Original Article

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Pathology Section

Utility of Intra-operative Cytological Diagnosis in Cases of Ovarian Tumours

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ABSTRACT

Introduction: Intraoperative cytology of ovarian tumors is an easy, inexpensive and reliable method of investigation. Its accuracy is comparable with histological diagnosis.

Aim: To assess the overall accuracy of intraoperative cytological diagnosis of ovarian tumours, to compare with histological findings and to review the cases that showed disparity in intraoperative diagnosis with final histological diagnosis.

Materials and Methods: A total number of 78 cases of ovarian tumors were studied. The imprint smears were obtained intraoperatively. The ovarian tissues were subjected for histological examination.

Results: Out of 78 cases, 71 cases were neoplastic (31 cases were malignant, 3 cases are borderline tumors and 37 cases were benign in nature). The sensitivity and specificity were 94.87% and 100% respectively.

Conclusion: Imprint cytology is an alternative method to frozen section in the diagnosis of ovarian tumors particularly in a developing country like ours. This procedure has the similar advantages as frozen section by providing a rapid preliminary diagnosis which may guide the surgeons for their next step of management.

Keywords: Cytology, Frozen section, Germ cell origin, Neoplasm

INTRODUCTION

Ovarian neoplasm comprises of a heterogeneous group of tumours having epithelial, stromal and germ cell origin. Macroscopically all these tumours appear as solid-cystic, but histologically they are quite different. The management of the patients depends largely on the pathological diagnosis.

Pre-operative evaluation by sonography and CT-scan do not always predict the exact nature of the ovarian lesions. The fine needle aspiration diagnosis is difficult to establish because of the difficulty in procedure due to its location [1]. Sonographic guided FNA can be done, but the fear of spillage and the seeding of the tumour cells withdraws the pathologists to perform the risky procedure. But intraoperative pathological diagnosis is very helpful and guides the surgeon to plan the extent of surgery and for further management of the patient. Till date, frozen section and intraoperative cytology are the methods used for intraoperative pathological diagnosis.

Frozen section is the most widely used technique with an accuracy of 86–97% [2,3]. But the disadvantages are, it is an expensive and complex process, possibility of tissue loss, difficulty in recognition of borderline tumours and to distinguish between primary and metastatic ovarian tumours [4,5].

In 1927 Dudgeon and Patrick [6] introduced the intraoperative cytological interpretation. Since, then many reports are obtained regarding the accuracy of intaroperative cytological impressions in various organs [7,8]. Various techniques used to obtain the cytological samples during surgery are needle sampling, touch imprint, scraping and smearing the cut surface of the tumour on glass slides. The advantages of this procedure are, it is simple, inexpensive and rapid, and there is no tissue loss, well preserved cellular details. This procedure takes about 10 minutes. It helps the surgeons by providing the provisional diagnosis and the extent of surgery can be planned quickly.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Pathology, AHRCC, Cuttack, Odisha, India, for 3 years between the period of January 2013 – December 2015. We included 78 cases that are clinically suspected as ovarian tumours. The patients age ranged from 5 to 65 years. Detailed clinical history, laboratory data and radiological data were collected. The intraoperative findings were noted. Depending on the morphological consistency such as solid, solid – cystic or cystic nature, scraping and touch imprints were taken. In touch preparation, a clean glass slide was touched to the cut end of the specimen. For solid and harder in consistency tumours, the surface was scraped with a scalpel and the collected materials were smeared gently over 2-3 slides. Crush technique was used for necrotic and friable tissues. The samples were fixed in 95% ethyl alcohol and stained rapidly in H & E stain. The slides were dipped in Hematoxylin for 2-3 minutes and then rinsed with water followed by counter stain with Eosin by 3-4 slow dips. Then the slides were air dried, put in xylene and mounted

with DPX covered by a cover slip. The entire procedure took around 4 - 5 minutes. All the cytological findings were analysed and evaluated independently. The macroscopic examination of the tumours and cytological impression was immediately conveyed to the surgeons to reduce the time interval and operative delay. Rest specimens were sent for histopathological study. Histopathological impressions were also recorded. The data obtained was entered in Microsoft excel sheet and analysed using SPSS. Descriptive statistics were computed and presented as frequencies.

