INTRODUCTION

Serous epithelial tumors of ovaries are the most common among epithelial tumors of ovary. These can be divided into benign, borderline, and malignant categories. Borderline serous tumors (BSTs) constitute 10–15% of the total serous tumors.[1] The presence of peritoneal or omental implants predicts a poorer prognosis. Peritoneal fluid cytology is done as a part of staging in cases with ovarian neoplasms and has been shown to predict omental involvement in cases with serous adenocarcinoma of ovary.[2–3] However, it may be difficult to identify tumor cells in peritoneal fluids in patients with ovarian BST. Moreover, misdiagnosing these tumor cells as metastatic carcinomas in peritoneal fluid will upstage these tumors and result in more radical treatment.[4–5] The morphologic features of BST in pelvic washing cytology using ThinPrep® have been described in an occasional study.[6] We study here the morphologic features of BST in ascitic fluid in cases with positive as well as negative ascitic fluid cytology. Cases of serous adenocarcinomas were
also included to compare the morphological features. The advantage of liquid-based cytology (LBC) using SurePath technique™ over conventional smears is also seen.

MATERIAL AND METHODS

We retrieved cases of ovarian BST who also had ascitic fluid cytology examination in the past 3 years (2013–2015). Out of total 30 cases of BST diagnosed on histopathology, five cases had ascitic fluid cytology reported positive for tumor cells or suspicious for neoplasm (BST+). Of these, four samples were of ascitic fluid sent preoperatively for cytological examination and one was peritoneal washing sent intraoperatively. All these five cases were included in the study. The cases in which ascitic fluid cytology had been reported as negative for malignant cells were also reviewed (BST-). The study was done by two cytopathologists (SS and PD) independently without prior knowledge of the status of omental involvement. Out of 25 BST- cases, two cases were found to be positive for neoplasm and the diagnosis was revised. In all the cases, smears had been prepared using LBC with SurePath™ technique stained with Papanicolaou (PAP) stain along with one May-Grunwald-Giemsa (MGG) stained conventional smear for each case. We also included seven cases of histologically proven serous adenocarcinoma with positive ascitic fluid cytology for comparison.

The following information were noted in each case: Age, site of tumor, diagnosis in histopathology, ascitic fluid, and omental involvement. Both the architectural and nuclear features of the cells were studied in each case. The architectural features included cellularity (more than 10 or <10 clusters), papillary fragments with and without fibrovascular cores, papillary fragment borders (smooth or irregular), three-dimensional clusters (3D clusters: Cohesive clusters with nuclear overlapping and crowding), cluster size (> or <5 cells per group), presence of single atypical cells, presence of psammoma bodies, intraepithelial inflammatory cells in the form of neutrophils or lymphocytes, and background (clean, bloody, or necrotic). The nuclear features included in the study were nuclear size (>2× or <2× the size of a neutrophil), nuclear pleomorphism (mild 2–3×, moderate 3–4×, or severe >4× size of neutrophils), nuclear membrane contour (regular or irregular), nuclear chromatin (fine or coarse), nucleoli (absent, small, or macronucleoli), presence or absence of cytoplasmic vacuolization, and intercellular windows. The morphological features were studied in both MGG and LBC smears (PAP’s stain). The histopathology findings in omentum were also recorded for each case.

RESULTS

The details of the cases included in the study are shown in Table 1. On histopathology, bilateral tumors were more commonly seen in the BST + and serous carcinoma group, whereas, in the BST- group, most cases had unilateral tumor. On histopathologic examination, omentum was reported free of tumor in four cases, two cases had non-invasive implants, and in one case, omentum was not received. Among the BST- group, one case had both invasive and non-invasive implants, and in all other cases (six cases), omentum was free of tumor. Out of seven cases of serous carcinomas with positive ascitic fluid cytology, six cases were high-grade serous carcinoma of ovary and one case was low-grade serous carcinoma. Six cases had metastatic carcinoma in omentum, while there was no omental metastasis in one case although parastomum was involved.

The cytoarchitectural and nuclear features studied are shown in Tables 2 and 3. All BST- cases showed low overall cellularity, with absent papillary fragments and 3D clusters. Monolayered sheets of mesothelial cells were seen in all cases. None of
the cases showed psammoma bodies or intraepithelial inflammatory cells. The nuclear size was less than 2 times the size of neutrophil, with mild to minimal pleomorphism, regular nuclear membrane, fine chromatin, absent, or small nucleoli. The background was clean in all LBC smears and bloody in conventional MGG stained smears [Figure 1].

