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Cyto-Diagnosis of Ascitic Fluid in Ovarian Tumor: A Combined Approach in Routinely Stained Cytology Smears and Modified Cell Block Technique with Histopathological Correlation

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Abstract

Background: The importance of cytological examination of ascitic fluid is widely recognized and well documented. It helps for staging and prognosis of the disease in malignancy and also gives information about inflammatory lesions. Problem in diagnosis arise in every day practice for the differentiation of reactive mesothelial cells and malignant cells by conventional smears (CS) method. Cell block (CB) method provides better architectural patterns, morphological features and helps to differentiate reactive mesothelial cells and malignant cells and thereby increases the efficacy of cytodiagnosis.

Objectives - To compare the accuracy of either conventional smear or cell block technique of ascitic fluid or combination of each in relation to histologic type for the diagnosis of ovarian tumors.

Methods – Asciticfluid will be subjected for cytological evaluation by CS and CB techniques. Gross examination of ascitic fluid will be done for cell count and cell type.
Conventional Smear Technique: About half of ascitic fluid sample received will be centrifuged at 2500 rpm for 15 minutes and a minimum of 2 smears were prepared from the sediment and stained with routine hematoxylin and eosin stain, Leishman stain and Papanicolaou stain.

Cellblock technique: Remaining fluid will be immediately fixed in 10% alcohol-formalin in

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1:1 proportion for 1 hour and centrifuged at 2500 rpm for 15 minutes. 3 ml of 10% alcohol-formalin will be added and kept for 24 hours. Cell button will be processed to prepare H and E stained sections. Special stains will be done wherever required.

Results -The observations will be made pertaining to the objectives and will be tabulated. These observations will be subjected to statistical tests for its significance and conclusions.

Conclusion - Conclusion will be drawn from the results obtained from the study.

INTRODUCTION:

Accumulation of large volumes of fluid in abdominal cavity is ascites.[1] Several litres of peritoneal fluid are produced regularly under normal conditions, and it is not collected but consumed effectively. Ascites is most common symptom of ovarian carcinoma which presents at advanced stage and associated with poor prognosis. Ascites can be exudative and transudative. Ninety percent of ascitic fluids are made up of transudates and they are caused by disorders of non-malignant aetiology. This fluid, with a limited number of cells and a low albumin content, is transparent. Typically, an exudate is malignant, cloudy, with a larger number of cells and a higher protein content than a transudate. [1]

It is suspected that the pathogenesis of malignant ascites is multifactorial and that increased vascular permeability, lymphatic drainage blocking, increased hydrostatic pressure differential and decreased oncotic pressure difference are the most important pathogenetic mechanisms. Ascites is the most frequent complaint in ovarian carcinoma patients. Ascites was the first detectable symptom of malignancy for 54 percent of patients with peritoneal carcinomatosis. Mortality rate of ovarian cancer is 75%.[2]

One of the most dangerous malignancies in women's reproductive system cancers is primary ovarian carcinoma (POC). This tumour also produces no noticeable symptoms in the early stages, unlike other gynecologic cancers such as cervical, endometrial, and vulvar cancer. However, when the tumour enlarges, pelvic bulk and/or fullness is a significant symptom in women with illness. Clinically, ovarian carcinoma can readily spread into the peritoneal cavity, illustrating the fact that the ovary is an intra-abdominal organ. Therefore, the bulk of this tumour is level III or higher at initial diagnosis. As a consequence, for most patients, curative and complete surgical resection is not an option.[3]

- Histogenesis of ovarian cancer: [3]