RESULTS

Out of 78 cases 71 (91%) cases were neoplastic. Among 71 neoplastic cases, 52.11% cases were benign and 43.6% were malignant [Table/Fig -1]. In scrape cytology smears, the cellularity was high as compared to other sample collection procedures.

Type of Tumour	No of Cases (%)
Benign	37 (52.11)
Borderline	03 (4.22)
Malignant	31 (43.66)

[Table/Fig-1]: Nature of tumour.

Serous tumours were observed as the most common ovarian tumour (37.1%) followed by mucinous tumours (24.3%) [Table/Fig-2]. Germ cell tumours (12.8%) were third in frequency.

Origin of the Tumour (%)	Type of Tumour	No of Cases (%)
Serous (37.17%)	Benign	08 (10.25)
	Borderline	01 (1.2)
	Malignant	20 (25.6)
Mucinous (24.35%)	Benign	12 (15.3)
	Borderline	02 (2.5)
	Malignant	05 (6.4)
Germ cell tumour (12.82%)	Teratoma-mature	02 (2.5)
	Teratoma – immature	02 (2.5)
	Teratoma- Struma ovarii	01 (1.2)
	Teratoma with carcinoma	02 (2.5)
	Dysgerminoma	03 (3.8)
Endometriod (1.28%)		01 (1.2)
Sexcord stromal tumor (7.69%)	Fibroma & thecoma	05 (6.4)
	Leydig cell tumour	01 (1.2)
Metastatic tumour (6.41%)	Krukenberg	05 (6.4)
Lymphoma (1.28%)	NHL	01 (1.2)
Others (8.97%)	Inflammatory lesion	03 (3.8)
	Corpus luteal cyst and chocolate cyst	04 (5.1)
Total		78

[Table/Fig-2]: Histological classification of ovarian tumors.

Malignant serous tumours were more as compared to the benign counterpart. Metastatic tumours involving both the ovaries in 6.41% cases. One rare Leydig cell tumour was diagnosed cytologically in a 5 year old girl child.

The inflammatory conditions like tuberculosis was misdiagnosed clinically as ovarian tumours in 3.8% cases. 5.1% cases of corpus luteal cysts were found. Most of the tumours were unilateral (80.75%) and cystic in nature [Table/Fig-3]. But the surface epithelial cancers and germ cell tumours were mostly solid-cystic and solid in consistency.

Laterality	No of cases (%)
Unilateral	63 (80.75%)
Bilateral	08 (10.25%)

[Table/Fig-3]: Laterality of tumours.
*Total numbers of tumours 71.

All the cases were subjected to histological examination. Agreement between cytological and histological diagnosis was found in 89.74% cases [Table/Fig-4]. Disparity was observed in case of dysgerminoma which on histopathological examination came out to be lymphoma. One tumour with extensive necrosis was diagnosed as serous cystadenocarcinoma, but histologically it was a mucinous adenocarcinoma. One case of endometrioid carcinoma was diagnosed as papillary serous cystadenocarcinoma on cytology. A benign cystadenoma was diagnosed as borderline tumour on histology. Two cases

Disease	Histological Diagnosis	Cytological Diagnosis
Benign Serous Tumour	8	10
Borderline Serous Tumour	1	-
Malignant Serous Tumour	20	22
Benign Mucinous Tumour	12	11
Borderline Mucinous Tumour	2	3
Malignant Mucinous Tumour	5	3
Endometrial Carcinoma	1	-
Dysgerminoma	3	4
Mature Teratoma	2	2
Immature Teratoma	2	2
Teratoma with Struma Ovarii	1	1
Teratoma with Carcinoma	2	2
Leydig Cell Tumour	1	1
Fibroma	5	5
Metastatic	5	5
Lymphoma	1	-
Benign cyst	4	4
Inflammation	3	3
Total	78	78

[Table/Fig-4]: Agreement between cytological and histological diagnosis.

of benign cysts were confirmed as chocolate cyst and corpus luteal cyst on histological study respectively. All the cases of benign serous and mucinous cystadenomas, teratomas, metastatic tumours and non-neoplastic lesions showed 100% accuracy with histopathological examination.

