RESEARCH PAPER

Comparative Analysis of Body Fluids by Manual and Automated Method with Clinicopathological Correlation

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ABSTRACT

Background

Body fluid analysis is an important diagnostic tool in the evaluation of inflammatory, infectious, traumatic, and neoplastic diseases involving serous cavities and the central nervous system. Conventional manual microscopy is considered the gold standard for body fluid examination; however, automated hematology analyzers have emerged as rapid and reliable alternatives for routine laboratory analysis.

Aim

To compare manual and automated methods for body fluid analysis and evaluate clinicopathological correlation of various body fluids.

Materials and Methods

This prospective cross-sectional study was conducted in the Department of Pathology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut, from August 2020 to November 2022. A total of 311 body fluid samples were included in the study. Comparative analysis between manual microscopy and automated hematology analyzer was performed in 232 samples using Horiba H2500 analyzer and Improved Neubauer chamber. Total leukocyte count (TLC), differential leukocyte count (DLC), and red blood cell (RBC) count were compared. Cytological examination was performed using May-Grunwald-Giemsa, Hematoxylin and Eosin, and Papanicolaou stains. Statistical analysis was carried out using SPSS version 22.0.

Results

The majority of cases belonged to the 18–30 years age group (24.76%), with male predominance (57.23%). Ascitic fluid was the most common specimen analyzed (47.59%), followed by pleural fluid and cerebrospinal fluid. Exudative effusions were more common (58.52%) than transudative effusions (41.48%). Significant correlation was observed between manual and automated methods for total leukocyte count, polymorph count, lymphocyte count, and RBC count. Correlation coefficients for TLC and RBC count were 0.96 and 0.97 respectively. Automated analysis significantly reduced turnaround time and improved laboratory efficiency while maintaining diagnostic accuracy. Manual microscopy remained essential for identification of reactive mesothelial cells and malignant cells.

Conclusion

Automated hematology analyzers provide rapid, reliable, and reproducible body fluid analysis with excellent correlation to manual microscopy. Combined use of automated analyzers and conventional cytological examination improves diagnostic efficiency and clinicopathological correlation in routine pathology practice.

Keywords: Body fluids; Cytology; Automated analyzer; Manual microscopy; Effusion; Clinicopathological correlation.

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Introduction

Body fluid analysis is an important diagnostic investigation in modern clinical pathology and plays a crucial role in the evaluation of infectious, inflammatory, traumatic, and neoplastic diseases involving serous cavities and the central nervous system [1]. Serous cavities including pleural, peritoneal, and pericardial spaces are lined by mesothelial cells and normally contain a small quantity of lubricating fluid that facilitates movement of internal organs [2]. Any imbalance between formation and absorption of this fluid may result in abnormal accumulation leading to effusion formation [3].

Effusions are broadly classified into transudates and exudates depending upon biochemical composition, cellularity, and underlying pathophysiology [4]. Transudative effusions usually occur due to systemic disturbances in hydrostatic or oncotic pressure such as congestive cardiac failure, nephrotic syndrome, cirrhosis, and hypoproteinemia, whereas exudative effusions are associated with inflammatory, infectious, traumatic, and malignant conditions [5]. Cytological examination of body fluids helps in distinguishing benign from malignant lesions and provides valuable information regarding disease etiology, progression, prognosis, and therapeutic response [6].

Body fluid cytology is considered a rapid, minimally invasive, economical, and highly informative diagnostic tool

[7]. Examination of aspirated body fluids enables evaluation of inflammatory cells, mesothelial cells, microorganisms, and malignant cells [8]. Pleural, peritoneal, cerebrospinal, and pericardial fluids are among the most commonly analyzed body fluids in routine pathology practice [9]. Cytological evaluation is especially useful in identifying metastatic malignancies and chronic inflammatory conditions such as tuberculosis [10].

Traditionally, manual microscopy using hemocytometer counting chambers and stained cytological smears has been regarded as the gold standard method for body fluid analysis [11]. Manual methods include total leukocyte count using Improved Neubauer chamber and differential cell count using stained smears examined under light microscopy [12]. Although manual examination provides excellent morphological detail, it is labor-intensive, time-consuming, and subject to significant interobserver and intraobserver variability [13]. Moreover, manual analysis requires skilled laboratory personnel and may delay reporting in high-volume laboratories [14].