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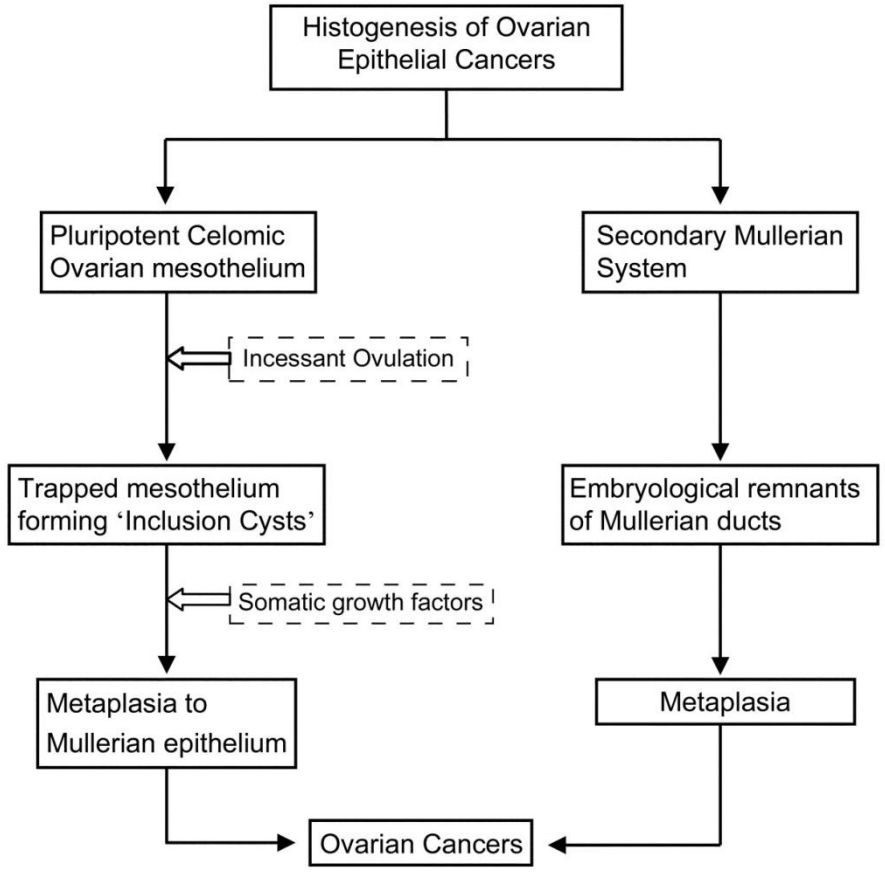

- Themalignant or reactive mesothelial cells identification by conventional cytology smear is a diagnostic problem.[4]
- It has been observed that the cellularity and morphology is better appreciated with cell block technique and it is also more sensitive than conventional cytology smear.[4]
- Cell block technique along with routine cytology smear method is considered more useful in evaluation of ascitic fluid for the diagnosis of ovarian cancer.[5]
- Sometimes the tumors arise from non-ovarian organs as the metastatic lesions and most common site of metastasis is ovary for many cancers.[5]
- Hence histological findings in such cases is helpful to distinguish between ovarian cancer and other metastatic lesions.[5]
- Conventional smears limitations include: [6]
    1. Morphological indistinct details
    2. Cells overlapping or overcrowding
    3. Abundance of inflammatory cells
    4. Cell changes/ losses
- Cell-block technique overcomes these limitations by providing better architecture and morphological details.
- Advantages of cell block
    1. Cellular material can be concentrated in a small area that can be evaluated at once in microscope.
    2. Architectural pattern is preservedlike papillae, clusters and cell balls.

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3. Intact cell membranes and crisp chromatin details.
4. Cytology and histology gap is bridged by this technique.
5. There is adequate details on cellularity, nucleus and cytoplasm.
6. Loose cells, cell aggregates and microscopic tissue fragments are easily recoverable.
7. IHCand histochemical stains can be done in cell- block sections.
8. Now a days, many laboratories are readily adapting this method since it is simple and reproducible. [7]

Maseki et al[5] in their study concluded that the histologic type based on subsequent biopsy, surgery and autopsy was accurately diagnosed with cell block technique and was consistent with clinical diagnosis and final pathologic diagnosis. A previous study[8] observed that 20% suspicious malignant case which was detected by conventional smear was confirmed by cell block technique. Sensitivity and specificity of cell block technique was concluded 88.88% and for conventional smear method that was 86.98%. Hence malignant lesion detection was significantly better with cell block technique. Another study[4] conducted on 44 samples of peritoneal fluid over a period of 20 months in the age group 21 to 80 years, 51 to 60 most commonly affected. In the conventional smear method and cell block method, findings such as cellularity, morphology, cytoplasmic and nuclear details were observed and all this findings were better appreciated by cell block technique. The detection of malignancy was 13.63% more with cell block technique. Another research[1]reported that the sensitivity and specificity of the cytological test was 90% and 96.5% respectively. Hence they concluded that peritoneal cytology of ovarian tumors is highly specific and sensitive for the detection of ovarian malignancies.

