

Efficacy of Imprint Cytology in the Diagnosis of ovarian Tumors

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Abstract

Objective: The study is mainly addressed to the gynaecologists and surgeons, and the purpose of the study was to determine the efficacy of intraoperative imprint cytology in the diagnosis of ovarian masses.

Methodology: A prospective, non-randomized, cross-sectional, descriptive study is done in the Department of Pathology, Shaheed Zulfiqar Ali Bhutto Medical University, Pakistan Institute of Medical Sciences, Islamabad. Duration of the study was 3 years from July 2015 to July 2018. An unfixated ovarian sample received in the pathology department, was immediately used for preparing imprint smears on glass slides. The slides were fixed in 95% ethyl alcohol. Staining was done using hematoxylin and Eosin stains. The results of intraoperative touch cytology were compared with histopathological diagnosis. The statistical efficacy of imprint cytology was determined by comparing it with the histopathological findings.

Results: Data was analyzed using descriptive statistics. The results of the imprint cytology and histopathology were compared. Sensitivity and specificity were 82.2% and 89.4 % respectively. Positive Predictive value was 92.7 %. The negative predictive value was 75%.

Conclusion: Imprint cytology is a simple and cost-effective diagnostic strategy, especially useful in resource limited countries like Pakistan, in making a quick intraoperative diagnosis for the optimal treatment regimens.

Keywords: Imprint cytology (IC), Fine needle aspiration cytology (FNAC), Ovarian tumors

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Introduction

The intraoperative assessment of ovarian masses in cancer suspicious cases is challenging due to diverse cytological features, yet has become one of the important goals for optimal surgical intervention. Ovarian carcinoma remains the cancer with the highest mortality rate among gynecologic malignancies, affecting around 2% of women in the developed world, and 25-30% of them are in the reproductive age group.^{1,2} Ovarian cancer is the 3rd most common malignancy in Pakistan, comprising 18 to 23% of all female cancers and almost 34% of gynaecological

cancers in Pakistan.³

There is a variation in the age groups, but most of the tumors are present in the reproductive age group.⁴ To determine the morphologic subtype of the ovarian tumors is crucial for the appropriate treatment strategy, to preserve fertility, as well as to assess their clinical behavior and outcome.^{5,6} The high degree of variability in the morphological patterns is a major challenge for the Pathologists. Conventionally frozen sections are utilized for intraoperative consultations for on-the table diagnosis.⁷ There are some additional benefits of

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imprint cytology which are not at the expense of accuracy. This includes sampling of multiple foci, rapidity of preparation, good preservation of cellular details, and minimal chances of contamination.⁸ At places where the amenities of frozen section are unavailable, intraoperative imprint cytology is a useful technique for on the table diagnosis of neoplastic and non-neoplastic pathological lesions.^{9,10} FNAC methodology requires experienced staff and expensive machinery. Also, the methodology is argumentative due to the probability of tumour seeding and dissemination.¹¹

The aim of the current study is to determine the role of imprint cytology for accurate diagnosis of surgically removed ovarian masses.

Methodology

A prospective, non-randomized, cross-sectional, descriptive study was conducted in the Department of Pathology at Shaheed Zulfiqar Ali Bhutto Medical University, Pakistan Institute of Medical Sciences, Islamabad. The duration of the study was 3 years from July 2015 to July 2018. A hundred cases of ovarian mass were included by consecutive (non-probability) sampling. The sample size was calculated by using WHO sample size calculator, taking: Sensitivity=96%¹², Specificity 85%¹⁰, Expected prevalence 15%¹³, Desired precision=10%, confidence level=95%, Sample size=99. Inclusion criteria: Cases with clinical or radiological suspicion of malignancy were included in the study. Exclusion criteria: Asymptomatic benign cysts mostly found incidentally, were excluded. After gross examination of the specimen, the excess of fluid was gently blotted with dry gauze to remove any blood or cyst fluid. The tissue was touched on the glass slides to take impressions. Slides were stained with hematoxylin and Eosin stain. The specimen was fixed in 10% formalin and processed for histopathology slides.

A provisional diagnosis was made on Imprint cytology. The results were compared with the histopathological diagnosis. Data was analyzed using SPSS version 12.0. Descriptive statistics were calculated. The results were used to determine sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy.

Data was analyzed using descriptive statistics:
 True Positive cases (TP): 51
 False Positive cases (FP) : 4
 True Negative cases (TN) : 34

False Negative cases (FN) : 11

$$\text{Positive Predictive value (PPV)} = \frac{TP}{TP+FP} \times 100 = 92.7 \%$$

$$\text{Negative Predictive value (NPV)} = \frac{TN}{TN+FN} \times 100 = 75 \%$$

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100 = 82.2 \%$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100 = 89.4 \%$$

Statistical Analysis

	Cytological Diagnosis	Histological Diagnosis
Cytological Diagnosis	Pearson Correlation	1
	Sig. (2-tailed)	.827(**)
	N	100
Histological Diagnosis	Pearson Correlation	.827(**)
	Sig. (2-tailed)	.000
	N	100

** Correlation is significant at the 0.01 level (2-tailed).

Level of significance:

α = 1% = 0.01 (level of significance)

Test statistic:

P < α = <0.01

Showing cytological diagnosis has a positive relationship with histological diagnosis.