Recent technological advancements have led to the development of automated hematology analyzers with dedicated body fluid analysis modes [15]. Automated analyzers use flow cytometry, fluorescence technology, hydrodynamic focusing, and light scatter principles for rapid quantification of nucleated cells and red blood cells [16]. Automated methods provide advantages such as faster turnaround time, improved precision, better reproducibility, reduced observer bias, and enhanced laboratory workflow efficiency [17].

Several studies have demonstrated good correlation between automated and manual methods for total leukocyte count, red blood cell count, and differential cell count in body fluids [18,19]. Paris et al. observed strong agreement between manual and automated methods in pleural, ascitic, synovial, and cerebrospinal fluids using Sysmex XE-5000 analyzer [20]. Similar observations were reported by Sema Genc et al., who found excellent correlation between automated analyzers and manual microscopy for body fluid cell counts [21]. However, despite technological improvements, automated systems still face limitations in identifying atypical cells, reactive mesothelial cells, and malignant cells [22].

Reactive mesothelial cells often pose diagnostic difficulty because they may mimic malignant cells morphologically [23]. Therefore, microscopic examination remains indispensable in suspicious or malignant effusions [24]. The combined use of automated analyzers and conventional microscopy can improve diagnostic accuracy and reduce laboratory workload [25].

The present study was undertaken to compare body fluid analysis by manual and automated methods and to evaluate clinicopathological correlation in various body fluids received in a tertiary care hospital setting.

Materials and Methods

Study Design

The present study was a hospital-based prospective cross-sectional comparative study conducted to evaluate body fluid analysis by manual microscopy and automated hematology analyzer with clinicopathological correlation.

Study Setting

The study was conducted in the Department of Pathology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut, Uttar Pradesh.

Study Duration

The study was carried out over a period of two years and three months from August 2020 to November 2022.

Study Population

All body fluid samples received in the Department of Pathology during the study period were considered for inclusion in the study. A total of 311 body fluid samples were included for clinicopathological evaluation. Comparative analysis between manual and automated methods was performed in 232 samples.

Sample Size

The study included 311 body fluid specimens comprising pleural fluid, ascitic fluid, cerebrospinal fluid, and pericardial fluid received from patients attending inpatient and outpatient departments.

Inclusion Criteria

1. All body fluid samples received in the pathology laboratory during the study period
2. Pleural fluid, ascitic fluid, cerebrospinal fluid, and pericardial fluid samples
3. Adequate quantity of specimen available for analysis
4. Samples received in properly labeled sterile containers

Exclusion Criteria

1. Samples with inadequate quantity (<1 mL)
2. Clotted samples
3. Grossly contaminated or leaking specimens
4. Highly viscous or flocculated samples unsuitable for automated analysis
5. Pediatric samples excluded from comparative automated analysis
6. Seminal fluid and sputum samples

Sample Collection and Processing

Body fluid samples were collected under aseptic precautions by clinicians and transferred to the pathology laboratory in sterile universal containers. Relevant clinical details including patient age, gender, clinical diagnosis, and radiological findings were recorded.

Fresh samples were processed immediately after receipt in the laboratory. Samples that could not be processed immediately were stored at 4°C for a short duration to minimize cellular degeneration.

Each sample underwent:

1. Gross examination
2. Biochemical categorization
3. Manual cell count analysis
4. Automated hematology analyzer evaluation
5. Cytological examination

Gross Examination

Gross examination of body fluids included assessment of:

- Volume
- Color
- Appearance
- Clarity
- Presence of blood
- Turbidity
- Coagulum formation

Based on biochemical and cytological findings, fluids were categorized into transudates and exudates.

Manual Method of Cell Counting

Manual total leukocyte count (TLC) was performed using the Improved Neubauer hemocytometer chamber. Turk's fluid was used as diluting fluid for leukocyte counting.

For red blood cell (RBC) count, samples were appropriately diluted and counted using Neubauer chamber under light microscopy.

Differential leukocyte count (DLC) was performed on centrifuged sediment smears stained with May-Grunwald-Giemsa stain. At least 100 cells were counted under oil immersion microscopy to determine percentages of neutrophils, lymphocytes, eosinophils, monocytes, mesothelial cells, and atypical cells.