Although it was difficult to differentiate between BST+ and serous carcinoma in ascitic fluid, there were few distinguishing features such as papillary fragment borders, nuclear pleomorphism, nuclear chromatin, and nucleoli. Most cases with BST+ had regular papillary fragment borders with nuclei showing mild-to-moderate pleomorphism, fine nuclear chromatin with small nucleoli [Figure 2a] as compared with serous carcinomas all of which had irregular borders with moderate-to-severe nuclear pleomorphism, coarse chromatin, and macronucleoli [Figure 2b]. Psammoma bodies were more commonly found in BST+ group, while occasional psammoma bodies were seen in one case with low-grade serous carcinoma of ovary [Figure 2a and b]. There was no difference between the two groups based on cellularity, papillary fragments with and without fibrovascular cores, 3D clusters, or size of clusters. Intraepithelial inflammatory cells and scattered atypical cells were seen in both BST + and serous carcinomas [Figure 3]. Both groups with BST+ and serous carcinomas showed nuclear size >2 times the size of neutrophil, irregular nuclear membrane contours, and cytoplasmic vacuoles.

3-D clusters and nuclear details were better appreciated in LBC smears as compared with conventional MGG smears in all cases. In addition, LBC smears provided a clean background, thus increasing the detection of tumor clusters [Figure 1 compares conventional and LBC smears in a case of BST-, BST+, and serous carcinoma. One case in which the diagnosis was revised showed 3-D papillary clusters in LBC smears. However, MGG stain showed only mesothelial cell clusters.

**DISCUSSION**

BST is defined by the World Health Organization as serous neoplasms that show epithelial proliferation greater than

| Architectural features | BST- (n=7) | BST+ (n=7) | Serous carcinomas (n=7) |
|------------------------|-----------|-----------|------------------------|
|                        | LBC       | Giemsa    | LBC       | Giemsa    | LBC       | Giemsa    |
| Cellularity            |           |           |           |           |           |           |
| >10 clusters           | 2         | 1         | 5         | 3         | 6         | 6         |
| <10 clusters           | 5         | 6         | 2         | 4         | 1         | 1         |
| Papillary fragments    |           |           |           |           |           |           |
| With FV core           | 0         | 0         | 6         | 3         | 6         | 7         |
| Without FV core        | 0         | 0         | 7         | 7         | 7         | 7         |
| Papillary fragment borders |         |           |           |           |           |           |
| Regular                | 0         | 0         | 5         | 5         | 0         | 0         |
| Irregular              | 0         | 0         | 2         | 2         | 7         | 7         |
| 3D clusters            |           |           |           |           |           |           |
| Present                | 0         | 0         | 7         | 4         | 7         | 7         |
| Absent                 | 7         | 7         | 0         | 3         | 0         | 0         |
| Size of clusters       |           |           |           |           |           |           |
| >5 cells               | 7         | 7         | 7         | 7         | 7         | 7         |
| <5 cells               | 0         | 0         | 0         | 0         | 0         | 0         |
| Single atypical cells  |           |           |           |           |           |           |
| Present                | 0         | 0         | 2         | 2         | 7         | 7         |
| Absent                 | 7         | 7         | 5         | 5         | 0         | 0         |
| Psammoma bodies        |           |           |           |           |           |           |
| Present                | 0         | 0         | 4         | 3         | 0         | 1         |
| Absent                 | 7         | 7         | 3         | 4         | 7         | 6         |
| Intraepithelial inflammatory cells |           |           |           |           |           |           |
| Present                | 0         | 0         | 4         | 0         | 1         | 0         |
| Absent                 | 7         | 7         | 3         | 7         | 6         | 7         |
| Background             |           |           |           |           |           |           |
| Clean                  | 7         | 1         | 7         | 0         | 7         | 0         |
| Bloody                 | 0         | 6         | 0         | 7         | 0         | 7         |

BST+: Borderline serous tumors with positive ascitic fluid cytology, BST-: Borderline serous tumors with negative ascitic fluid cytology, LBC: Liquid-based cytology, FV: Fibrovascular, 3D: 3 dimensional
that seen in serous cystadenomas, as evidenced by cellular stratification, cytological atypia, and epithelial tufting, but which exhibit no evidence of “destructive stromal” invasion and can show extraovarian implants. It can occur at any age, but common in women of reproductive age. This tumor is bilateral in one-third of cases. Peritoneal wash (PW) cytology is routinely done as a staging procedure. Positive PW cytology increases the FIGO staging of ovarian carcinoma from IA/IB or IIA/IIIB to IC or IIC, respectively. Histopathologic examination of peritoneal surfaces and omental involvement has been considered the gold standard for staging of ovarian carcinomas. However, PW cytology is also fairly sensitive and highly specific to detect the presence of peritoneal implants. 