DISCUSSION

FNA of ovary was a well known procedure as a diagnostic tool since 20 years. Though, initially it was not so acceptable procedure, now days it plays a major role as a diagnostic as well as therapeutic tool, especially for clinically suspicious ovarian masses. The initial drawbacks for accepting this procedure were-most of the lesions were benign in nature, the potential risk of tumour spillage during intervention, and the diagnostic reliability.

But intraoperative cytological procedure eliminates the above risks and the most important aspect is it guides the surgeon's hand.

Dudgeon and Patrick [6] introduced the intraoperative cytological examination assessing the ovarian masses. Though frozen section was the most widely accepted procedure for the diagnosis of malignant tumours, the results were always not reliable particularly if the size of the tumour is too large and the tumour has multiple components of various cell origins. In comparison to frozen section, Nagai et al.,[9] has shown that the diagnostic accuracy of IOC is comparable with frozen section. There are a lot of advantages of intraoperative cytology (IOC) such as—

1) Easy and adequate.

2) Well preserved cellular architecture and details of individual cells.

3) No loss of tissue as occurs during cryostat procedure.

4) Examination of different types of tissues such as adipose tissue, necrotic and calcified tissue.

5) Sampling of multiple suspicious sites like omentum and pouch of Douglas to confirm the presence of tumour dissemination.

6) No special requirement of any trained staff or equipments.

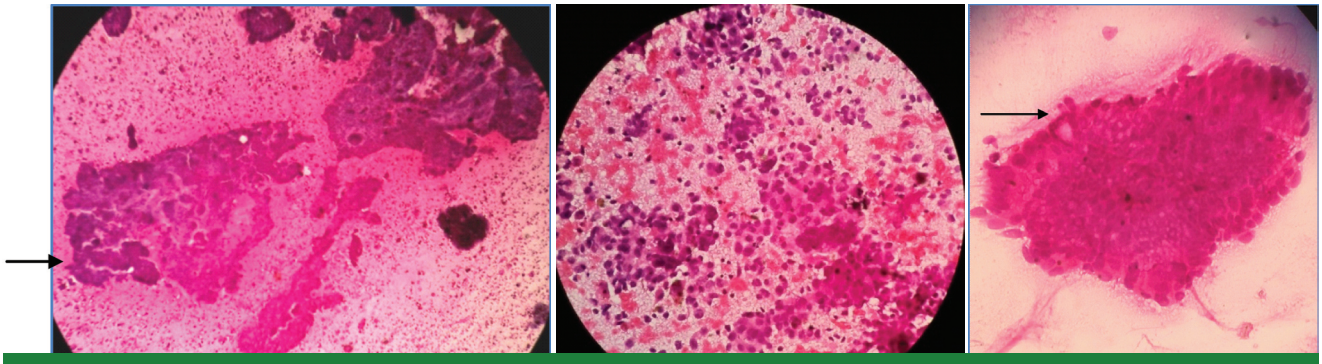
7) Results within minutes.

Certain limitations are also observed in IOC preparations particularly in case of borderline malignant tumours, for which histological examination is mandatory for the architectural evaluation. In a developing country like India, frozen section is a costly procedure which may not be available at all set up. Therefore, IOC plays the simplest and easiest method of diagnostic utility [10].

Out of all the procedures scrape cytology yields maximum number of cells. We used rapid H & E staining technique which preserves the architectural and cellular details very well. Sidham et al.,[11] had also shown that H & E stain is superior than papanicolaou and other polychromatic stains. Similar results were also achieved by Rao et al.,[12] and Tushar et al.,[13].