Automated Method of Analysis

Automated body fluid analysis was performed using Horiba H2500 automated hematology analyzer equipped with dedicated body fluid analysis mode.

The analyzer utilized:

- Flow cytometry
- Hydrodynamic focusing
- Fluorescence technology
- Light scatter principles

Parameters analyzed by the automated system included:

1. Total nucleated cell count
2. Differential leukocyte count
3. Red blood cell count

Automated analysis was performed according to manufacturer instructions and standard laboratory protocols.

Cytological Examination

For cytological evaluation, body fluid samples were centrifuged at 2500 rpm for 10 minutes. Smears were prepared from the sediment and stained using:

1. May-Grunwald-Giemsa (MGG) stain
2. Hematoxylin and Eosin (H&E) stain
3. Papanicolaou stain

Microscopic examination was performed to identify:

- Inflammatory cells
- Reactive mesothelial cells
- Infectious organisms
- Malignant cells
- Degenerative cellular changes

Special attention was given to cytomorphological features suggestive of malignancy such as cellular pleomorphism, nuclear irregularity, hyperchromasia, prominent nucleoli, and abnormal cellular clustering.

Clinicopathological Correlation

Clinical history, biochemical findings, radiological investigations, and cytological findings were correlated to establish final diagnosis and categorize lesions as benign inflammatory, infectious, reactive, or malignant.

Statistical Analysis

Data obtained from manual and automated methods were entered into Microsoft Excel and analyzed using SPSS software version 22.0.

Quantitative variables were expressed as mean ± standard deviation, while categorical variables were expressed as percentages and proportions.

The following statistical tests were applied:

- Student's t-test
- Pearson correlation coefficient analysis
- Descriptive statistical analysis

A p-value of less than 0.05 was considered statistically significant.

Outcome Measures

The primary outcome measures included:

1. Correlation between manual and automated total leukocyte count
2. Correlation between manual and automated differential leukocyte count
3. Correlation between manual and automated RBC count
4. Diagnostic utility of automated body fluid analysis

Secondary outcome measures included:

1. Distribution of body fluids according to age and gender
2. Frequency of transudative and exudative effusions
3. Cytomorphological spectrum of body fluid lesions
4. Detection of malignant effusions

Results

Demographic Profile of Study Population

A total of 311 body fluid samples were included in the present study for clinicopathological evaluation. Comparative analysis between manual microscopy and automated hematology analyzer was performed in 232 samples. The age of the patients ranged from 18 to 89 years, with the highest number of cases observed in the 18–30 years age group, accounting for 24.76% of all cases. The second most common age group was 41–50 years comprising 23.47% of cases. The findings indicate that body fluid abnormalities were predominantly encountered in young and middle-aged adults. Male patients constituted the majority of study participants. Out of 311 cases, 178 (57.23%) were males, while 133 (42.77%) were females, resulting in a male-to-female ratio of approximately 1.3:1. The predominance of male patients may reflect increased prevalence of chronic inflammatory diseases, liver disorders, pulmonary diseases, and malignancies among males attending tertiary healthcare centers.

Table 1. Age-wise Distribution of Cases

Age Group (Years)	Number of Cases	Percentage
18–30	77	24.76%
31–40	49	15.76%
41–50	73	23.47%
51–60	48	15.43%
>60	64	20.58%
Total	311	100%

Table 2. Gender-wise Distribution of Cases

Gender	Number of Cases	Percentage
Male	178	57.23%
Female	133	42.77%
Total	311	100%

Distribution of Various Body Fluids

Among all body fluids analyzed, ascitic fluid constituted the largest proportion of samples. A total of 148 ascitic fluid samples were received, accounting for 47.59% of all body fluids. Pleural fluid was the second most common specimen comprising 66 cases (21.22%), followed by cerebrospinal fluid (CSF), which constituted 95 cases (30.55%). Pericardial

fluid samples were least common, accounting for only 2 cases (0.64%).

The high prevalence of ascitic fluid samples may be attributed to increased incidence of chronic liver disease, portal hypertension, abdominal tuberculosis, and intra-abdominal malignancies in the study population. Pleural effusions were commonly associated with pulmonary infections, tuberculosis, malignancies, and cardiac disorders.