Due to scarce literature in Indian population, the present study will be conducted to assess combined approach in routinely stained cytology smears and modified cell block technique with histopathological correlation in ovarian tumor.

MATERIAL AND METHODS:

The following material and methods will be adopted for present study:-

1. **Recording of preliminary data in proforma with following details:-**
   - I. Name
   - II. Age
   - III. Gender
   - IV. Ward
   - V. OPD
   - VI. Unit incharge
   - VII. MRD
   - VIII. Complains
   - IX. Comorbid conditions

   Place of study- Department of Pathology JNMC, sawangimeghe, wardha.
2. **Duration of study** – Two years
3. **Study Design** – Prospective study design.
4. **Sample size** – Sample size formula with desired error of margin

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$n=\frac{Z\alpha/2^2\ p\ (1-p)}{d^2}$

Where,

$Z\alpha/2$ is the level of Significance at 5% i.e. 95%

Confidence interval = 196

p = Prevalence of ovarian cancer in Wardha district = 4.4%[9] = 0.044

d = Desired error of margin is = 6% = 0.06

$n = \frac{1.96^2 x 0.044 x (1-0.044)}{0.062}$

= 44.88

= 45 patients needed in the study

5. Techniques- Sample processing

Conventional cytology- cytocentrifugation of sample followed by giemsa, pap and H&E stain.

Cell block preparation-Thromboplastin-plasma method

This method is useful when the quantity of the sediment obtained is scant

Equipment and reagents

1. 10% buffered formalin
2. Whatman's No, 1 filter paper/lens paper
3. Tissue cassette
4. Applicator sticks
5. Screw capped disposable plastic transparent centrifuge tube
6. Pasteur pipette
7. Thromboplastin
8. Pooled plasma

Procedure

- Centrifuge the sample
- Discard the supernatant
- Add to the sediment pooled plasma (2 drops) and mix well
- Add thromboplastin (4 drops) and mix again
- Allow to settle for five minutes and to form the clot
- The clot in the test tube is slid onto the filter paper pre- moistened with formalin fixative
- Wrap the sediment securely in the filter paper and place it into a labelled tissue cassette
- Put the cassette into a jar containing fixative atleastfor 4 hours
- Process as tissue biopsy by conventional histopathological technique
- Section of cell block obtained at 5 micron
- Stain by H&E stain

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Histopathological correlation

Those samples which will be operated in our hospital and available for histopathological examination will be taken for histopathological correlation.

Subject characteristics- Described below for inclusion and exclusion

Inclusion criteria:

- All females of all age group admitted in JNMC during thesis period with ovarian tumor and ascites as suspected by clinical examination and ultrasonography.

Exclusion criteria:

- All ovarian conditions with non-neoplastic ovarian pathology.

Investigations: As fluid is tapped using all aseptic precautions with patient's consent under radiological guidance.

Conventional smear prepared and stained with - with remaining fluid cell- block were prepared by technique-

And histopathological correlation is done in those cases which were operated in our hospital.

Statistics:

Statistical Tools: The correlation will be carried out by statistical tests along with values of significance compatible to said objectives. (p-value), Fischer exact test, Univariate comparisons.

Consent: The investigations over the blood sample in AVBRH are carried out by informal consent. The investigations specified in this work do not involve infringement and harm to human subjects participating as a patients in present study.

Ethics: Study doesn't involve major or minor issues offending to human subjects.