We studied 78 cases 91% cases were neoplastic. The non-neoplastic cases were clinically suspected as ovarian tumours. Predominantly 37.1% cases were of serous in nature and most cases were malignant. Verma and Bhatia [14] and Tushar et al. [13] etc., had also found similar results. Cystadenomas were cystic in nature containing clear fluid. Malignant tumours were mostly observed in 4th to 6th decade. IOC smears showed disparity in one case of benign serous cystadenoma which on histological examination came out as borderline serous cystadenoma. Presence of focal stratification, mild atypia and minimal invasion for the diagnosis of borderline tumours may be missed in cytological examination which is better diagnosed in histological examination. One case of papillary serous adenoma on cytology was wrongly diagnosed as mucinous adenoma [Table/Fig-5&6]. As most of the ovarian tumours are papillary serous tumours, usually any papillary architecture pulls the diagnosis towards it unless we get other specific findings. In the above said case, the mucinous component was not observed in cytological smears.

Mucinous tumours were the second most common tumours 24.35% [Table/Fig-7]. All the benign mucinous cystadenomas



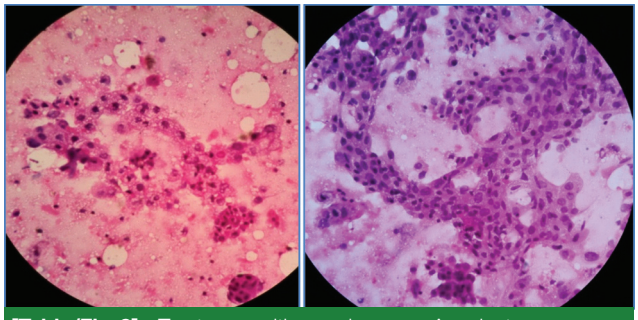
[Table/Fig-5]: Serous cystadenocarcinoma:- Mostly cells are arranged in papillary (arrow) and glandular architecture. H & E 100X.
[Table/Fig-6]: Serous cystadenocarcinoma; Cells show high N:C ratio having moderate amount of cyanophillic cytoplasm over a dirty and necrotic background. H & E 400X.
[Table/Fig-7]: Mucinous adenoma:- cyto smears revealed the tall, columnar cells in sheets having basally located nuclei, abundant amount of empty looking mucin (arrow). Background is highly viscous in nature. H & E stain 400X.

were diagnosed correctly. One borderline tumour was diagnosed due to the presence of prominent nuclear atypia, focal epithelial crowding and clumped chromatin. The histological examination revealed minimal stromal invasion. One case of malignant mucinous cyst adenocarcinoma was diagnosed as borderline tumour cytologically. Two cases of mucinous cystadenocarcinoma were diagnosed correctly on cytological examination.

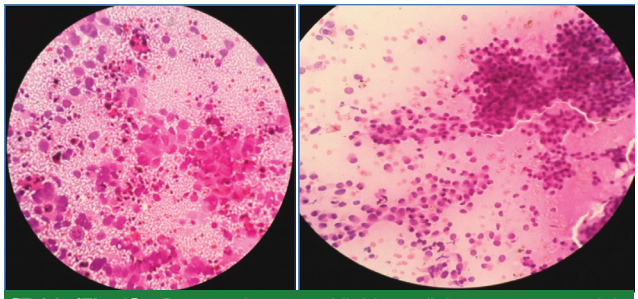
Disparity was observed in a case of endometrioid carcinoma which was wrongly diagnosed as serous carcinoma as both the tumours carry many overlapping features. Well differentiated villoglandular endometrioid adenocarcinoma has papillae like structure; and the absence of prominent rosette like or glandular structure may mislead the cytological diagnosis towards serous carcinoma. Histopathological examination study revealed complex tubulopapillary architecture lined by stratified atypical non-mucinous epithelium with focal squamous differentiation.

Among the germ cell tumours, teratoma was the most common 8.7%. These cases showed benign squamous cells, cyst macrophages, and foreign body type of giant cells in response to keratin, glandular epithelium and fat. Two cases of teratoma had malignant squamous cells and malignant glandular epithelium respectively which was diagnosed correctly [Table/Fig-8]. These 2 cases were mostly observed in 4th to 6th decade patients. One case of grade III immature teratoma with metastasis to paraaortic lymph nodes was wrongly diagnosed as well differentiated mucinous adenocarcinoma. Cytologically examination showed mucinous component only and no other elements. Dysgerminoma showed 100% accuracy on cytology [Table/Fig-9]. However, one case of lymphoma was diagnosed as dysgerminoma cytologically due to the presence of lymphocytes and monotonous population of cells in the background. As lymphoma is a rare tumour of ovary, cytologically it was diagnosed as dysgerminoma. Final diagnosis was confirmed as a case of B-NHL.