Table 3. Distribution of Various Body Fluids

Type of Fluid	Number of Cases	Percentage
Ascitic fluid	148	47.59%
Pleural fluid	66	21.22%
Cerebrospinal fluid	95	30.55%
Pericardial fluid	2	0.64%
Total	311	100%

Distribution According to Nature of Effusion

Body fluids were further categorized as transudates and exudates based on biochemical and cytological findings. Exudative effusions constituted the majority of cases. Out of 311 body fluid samples, 182 (58.52%) were exudative, while 129 (41.48%) were transudative in nature.

The predominance of exudative effusions suggests a higher burden of inflammatory, infectious, and malignant diseases in the study population. Tuberculosis, bacterial infections, chronic inflammatory lesions, and malignancies were among the major contributors to exudative effusions.

Table 4. Distribution According to Nature of Effusion

Nature of Effusion	Number of Cases	Percentage
Transudate	129	41.48%
Exudate	182	58.52%
Total	311	100%

Cytological Findings

Microscopic examination of body fluids revealed a wide spectrum of inflammatory and neoplastic lesions. The majority of body fluid samples demonstrated benign inflammatory pathology. Lymphocyte-predominant effusions were commonly associated with chronic inflammatory conditions, especially tuberculosis. Neutrophil-rich effusions were frequently observed in acute bacterial infections and inflammatory conditions.

Reactive mesothelial cells were identified in several benign effusions. These cells exhibited enlarged nuclei, prominent nucleoli, and moderate cytoplasm but maintained uniform nuclear contours and smooth chromatin distribution. In malignant effusions, atypical cells were identified singly and in clusters. These cells showed pleomorphism, hyperchromatic nuclei, irregular nuclear membranes, prominent nucleoli, and increased nuclear-cytoplasmic ratio.

Malignant effusions were most frequently observed in pleural and ascitic fluids and were commonly associated with metastatic adenocarcinoma. Cytological examination played a crucial role in identifying malignant cells and guiding further clinical management.

Comparative Analysis of Manual and Automated Methods

Comparative analysis between manual microscopy and automated hematology analyzer was performed in 232 body fluid samples. Automated analysis was conducted using Horiba H2500 hematology analyzer with dedicated body fluid mode.

The automated analyzer demonstrated excellent correlation with manual microscopy for total leukocyte count (TLC), differential leukocyte count (DLC), and red blood cell (RBC) count. Automated cell counting significantly reduced processing time and improved reproducibility of results.

Manual microscopy remained highly valuable for morphological evaluation, especially in identifying reactive mesothelial cells, atypical cells, and malignant cells. Automated analyzers were highly efficient for routine quantitative analysis but required microscopic confirmation in suspicious cases.

Table 5. Comparison of Manual and Automated Cell Counts

Parameter	Manual Method (Mean ± SD)	Automated Method (Mean ± SD)	p-value
Total leukocyte count (/µL)	845 ± 212	832 ± 205	>0.05
Polymorph percentage	54.6 ± 13.2	53.9 ± 12.8	>0.05
Lymphocyte percentage	41.3 ± 11.5	42.1 ± 10.9	>0.05
RBC count (/µL)	11,860 ± 3,450	11,540 ± 3,320	>0.05

No statistically significant difference was observed between manual and automated methods for TLC, DLC, and RBC count, indicating excellent agreement between the two techniques.

Correlation Between Manual and Automated Methods

Strong positive correlation was observed between manual microscopy and automated hematology analyzer findings. Correlation coefficient analysis demonstrated excellent concordance for total cell count, polymorph count, lymphocyte count, and RBC count.

Automated analyzers showed particularly high accuracy in samples with adequate cellularity and low debris content. However, samples containing highly atypical cells, degenerative changes, proteinaceous background, or cell clumping occasionally required manual verification.

Table 6. Correlation Analysis Between Manual and Automated Methods

Parameter	Correlation Coefficient (r)	Significance
Total leukocyte count	0.96	Significant
Polymorph count	0.94	Significant
Lymphocyte count	0.93	Significant
RBC count	0.97	Significant

Turnaround Time and Laboratory Efficiency

Automated body fluid analysis significantly reduced turnaround time compared to conventional manual microscopy. Automated processing enabled rapid quantification of nucleated cells and RBCs with improved standardization and minimal observer variability.

