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Original Research Article

Cytological and microbiological profiling of body fluids: a study from Acharya Harihar Post Graduate Institute of Cancer, Cuttack, Odisha, India

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ABSTRACT

Background: Body fluids play a crucial role in the human body, serving as the medium for biochemical reactions essential to cellular metabolism. They include intestinal fluids, saliva, tears, and gastric juice. The concentration of substrates in cellular fluids significantly influences the rate of metabolic reactions. Body fluids are typically found in the central nervous system (CNS), pericardial, pleural, peritoneal, and synovial spaces, comprising up to 60% water.
Methods: The present study was conducted at Acharya Harihar Post Graduate Institute of Cancer (AHPGIC), Cuttack, over one month. A total of 25 fluid samples were collected from the Department of Pathology, AHPGIC. Pleural fluid was obtained through thoracocentesis, while peritoneal and ascitic fluids were collected via paracentesis. The fluids were drained using a syringe and aliquoted into tubes containing EDTA for cell counts and heparin for further studies. The collected samples were then transported in sterile containers and stored under refrigeration for further examination. For microbiological analysis, the collected fluids were inoculated in different culture media to facilitate the growth of microorganisms.
Results: Among the 25 fluid samples analyzed, malignant cells were identified in pleural and ascitic fluids. However, Gram-positive and Gram-negative bacteria, as well as acid-fast bacteria, were not observed in any of the samples through direct staining.
Conclusions: In culture, the growth of Gram-positive cocci was detected in peritoneal fluid, while Gram-negative bacilli were found in ascitic fluid.

Keywords: Cytology, Body fluid, Microbiology

INTRODUCTION

Body fluids play a vital role in physiological processes and are composed of 50-60% water, distributed across intracellular and extracellular compartments. These fluids are not derived from blood or urine and are considered nonstandard body fluids (BFs). They are found in various anatomical locations, such as cerebrospinal fluid (CSF) in the central nervous system, pericardial fluid around the heart, pleural fluid surrounding the lungs, peritoneal fluid encasing the abdominal organs, and synovial fluid within joints like the elbow and knee. An increased quantity or abnormal presence of body fluids often indicates an underlying disease process. The various body cavities are sterile sites, and these body cavities are filled with sterile body fluids. The purpose of these body fluids is to bath

the organs and membranes for the protection of vital organs, transportation of nutrients, regulation of body temperature, and reducing friction.[1,2] CSF, serous fluids (SEF), and synovial fluid (SYF) are the three main categories of bodily fluids. Pleural fluid (PLF), peritoneal fluid (PNF), and pericardial fluid (PCF) are examples of serous fluids. Other fluids with clinical significance include bronchoalveolar lavage (BAL) and peritoneal dialysate.

The laboratory evaluation of body fluids includes gross examination, biochemical analysis, microbiological testing, cell count, differential cell count, and cytological examination. In this study, two primary tests were conducted: microbiological and cytological analysis, with a focus on commonly received fluids such as pleural, peritoneal, and ascitic fluids. These fluids act as natural lubricants, facilitating the movement of organs and reducing friction. Pleural fluid, present in the pleural space between the lung and chest wall membranes, serves as a lubricant that enables smooth lung expansion during breathing.[3] Under normal conditions, mesothelial cells produce approximately 15 ml of pleural fluid daily. However, excessive accumulation, known as pleural effusion, may occur due to increased fluid production or impaired absorption. Pleural effusions are classified into transudates and exudates. Transudative effusions result from increased hydrostatic pressure, as seen in congestive heart failure, or decreased oncotic pressure associated with hypoproteinemia in renal disease. In contrast, exudative effusions arise from localized inflammation, infections (pleuritis, pneumonia), pulmonary infarction, or malignancies that damage the pleural lining, disrupting the filtration process.[4]