Metastatic carcinoma in ascitic fluids in case of ovarian serous carcinomas correlates with a poor prognosis, however, the same is not true in case of borderline ovarian tumors. Hence, there is a need to differentiate the type of tumor cells in peritoneal fluid specimens. In borderline ovarian tumors, the type of implants is important to predict the course of disease and to determine if additional therapy is required. Patients with invasive implants have a higher risk of relapse and mortality compared to those with non-invasive implants. Although it is not possible to determine the type of implants on ascitic fluid cytology, a combination of cytoarchitectural features may give a clue to distinguish BST implants from those of serous carcinoma. This is of more importance in ascitic fluid samples received for cytologic examination before surgery where the primary diagnosis is not available. The use of ThinPrep in diagnosis of BST in PW cytology has been described earlier. We describe the morphologic features of BST and compared with those of serous carcinomas, highlighting the advantage of LBC using SurePath technique over conventional smears. All cases in BST showed mesothelial cells, in a background of blood. LBC smears in all cases provided a clean background with better morphologic appreciation. These cells were in the form of monolayered sheets with nil to mild nuclear pleomorphism, fine nuclear chromatin, absent to occasional small nucleoli, regular nuclear margins, and intercellular windows. Papillary clusters, psammoma bodies, atypical cells, or intraepithelial inflammatory cells were not seen.

A combination of cytoarchitectural and nuclear features can help in suspecting BST in ascitic fluid. Papillary fragments with regular nuclear margins, 3-D clusters, nuclear size >2 times the size of neutrophil, mild nuclear pleomorphism, presence of nucleoli, cytoplasmic vacuoles, and absence of

| Table 3: Nuclear features studied in BST with positive and negative ascitic fluid cytology and comparison with those of serous carcinomas. |
|--------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Nuclear features                             | BST- (n=7) LBC | BST- (n=7) Giemsa | BST+ (n=7) LBC | BST+ (n=7) Giemsa | Serous carcinomas (n=7) LBC | Serous carcinomas (n=7) Giemsa |
| Nuclear size                                |                |                  |                |                  |                            |                            |
| >2 times the size of neutrophil              | 0              | 0                | 7              | 7                | 7                           | 7                           |
| <2 times the size of neutrophil              | 7              | 7                | 0              | 0                | 0                           | 0                           |
| Nuclear pleomorphism                         |                |                  |                |                  |                            |                            |
| Nil                                         | 5              | 5                | 0              | 0                | 0                           | 0                           |
| Mild                                        | 2              | 2                | 6              | 6                | 1                           | 1                           |
| Moderate                                    | 0              | 0                | 1              | 1                | 5                           | 5                           |
| Severe                                      | 0              | 0                | 0              | 0                | 1                           | 1                           |
| Nuclear membrane contours                    |                |                  |                |                  |                            |                            |
| Regular                                     | 7              | 7                | 2              | 2                | 0                           | 0                           |
| Irregular                                   | 0              | 0                | 5              | 5                | 7                           | 7                           |
| Nuclear chromatin                           |                |                  |                |                  |                            |                            |
| Fine                                        | 7              | 7                | 7              | 7                | 3                           | 3                           |
| Coarse                                      | 0              | 0                | 0              | 0                | 4                           | 4                           |
| Nucleoli                                    |                |                  |                |                  |                            |                            |
| Absent                                      | 3              | 3                | 0              | 2                | 0                           | 0                           |
| Small                                       | 4              | 4                | 7              | 5                | 2                           | 2                           |
| Macronucleoli                               | 0              | 0                | 0              | 0                | 5                           | 5                           |
| Cytoplasmic vacuoles                        |                |                  |                |                  |                            |                            |
| Present                                     | 0              | 0                | 5              | 5                | 7                           | 7                           |
| Absent                                      | 7              | 7                | 2              | 2                | 0                           | 0                           |
| Intercellular windows                       |                |                  |                |                  |                            |                            |
| Present                                     | 7              | 7                | 0              | 0                | 0                           | 0                           |
| Absent                                      | 0              | 0                | 7              | 7                | 7                           | 7                           |