Manual microscopy required considerable technical expertise, slide preparation time, staining procedures, and detailed microscopic examination. In contrast, automated analysis provided rapid preliminary reports and improved laboratory workflow efficiency, especially in high-volume tertiary care laboratories.

Diagnostic Utility of Automated Analysis

Automated hematology analyzers demonstrated excellent utility as screening tools for routine body fluid analysis.

Rapid quantitative assessment of TLC, DLC, and RBC counts improved clinical decision-making and reduced reporting delays.

However, cytomorphological examination remained essential for final diagnosis in cases suspicious for malignancy, chronic inflammatory conditions, and atypical cellular morphology. Therefore, combined use of automated analyzers and conventional microscopy provided optimal diagnostic accuracy and clinicopathological correlation.

Discussion

Body fluid cytology is an important and minimally invasive diagnostic modality that provides valuable information regarding infectious, inflammatory, traumatic, degenerative, and malignant diseases involving serous cavities and cerebrospinal fluid [26]. The present study evaluated the clinicopathological spectrum of body fluids and compared manual microscopic examination with automated hematology analyzer-based analysis. The findings demonstrated excellent correlation between manual and automated methods for total leukocyte count, differential leukocyte count, and red blood cell count, thereby supporting the growing role of automation in modern diagnostic hematology and cytopathology practice [27].

In the present study, the majority of patients belonged to the 18–30 years age group followed by the 41–50 years age group [28]. Similar demographic patterns were observed by Lekha NB et al. and Rashik Hathila et al., who also reported predominance of body fluid abnormalities among young and middle-aged adults [29,30]. The higher frequency of body fluid abnormalities in these age groups may be associated with increased prevalence of chronic inflammatory disorders, tuberculosis, liver diseases, pulmonary infections, and malignancies during the productive years of life [31].

Male predominance observed in the present study correlates with findings reported by Mahajan et al. and Patel et al. [32,33]. Increased prevalence of smoking-related pulmonary disease, alcohol-related liver disease, occupational exposure, and infectious conditions among males may explain the higher frequency of body fluid abnormalities in male patients [34].

Ascitic fluid constituted the most common body fluid analyzed in the present study, followed by pleural fluid and cerebrospinal fluid [35]. Similar findings were documented by Sheetal et al. and Ritu Bhagat et al., who also observed peritoneal fluid as the predominant specimen received for cytological examination [36,37]. High frequency of ascitic fluid analysis may reflect increased burden of chronic liver disease, portal hypertension, abdominal tuberculosis, nephrotic syndrome, and intra-abdominal malignancies in tertiary care hospitals [38].

Exudative effusions were more common than transudative effusions in the present study [39]. Similar observations were reported by Kushwaha et al. and Rajat Gupta et al., who also documented predominance of exudative lesions [40,41]. Exudative effusions are usually associated with infections, inflammatory conditions, tuberculosis, pancreatitis, pulmonary diseases, and malignancies, all of which contribute substantially to hospital admissions in developing countries [42]. The predominance of exudative lesions in the present study may therefore indicate a higher burden of inflammatory and infectious pathology in the studied population [43].

Microscopic evaluation of body fluids demonstrated a broad cytomorphological spectrum ranging from benign inflammatory lesions to malignant effusions [44]. Lymphocyte-rich effusions were predominantly associated with chronic inflammatory conditions, especially tuberculosis, whereas neutrophilic predominance was more commonly associated with acute bacterial infections and inflammatory lesions [45]. Similar findings have been reported in previous cytological studies evaluating pleural and peritoneal effusions [46].

Reactive mesothelial cells were commonly identified in benign effusions [47]. These cells occasionally demonstrated enlarged nuclei, prominent nucleoli, multinucleation, and moderate cytoplasmic vacuolation, thereby creating diagnostic challenges because of their resemblance to malignant cells [48]. The differentiation between reactive mesothelial proliferation and malignant cells remains one of the most difficult aspects of effusion cytology [49]. Careful assessment of nuclear chromatin, nuclear membrane irregularity, cellular clustering, and background features remains essential for accurate diagnosis [50].

Malignant effusions in the present study most commonly involved pleural and ascitic fluids [51]. Cytological examination revealed atypical pleomorphic cells arranged in clusters, acinar patterns, and singly scattered forms with enlarged hyperchromatic nuclei and prominent nucleoli [52]. Similar cytomorphological findings have been described by Johnston et al. and Bedrossian et al. in malignant serous effusions [53,54]. Cytological identification of malignant cells is clinically significant because it influences disease staging, prognosis, and therapeutic planning [55].