Results

The smears were evaluated for cellularity, pattern, and morphology. Out of 100 cases, 38 (38%) were benign, 5(5%) were borderline and 57 (57%) were malignant. Age range of the patients was 1 to 90 years. Majority patients presented between 21 to 30 years (Figure 1).

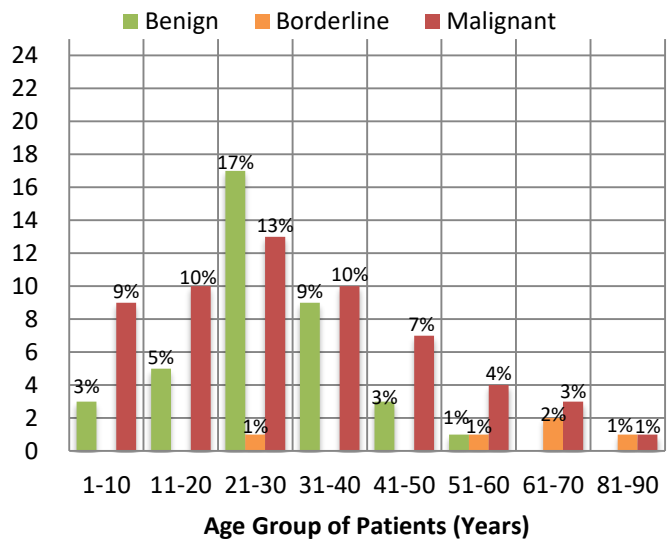


Figure 1: Age Distribution of Benign, Borderline and Malignant Tumors

Tumors were further subdivided into four major categories: surface epithelial tumor 48%, germ cell tumors 44%, sex-cord stromal tumor 5% and undifferentiated tumor 3% of total cases on Histopathology (Figure 2).

Accuracy was 100% for mature Teratoma, showing anucleated squames, amorphous material, keratin flakes and mixed inflammatory infiltrate. Smears benign serous neoplasms revealed aggregates of columnar epithelial cells forming papilla having bland nuclei in a hemorrhagic background. (Figure 3) Three cases were misdiagnosed as serous carcinoma due to cellular stratification and focal nuclear atypia. Benign mucinous tumors revealed mucinous columnar cells with eccentric bland nuclei in a viscous background. (Figure 4) One of the cases was labeled positive falsely due to obscuring artifact by abundant mucin and inflammatory infiltrate. Five cases revealed bland ovoid to spindle cells showing moderate amount of cytoplasm and bland nuclei, correctly labeled as benign stromal tumors on cytology were diagnosed as fibro-thecoma on histopathology (Table II).

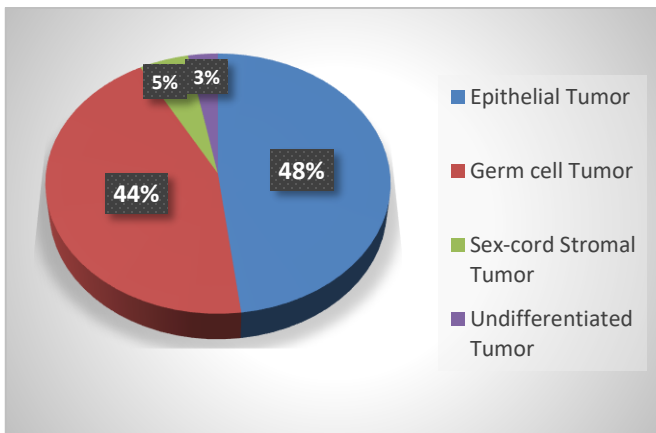


Figure 2: Histologic type of tumors

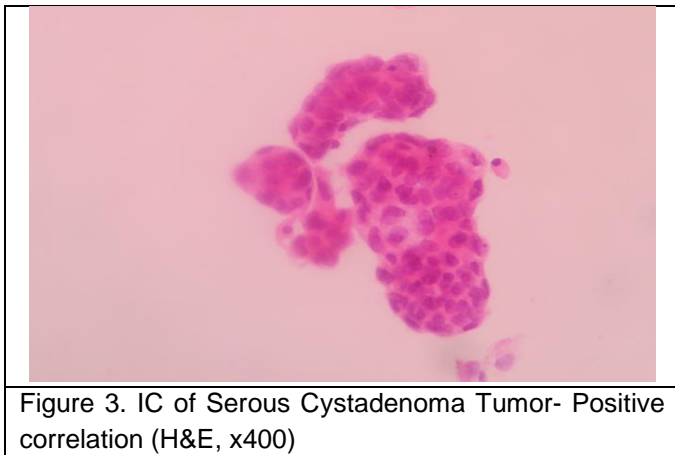


Figure 3. IC of Serous Cystadenoma Tumor- Positive correlation (H&E, x400)