BST+: Borderline serous tumors with positive ascitic fluid cytology, BST-: Borderline serous tumors with negative ascitic fluid cytology, LBC: Liquid-based cytology
intercellular windows should lead to suspicion of neoplasm. In our study, psammoma bodies were more commonly found in SBT. LBC smears provide a clean background and are useful in better appreciating the 3-D clusters, papillary fragments with fibrovascular cores, intraepithelial inflammatory cells, and nucleoli. Cases with serous carcinoma also showed 3-D papillary clusters but most of them had irregular nuclear margins, moderate-to-severe nuclear pleomorphism, coarse chromatin, and prominent nucleoli. Single atypical cells were seen in all cases and psammoma bodies were absent in all high-grade serous carcinomas. Occasional psammoma bodies were seen in one case of low-grade serous carcinoma. However, since most of the serous carcinomas included in our study were high-grade serous carcinomas in ascitic fluid need to be studied further.

All cases with positive ascitic fluid cytology were not associated with omental implants in our study and vice versa. In the BST- group, one case had invasive and non-invasive implants, however, ascitic fluid cytology showed no malignant cells. On the other hand, in the BST+ group, only two cases showed non-invasive omental implants. No omental implants were noted in three cases, and in one case, omentum was not received. Similarly, in the cases with serous carcinoma, one case showed no omental metastasis, however, parametrium was involved by tumor. The absence of omental implants can be attributed to inadequate tissue sampling. Thus, according to our study, ascitic fluid cytology is sensitive in detection of peritoneal implants and can be positive even if omental
metastasis is not confirmed histologically. If strict criteria are present, ascitic fluid cytology may help in distinguishing or at least suspecting a BST against serous carcinomas before a histopathologic diagnosis is made. In a study by Cheng et al.,[9] 23% of patients had positive pelvic washes in the absence of omental implants, hence suggesting that PW cytology is a sensitive indicator to detect peritoneal implants. In another study by Mulvany,[10] 5 of 8 (63%) of borderline tumors were upstaged based on positive PW cytology and were suggested to be superior than histologic sampling of peritoneum.

The detection of high-grade serous carcinomas of ovary in ascitic fluid smears is usually not difficult. However, BST is less frequently seen in ascitic fluid cytology, and knowledge of the cytomorphologic features of these cases is required to prevent overlooking them as reactive mesothelial cells or diagnosing them as adenocarcinoma. LBC smears using SurePath technique™ aid in better detection of tumor cells as these smears provide a clean background with better appreciable morphologic details. Moreover, there is even distribution and representative transfer of cells, thus, increasing the possibility of the detection of tumor cells. In our study, one case which had been previously reported as negative was given a revised diagnosis of, positive for neoplasm, based on LBC smears, whereas Giemsa smears showed mesothelial cells only. This emphasizes the importance of LBC using SurePath technique™ and the need to know the morphologic features in these smears.
CONCLUSION

The most important cytomorphologic features to distinguish BST from serous carcinoma are papillary fragment borders, nuclear pleomorphism, nuclear chromatin, and nucleoli. Psammoma bodies were more commonly found in BST. Although it may be difficult to categorize these cases as BST, a possibility may be considered and correlated with histopathology. Ascitic fluid cytology along with omental histopathology may increase the sensitivity to detect peritoneal implants. LBC further aids in increased detection and provides better morphologic details. However, more studies with follow-up of these cases are required to find if there is a difference in prognosis in SBT patients with positive ascitic fluid cytology compared with those with negative cytology.

COMPETING INTEREST STATEMENT BY ALL AUTHORS

The authors do not have any competing interest.

AUTHORSHIP STATEMENT BY ALL AUTHORS

Dr Sudha Sharma has collected data, analysed and drafted the paper.
Dr Mohapatra has helped in drafting the manuscript.
Dr Gupts helped in data collection.
Dr Radhiaka helped in data analysis.
Dr Rajwanshi helped in data analysis.
Dr Dey helped in data collection, analysis and drafting the manuscript.

ETHICS STATEMENT BY ALL AUTHORS

This is a retrospective study and consent was taken before the peritoneal aspirate in each case.

The study was approved by the Departmental Ethical committee. We followed the Helsinki ethical rule in this paper.

LIST OF ABBREVIATIONS (In alphabetic order)

BST - Borderline serous tumors
FIGO - Federation of Gynecology and Obstetrics
LBC - Liquid based cytology
MGG - May Grunwald Giemsa
PAP - Papanicolaou
PW - Peritoneal wash
SBT - Serous borderline tumour
3-D - Three dimension

EDITORIAL/PEER-REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double-blind model (authors are blinded for reviewers and vice versa) through automatic online system.

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