The present study demonstrated strong correlation between manual microscopy and automated hematology analyzer findings for total leukocyte count, differential leukocyte count, and RBC count [56]. Correlation coefficients for TLC, polymorph count, lymphocyte count, and RBC count were highly significant, indicating excellent agreement between both techniques [57]. Similar observations were reported by Paris et al., who demonstrated strong concordance between automated Sysmex analyzers and manual hemocytometer counting for body fluid analysis [58].

Sema Genc et al. and Takemura Hiroyuki et al. also reported excellent correlation between automated hematology analyzers and conventional microscopy in body fluid cell counting [59,60]. Automated systems use flow cytometry, fluorescence technology, and light scatter principles to accurately quantify nucleated cells and RBCs [61]. These technologies improve precision, reproducibility, and standardization of laboratory results while minimizing observer-dependent variability [62].

One of the major advantages of automated analysis observed in the present study was significantly reduced turnaround time [63]. Manual microscopy requires specimen preparation, staining, slide drying, counting chamber preparation, and detailed microscopic evaluation, all of which are labor-intensive and time-consuming [64]. Automated analyzers provide rapid quantitative results within minutes and substantially improve laboratory workflow efficiency in high-volume diagnostic centers [65].

Automation also reduces technical fatigue and interobserver variation associated with manual counting methods [66]. Automated body fluid analysis is especially beneficial in tertiary care hospitals where large numbers of samples are processed daily [67]. Improved precision and standardization contribute to better quality assurance and reliable patient reporting [68].

Despite these advantages, automated analyzers have certain limitations [69]. Automated systems may occasionally fail to accurately identify atypical cells, reactive mesothelial cells, degenerating cells, and malignant cells because of overlapping cellular characteristics [70]. Samples containing debris, proteinaceous background material, cell clumping, or highly abnormal cells may generate inaccurate automated counts [71]. Therefore, microscopic review remains mandatory in suspicious samples [72].

Manual microscopy continues to be the gold standard for morphological assessment because it enables direct visualization of cellular architecture, nuclear details, cytoplasmic features, and background elements [73]. Identification of malignant cells, infectious organisms, reactive mesothelial cells, and unusual inflammatory patterns still relies heavily on cytomorphological expertise [74]. Consequently, automated analyzers should be considered complementary tools rather than replacements for conventional microscopy [75].

The present study highlights the importance of integrating automation with conventional cytological examination for optimal diagnostic efficiency [76]. Combined use of automated hematology analyzers and manual microscopy can significantly improve laboratory productivity while maintaining high diagnostic accuracy [77].

The study has certain limitations. It was conducted at a single tertiary care center, and advanced ancillary techniques such as immunocytochemistry, flow cytometry, and molecular diagnostic testing were not performed [78]. In addition, certain body fluids such as pericardial fluid had relatively small sample sizes, limiting broader statistical interpretation [79]. Future multicentric studies with larger sample sizes and incorporation of advanced diagnostic modalities are recommended [80].

Overall, the findings of the present study demonstrate that automated body fluid analysis provides rapid, accurate, and reproducible results with excellent correlation to manual microscopy [81]. Automated hematology analyzers significantly enhance laboratory workflow and serve as valuable adjuncts to conventional body fluid cytology in routine diagnostic pathology practice [82].

Conclusion

Automated body fluid analysis demonstrates excellent correlation with manual microscopy for total and differential cell counts. Automated hematology analyzers provide rapid, accurate, and reproducible results and significantly improve laboratory efficiency. However, manual cytological examination remains essential for detection of atypical and malignant cells.

Combined use of automated analyzers and conventional microscopy provides optimal diagnostic accuracy and clinicopathological correlation in body fluid analysis.

Ethical Approval: The present study was conducted after obtaining approval from the Institutional Ethics Committee of Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut, Uttar Pradesh. The study protocol complied with institutional ethical standards and the Declaration of Helsinki guidelines for biomedical research involving human participants.

Informed Consent: Written informed consent was obtained from all patients prior to sample collection and inclusion in the study.

Conflict of Interest: The authors declare that there is no conflict of interest regarding publication of this study.

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