One case of Leydig cell tumour in a 5-year-old child, was diagnosed correctly due to the presence of relatively small uniform cell having scanty cytoplasm, small and rounded



[Table/Fig-8]: Teratoma with carcinoma:- Anucleate squames, amorphous background and some inflammatory cells admixed with malignant appearing epithelial cells. H & E 400 X.



[Table/Fig-9]: Dysgerminoma:- Highly cellular smears mainly arranged in dispersed manner. Cells are large, vesicular nuclei, prominent nucleoli, and abundant fragile and vacuolated cytoplasm over a tigroid background. H & E 400X.

[Table/Fig-10]: Leydig cell tumour:- small, cuboidal cells having scanty cytoplasm arranged in a glandular pattern. H & E 400X.

blunt papillae with hyalinised core and the lining epithelium showed mildly atypical cuboidal cells [Table/Fig-10]. All fibromas showed 100% accuracy as there are benign spindle cells. Metastatic tumours (6.4%) were diagnosed correctly. A case of coexisting ovarian serous cystadenocarcinoma along with colorectal carcinoma was seen. The tumour markers CA 125 and CA19-9 both were raised indicating the possibility of double primary in origin. One Krukenberg tumour was there showing bilateral ovarian tumours.

Among the benign lesions, 5.1% case of chocolate cyst and corpus luteal cyst were diagnosed as benign cysts on cytololgy. In case of chocolate cyst, the definite presence of endometrial glands and hemosiderin laden macrophages are required this is usually obscured by extensive haemorrhage on cytology. Again corpus luteal cysts are mostly a gross and histological diagnosis rather than a cytological one.

In all of our disparity 10.25% cases, the diagnosis was correct in respect to the major tumour category i.e. benign, borderline or malignant [Table/Fig-4]. The failure in specification of tumour origin or correct designation of subtype ovarian tumour doesn't influence the surgical management much. In our cases there was no gross discordance which could have adversely affected the intraoperative management. Two cases were reported as benign cysts cytologically which were managed conservatively.

Sensitivity of the present study is 94.87% with 95% confidential limit of 82.68% - 99.37%. Specificity is 100%. Positive predictive value is 100% [Table/Fig-11]. Similar findings were observed in Tushar et al.,[13] Sidham et al.,[11] and Khunamornpong et al.,[10] studies [Table/Fig-12].

The limitation of our study is that multiple cytological sampling from various sites of a large tumour must have been taken to increase the chance of diagnostic accuracy. But due to shortage time limit this was not possible in all the cases. Some studies have shown that in case of heterogenous and large tumours, additional sampling by taking four smears from different sites instead of taking 1 or 2 may improve our diagnostic accuracy [15]. Again in case of in situ and borderline

	Value	95% Confidential limit
Sensitivity	94.87%	82.68 – 99.37%
Specificity	100%	90.97 – 100%
Disease prevelance	50%	38.46 = 61.54%
Positive predictive value	100%	90.51 – 100%
Negative predictive value	95.12%	83.47 – 99.40%
Negative likelihood ratio	0.05	0.01 – 0.20
True positive 37	**False negative 0**	
False positive 2	True negative 39	

[Table/Fig-11]: Statistical analysis of ovarian tumours.

Studies	Diagnostic accuracy
Khunamornpong S et al., [10]	95%
Shidham et al., [11]	98.4%
Tushar et al., [13]	93%
Present Study	94.87%

[Table/Fig-12]: Comparison with other studies.

ovarian tumours and the resection margin assessment in cytology preparation is less useful [16]. Usually metastatic tumours mimic primary neoplasms for which extensive sampling, detailed clinical history, relevant investigations and ancillary studies like immunohistochemistry may be required to reach at a specific diagnosis. By intraoperative cytology we can predict the primary origin of the tumour.