Microbiological examination of sterile body fluids is essential for detecting bacterial infections caused by pathogens such as Pseudomonas spp. and Acinetobacter spp.. Microbiological testing helps identify infectious agents, including bacteria, fungi, parasites, and viruses, which contribute to inflammatory and infectious diseases. Since body fluids are typically sterile, their analysis provides valuable diagnostic insights by bypassing the normal bacterial flora of the body surfaces. Therefore, complete microscopic and culture-based investigations should be performed on these specimens to detect viral, bacterial, mycobacterial, fungal, and parasitic infections. Although the bodily fluids are normally sterile, many bacteria can infiltrate the internal cavities, resulting in infection and physiochemical alterations. Clinicians can start early and focused antimicrobial therapy by identifying the organisms causing sterile bodily fluid infections early on and determining their susceptibility to antibiotics.[5]

Cytological analysis of body fluids is a relatively non-invasive technique that aids in identifying both malignant and non-malignant causes of effusions. It especially contributes to cancer research and staging of various tumors.[6] It plays a crucial role in cancer research, particularly in tumor staging and diagnosis. Cytology is an essential diagnostic tool for detecting malignant, infectious, inflammatory, and benign conditions. However, cytomorphological similarities between certain pathologies often pose diagnostic challenges.[7] Despite these challenges, cytological examination remains an invaluable method for differentiating between neoplastic and non-neoplastic conditions, including malignancies, inflammatory diseases, and infections caused by bacteria, fungi, and parasites.[8] It is a simple, rapid, cost-effective, and minimally invasive diagnostic approach with high accuracy and a low incidence of false-positive results.[9]

The cytological interpretation of individual cells that are exfoliated into these fluids is of paramount importance since they provide an insight into the diagnostic, prognostic and therapeutic aspect of various pathological processes in the body.[10] A high sensitivity and specificity of a cytological diagnosis of body fluids is presumably because the cell population present in the fluid sediment provides a more representative sample of a much larger surface area than that obtained by needle biopsy.[11,12] In a tertiary care hospital, the current study aims to assess the value of fluid cytology for a variety of clinical conditions, including cancer. A comprehensive diagnostic method is the cytological analysis of bodily fluids. First, it helps the clinician create a list of differential diagnoses and identify the aetiology of effusion. Secondly, it makes it possible to track the outcome of treatment and prognosis.[13]

Body fluids, including ascitic, pleural, synovial, cerebrospinal, and hydrocele fluid, are frequently submitted for microbiological culture in cases of suspected infections. These infections contribute significantly to morbidity and mortality. However, positive culture rates remain low due to the limited presence of pathogens and prior empirical antibiotic administration.[14] Early detection and rapid identification of microorganisms are crucial for timely and effective patient management.[15] Prompt diagnosis enables clinicians to initiate targeted therapy, reducing hospital stays and minimizing adverse effects.[16]

Periodically assessing regional microbial profiles and antibiotic susceptibility patterns is crucial because localised bacteriological data are crucial for optimising antimicrobial therapy. To guarantee that treatment decisions are supported by evidence, the results should be adequately conveyed to doctors.

Thus, the objective of the current study is to examine the aerobic bacteriological profile and antibiogram of bodily fluids. The selection of suitable empirical antibiotics can be aided by knowledge of frequently isolated organisms from a variety of sterile bodily fluids and their antimicrobial susceptibility testing (AST) pattern, which is displayed as an antibiogram. Infections with sterile bodily fluids have a relatively modest culture-positive rate, ranging from 10% to 30%.[17]

METHODS

Place of collection of samples

The samples were collected from Department of Pathology of Acharya Harihar Post Graduate Institute Of Cancer, Cuttack, which is a well-known cancer research centre of Odisha. The study was conducted for the period of one month from 5th February to 5th March 2024.

