percentage of ASC alone. As a result, this ratio acts as a stand-in indicator of degree of certainty. As indicated by Kumaran RJ and SolomonD, it is advisable to keep the ASC/SIL ratio below 2:1 or 3:1. A range of 0.4 to 5.1 is recommended by the College of American Pathologists laboratory accreditation program (8).

A high proportion of unsatisfactory Pap smears, a significant marker of inadequate screening, is primarily attributed to sampling errors. In this study, the unsatisfactory smear rate is elevated in contrast to the suggested CAP median of 0.5% (7), indicating a notable absence of a pathologist-clinician feedback system. It underscores the urgent need to implement and enforce such a system. Unlike other quality indicators in cervical cytology, an unsatisfactory smear rate provides a crucial aspect of quality assurance. By instituting a feedback system and providing ongoing training for sample collection teams, the rate of unsatisfactory smears can be significantly reduced to meet benchmark standards.

Various methods are available to rescreen slides to ensure the accuracy of negative Pap smear results. These methods include randomly rescreening a subset of 10% of negative cases, rapidly rescreening all negative cases, or even rapidly rescreening all smears, regardless of the initial result (14). However, high-volume laboratories may not have the capacity to rescreen all negative cases, so the choice of rescreening method depends on the laboratory workload and the availability of cytopathologists. ten percent of the total patients in this study were randomly screened, and the false-negative rate was only 0.09%. Although it is still difficult to completely eliminate screening and interpretation errors, rescreening techniques like these are a useful tool for reducing them, specifically in high-volume labs. Compared to previous studies, this one mayhave a healthier screening group, which could explain the lower detection rates of precancerous lesions.

To uphold internal quality indicators within our institution and among others, aim for high standards, we propose concentrating on several essential aspects: 1) Promptly providing feedback to clinicians regarding unsatisfactory Pap smears to ensure appropriate follow-up and confirmatory resampling for accurate diagnosis; 2) organizing regular training sessions for the sampling team and cytopathologists; 3) having the most senior cytopathologist rescreen all questionable cases before issuing the report; 4) addressing any differences and deciding on next steps by holding regular reviews of the cytology-histopathology correlation register and consulting with clinicians.

CONCLUSION:

The internal quality control indicators achieved in this study largely meet the standard values suggested by CAP/Bethesda. However, this situation presents a double-edged sword: while evaluating past performance enhances screening effectiveness, it also results in more false positives. Despite the high false positivity associated with ASC diagnosis, utilizing this reporting category reduces the risk of missing premalignancies, allowing for early intervention. Therefore,it is essential to maintain this reporting category. It is imperative for all laboratories to strive towards achieving established benchmarks for Pap smear quality. To optimize the effectiveness of cervical cancer screening programs, emphasis should be placed on well-trained sampling teams, skilled cytopathologists for interpretation, and a rigorous quality control system. In these programs, prioritizing sensitivity over specificity is crucial.

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