In summary, intraoperative cytology proved itself as an initial step towards the correct diagnosis of ovarian tumours, especially in a developing country like India, where frozen section is not possible at every set up. IOC plays an important role as a good complement to histology and for the patients; it is helpful by providing rapid preliminary diagnosis and surgical management. It has high accuracy though exact subtype of ovarian tumours may not be possible in every case. Intraoperative cytology is specifically helpful in young patients, who need conservative surgery to retain their fertility.

CONCLUSION

Intraoperative cytology plays an important role by guiding the surgeon within a short period of time. It is a preferred method of investigation due to its simple, inexpensive, sensitivity, specificity and positive predictive value.

REFERENCES

[1] Uguz A, Ersoz C, Bolat F, Gokdemir A, Vardar MA. Fine needle aspiration cytology of ovarian lesions. *Acta Cytol*. 2005; 49(2):144-48.

[2] Geomini P, Bremer G, Kruitwagen R, Mol BW. Diagnostic accuracy of frozen section diagnosis of the adnexal mass: a metaanalysis. *Gynecol Oncol*. 2005; 96(1):01-09.

[3] Medeiros LR, Rosa DD, Edelweiss MI, Stein AT, Bozzetti MC, et al. Accuracy of frozen-section analysis in the diagnosis of ovarian tumors: a systematic quantitative review. *Int J Gynecol Cancer*. 2005;15(2):192-202.

[4] Brun JL, Cortez A, Rouzier R, Callard P, Bazot M, et al. Factors influencing the use and accuracy of frozen section diagnosis of epithelial ovarian tumors. *Am J Obstet Gynecol*. 2008; 199(3):244.e1-e7.

[5] Stewart CJ, Brennan BA, Hammond IG, Leung YC, McCartney AJ. Accuracy of frozen section in distinguishing primary ovarian neoplasia from tumors metastatic to the ovary. *Int J Gynecol Pathol*. 2005; 24(4):356-62.

[6] Dudgeon, LS, Patrick, CV. A new method for the rapid microscopical diagnosis of tumors: with an account of 200 cases so examined. *Br J Surg*. 1927;15:250–61.

[7] Clarke MR, Landreneau RJ, Borochovitz D. Intraoperative imprint cytology for evaluation of mediastinal lymphadenopathy. *Ann Thorac Surg*. 1994;57(5):1206-10.

[8] Rahman K, Asif Siddiqui F, Zaheer S, Sherwani MK, Shahid M,et al. Intraoperative cytology-role in bone lesions. *Diagn Cytopathol.* 2010;38(9):639-44.

[9] Nagai Y, Tanaka N, Horiushi F, Okhi S, Seki K, Sekia S: Diagnostic accuracy of intra-operative imprint cytology in ovarian epithelial tumors. *Int J Gynecol Obstet*. 2001;72:159-64.

[10] Khunamornpong S, Siriaunkgul S. Scrape cytology of the ovaries: potential role in intraoperative consultation of ovarian lesions. *Diagn Cytopathol*. 2003; 28(5):250-57.

[11] Shidham VB, Dravid NV, Grover S, Kher AV. Role of scrape cytology in rapid intraoperative diagnosis. Value and limitations. *Acta Cytol.* 1984; 28(4):477-82.

[12] Rao S, Sadiya N, Joseph LD, Rajendiran S. Role of scrape cytology in ovarian neoplasms. *J Cytol*. 2009; 26(1):26-29.

[13] Tushar K, Asaranti K, Mohapatra PC: Intraoperative cytology of ovarian tumors. *J Obstet Gynecol India*. 2005; 55:345–49.

[14] Verma K, Bhatia A. Ovarian neoplasms. A study of 403 tumours. *J Obstetric Gynecol India*. 1981;3:106-11

[15] Khunamornpong S, Siriaukgul S. Scrape cytology of the ovaries: potential role in intra-operative consultation of ovarian lesion. *Diagno Cytopathol*. 2003;28:250-57.

[16] Stewart CJR, Brennan BA, Koay E, Naran A and Ruba S. Value of cytology in the intraoperative assessment of ovarian tumors. *Cancer Cytopathology*. 2010;118:127–36.

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