Sample collection

The process known as thoracocentesis was used to remove the pleural fluid from the body. An inner catheter is carefully advanced to prevent lung punctures, and an 18-gauge IV needle is placed into the pleural area above the ribs. The needle is then removed. A syringe and aliquot are used to drain or collect the fluid, which is then transferred into tubes with heparin for further analysis and EDTA for cell counts. Analysing peritoneal fluid is also frequently used to confirm or rule out bacterial peritonitis and suspected neoplastic illness. Paracentesis is the term for the process of draining fluid from the peritoneal cavity. For diagnostic purposes, a catheter or needle is placed into the peritoneal cavity.

Sample transportation

After collection of samples, the fluid was transported through a sterile bottle and keep it under refrigerator for further examination.

Identification of sample

Differentiating between transudates and exudates usually requires a thorough examination of the fluid for colour and turbidity. Typically, serous fluid is clear and pale yellow or straw in colour. Cloudy, turbid fluids indicate illness; bloody fluids indicate a traumatic tap; chest trauma or cancer; and a milky appearance indicates a chylous effusion, which is a chyle-contaminated pleural fluid. Similar to this, the peritoneal fluid's gross appearance can reveal details about its likely cause. Peritonitis frequently manifests as cloudy and purulent fluids; gallbladder or small intestine damage may be indicated by a green hue; trauma is suggested by severely hemorrhagic fluids; and lymphatic vascular leakage, possibly due to malignancy, is suggested by milky (chylous) fluids.

Cytological test of fluid

Pap smear test

The Pap smear test was performed by using Pap stain with different percentage of alcohol.

Microbiological profile

Microbiological profile includes Gram stain, acid-fast staining, and culture growth

Staining of body fluid

First centrifuged the body fluid then the supernatant part was discarded, and the palate then the sediment smear were made. Fix the smear by heat otherwise fix that by ethanol. After fix the smear sediment smear wear stained by gram stain and acid fast stain or ZN stain.

RESULTS

Different kinds of analysis were conducted on 25 number of fluid samples of different cancer patients.

Table 1: Cytological analysis of different fluids.

Type of fluid	Total fluid (25)	Malignant cell seen	Malignant cell not seen
Pleural	05	01	04
Peritoneal	06	00	06
Ascitic	14	03	11

Table 2: Pap stain results.

Types of fluid	Pus cell	Mesothelial cell
Plural	+++	+
Peritoneal	++	+
Ascitic	++	+

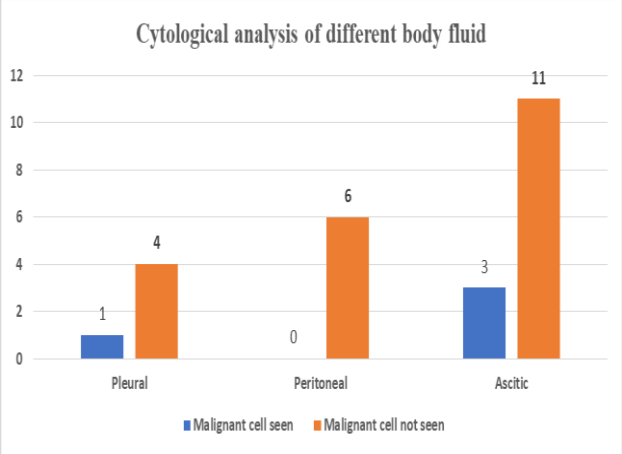

Figure 1: Cytological analysis of different body fluids.

Table 3: Gram staining results in different fluids.

Types of fluids	Gram positive Organism	Gram negative organism
Pleural	Nil	Nil
Peritoneal	Nil	Nil
Ascitic	Nil	Nil

Cytological analysis

In the present study the cytological analysis of different fluid shows. Out of 25 fluids the pleural fluid shows (1 number) the malignant case and ascetic fluid shows (3 numbers) malignant case shown in (Table 1). Pleural fluid (4 numbers) peritoneal fluid (6 numbers) of ascitic fluid (11 numbers) not showing any malignant case (Table 1 and Figure 1).