RESULTS:

In our analysis, we expect that the combined approach of routinely stained cytology smears and modified cell block technique of ascitic fluid would be a highly precise and responsive test for malignant ovarian tumours, especially in the advanced stages of malignancy that are present in our hospital for most of our patients.

DISCUSSION:

There has always been the scientific curiosity for the cell block studies. Over a period of time the cell block studies became popular diagnostic modality. It was established because of its advantages as an alternative or auxiliary diagnostic method in cytopathology laboratory. Several advantages and its ability to provide the cytoarchitect and cell detail at microscopy made it to be use in fluid cytology assessment especially in the situations of equivocal presence of malignant cells.Due to low symptomatology and lack of screening, more cases are diagnosed in later stage, presented with ascites. Hence, the cytology of ascitic fluid is very useful in the diagnosis of ovarian cancer.

In context of the Objectives selected for the study, review of literature has been brought out in the following paragraphs in context to cell block studies of ascitic fluid in ovarian

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carcinoma, distinguishing the conditions of overlapping cytomorphology of reactive atypical benign mesothelial cells and malignant cells.

Dey at al[8] did a study on use of cell block technique and compared it with conventional smear technique for the detection of malignancy in serious fluid. It was one year institutional based study carried on over 50 patients, most commonly between age group of 24-82 years, maximum patients belonged between 61 to 70 years of age. It was observed that 20% suspicious malignant case which was detected by conventional smear was confirmed by cell block technique. Sensitivity and specificity of cell block technique was concluded 88.88% and for conventional smear method that was 86.98%. Hence malignant lesion detection was significantly better with cell block technique.

Udasimath et al[4] did a study on diagnosis of malignant ascetic fluid effusion and role of cell block technique. Study was done on 44 samples of peritoneal fluid over a period of 20 months in the age group 21 to 80 years, 51 to 60 most commonly affected. In the conventional smear method and cell block method, findings such as cellularity, morphology, cytoplasmic and nuclear details were observed and all this findings were better appreciated by cell block technique. The detection of malignancy was 13.63% more with cell block technique.

Atla et al[1] did a cytological study on ovarian tumors of ascitic fluid for a period of 6 months in the year 2017. The obtained 106 samples from the patients of ovarian tumors presented with ascites in which they included 88 benign ovarian tumors (83%), most common amongst 21 to 40 years and 18 malignant tumors (17%). The purpose of the study was to test the accuracy of ascitic fluid cytology in the diagnosis of malignancy in ovarian tumors and to determine the accuracy of peritoneal cytology in relation to the histopathological type of ovarian tumors. The sensitivity and specificity of the cytological test was 90% and 96.5% respectively. Hence they concluded that peritoneal cytology of ovarian tumors is highly specific and sensitive for the detection of ovarian malignancies.

Maseki et al[5]did a study on cell block technique; weather it is useful in the diagnosis of histological type of ovarian tumor or not. The study was done between 2008 and 2017 where 15 peritoneal fluid samples were taken and cell blocks were made after conventional cytological smear. The study concluded that the histologic type based on subsequent biopsy, surgery and autopsy was accurately diagnosed with cell block technique and was consistent with clinical diagnosis and final pathologic diagnosis.

Kulkarni et al[10] in 2009, done study to assess the cell block feasibility of thromboplastin method and concluded that, in cell block method cellular material were concentrated in one field and architectural pattern were well observed.
Thapar et al[11] in 2010, concluded that cell block technique not only increases positive results of malignancy, but also showed better architectural pattern and it helped in diagnosis of primary site.

Studies on different cytodiagnosis techniques were reported [12-15]. Laishram et. al. assessed the utility of proliferative marker Ki-67 in surface epithelial ovarian tumor [16]. Few of the related studies were reviewed [17,18].

CONCLUSION:

In our analysis, we would conclude that the combined approach of routinely stained cytology smears and modified cell block technique of ascitic fluid would be a highly precise and responsive test for malignant ovarian tumours, especially in the advanced stages of malignancy that are present in our hospital for most of our patients. It significantly helps to support the diagnosis, to estimate the tumor's prognosis and risk of recurrence, which in turn helps patients to be better handled and treated.

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