Table 4: Acid fast staining results in different fluids.

Types of fluids	Acid fast bacteria
Plural fluid	Nil
Peritoneal fluid	Nil
Ascitic fluid	Nil

Table 5: The culture characteristics of different fluid found in Mac-Conkey Agar and blood agar medium.

Types of fluid	Culture growth
Plural fluid	No bacteria growth after 48 hours
Peritoneal fluid	*Staphylococcus aureus*
Ascitic fluid	*Acinetobacter spp.*

Pap smear analysis

In pap smear stain experiment, in plural fluid the more in number (+++) and peritoneal and ascetic fluid less in number (++) and mesothelial cell are very less number show in Table 2.

Gram stain

In gram staining plural, peritoneal, ascetic fluid the gram-positive and gram negative organisms are not seen in every staining of the fluid.

Acid fast stain

In acid fast staining plural, peritoneal, ascetic fluid, the acid fast organisms are not seen in every staining of fluid.

Cultural analysis

In cultural characteristics of different fluids found in Mac-Conkey agar were examined. The pleural fluid shows no bacteria but at the same time peritoneal fluid shows gram-positive *cocci,* and the ascitic fluid shows *Acenetobacter* in culture plates.

DISCUSSION

Main bodily fluids, such as peritoneal, pericardial, pleural, CSF, and synovial fluid, are often found in trace amounts within their respective body cavities together with their constituents in particular ratios. Both qualitative and quantitative alterations occur in these fluids during a disease process.[18] It is assumed that a cytological diagnosis of bodily fluids will have a high sensitivity and specificity since the cell population in the fluid sediment offers a more representative sample over a much greater area. When determining if malignant metastatic tumours to the cavities are present or not, cytological analysis of bodily fluid is quite valuable. This technique is helpful in identifying malignant cells that have spread to other bodily cavities because mesothelial and synovial tumours are uncommon. The technique is more useful for predicting outcomes than for detecting tumours early or stopping their growth.[19,20]

These fluids' cytological analysis aids in the diagnosis of both neoplastic and non-neoplastic diseases. Malignant cell identification through pathology and cytological investigation is crucial.

This study has several limitations that must be acknowledged. Firstly, the small sample size (n=25) limits the generalizability of the findings. A larger sample could provide a more comprehensive understanding of the cytological and microbiological profiles of body fluids in cancer patients.[21] Secondly, the study did not incorporate radiological or histopathological correlation with the cytological findings. These additional modalities could enhance diagnostic accuracy and help confirm the presence of malignancy, especially in borderline or suspicious cases.[22] Thirdly, the analysis was limited to conventional cytology techniques, such as Pap smear and gram staining, without employing advanced methods like immunocytochemistry, molecular diagnostics, or cell block techniques. These advanced tools have been shown to significantly improve the diagnostic yield, particularly in identifying malignancies and microbial pathogens.[23,24] Moreover, clinical and demographic data such as age, cancer type, stage, treatment history, or comorbidities were not included in the analysis. These factors may influence the composition and diagnostic yield of body fluid analysis and should be considered in future research.[25]

CONCLUSION

Ascitic fluid was the most often received fluid for analysis, according to the study's findings. Ascitic fluid contains the highest number of cancerous cells. There were more pus cells and some mesothelial cells in the fluid's pap stain. Gram staining does not reveal Gram-positive or Gram-negative bacteria, and acid fast staining does not reveal fluid containing acid-fast bacteria. *Staphylococci* and *Acinetobacter* were the only two species discovered using the fluid culture approach. Fluid cytology is a valuable diagnostic tool that can be used as a first-line diagnostic method since it is easy to use, reasonably painless, economical, time-efficient, and produces results quickly. A helpful supplementary diagnostic method for classifying both benign and malignant disorders is fluid cytology. For proper management, early detection and quick identification of

bacteria are essential, and microbiological analysis is very beneficial.

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