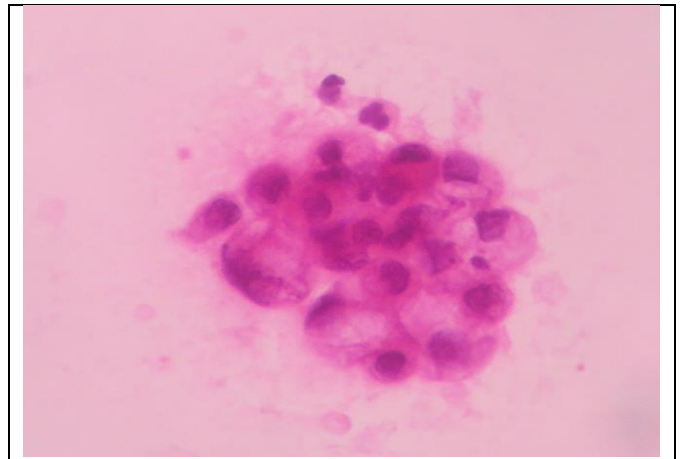


Figure 4. IC of Mucinous Cystadenoma- Positive correlation (H&E, x400)

Out of 38 benign ovarian tumors, 34 was correctly diagnosed on IC. (Table I)

Table I: Cytologic diagnosis of ovarian tumors

Total cases n=100	Histological diagnosis n=100	Accurate Cytological diagnosis n=85	Incorrect Cytological diagnosis n=15
Benign lesions	38 (38%)	34 (89.5%)	4 (10.5%)
Borderline epithelial neoplasms	5 (5%)	3 (60%)	2 (40%)
Malignant neoplasms	57 (57%)	48 (84.2%)	9 (15.8%)

The borderline tumors were considered as positive, sharing similar cytological features as malignant tumors. In case of borderline mucinous neoplasms, 50% were misdiagnosed as benign on imprints. The smears showed low cellularity with epithelial cells showing lack of clear-cut malignant features and extensive mucin in the background. Smears from two borderline serous tumors were diagnosed as serous adenocarcinoma on imprints due to overlapping papillary cell clusters showing moderate nuclear pleomorphism but in histopathology no definitive stromal invasion.

Smears from 1 out of 3 borderline serous tumors showed papillary configuration of low columnar cells. Nuclear abnormalities were inconspicuous, and nucleoli were small, therefore the case proved to be false negative on imprints.

Out of total 22 cases of serous cystadenocarcinoma, cytology of 21 cases were correctly diagnosed. The IC revealed columnar cells with high N:C ratio forming

papillary and glandular aggregates of cells with hyperchromatic nuclei in a turbid background. (Figure 5). One case (4.5%) was misdiagnosed on imprints due to low cellularity and lack of prominent cytologic atypia (Table II).

Table II: Cytological Correlation of Ovarian tumors				
Ovarian tumors n=100	Histologic al diagnosis n=100	Accurate Cytological diagnosis n=85	False positive cytology n=4	False negative cytology n=11
Benign tumors	n=38	n=34 (89.5%)	n= 4 (10.5%)	n=0
Mature teratoma	19 (50%)	19 (100%)	0	0
Serous cystadenoma	9 (23.8%)	6 (66.7%)	3 (33.3%)	0
Mucinous cystadenoma	5 (13.1%)	4 (80%)	1 (20%)	0
Thecoma fibroma	5 (13.1%)	5 (100%)	0	0
Borderline tumors	n=5	n=3(60%)	n=0	n=2 (40%)
Borderline serous tumor	3 (60%)	2 (66.7%)	0	1 (33.3%)
Borderline mucinous tumor	2 (40%)	1 (50%)	0	1 (50%)
Malignant tumors	n=57	n=48 (84.2%)	n=0	n=9 (15.8%)
Serous cyst adenocarcinoma	22 (38.5%)	21 (95.4%)	0	1 (4.5%)
Dysgerminoma	11 (19.2%)	11 (100%)	0	0
Mixed Germ cell tumor	8 (14%)	6 (75%)	0	2 (25%)
Immature teratoma	6 (10.5%)	2 (33.3%)	0	4 (66.6%)
Mucinous cyst adenocarcinoma	4 (7%)	2 (50%)	0	2 (50%)
Endometrioid carcinoma	3 (5.26%)	3 (100%)	0	0
Undifferentiated carcinoma	3 (5.26%)	3 (100%)	0	0

Dysgerminoma showed discohesive cells with well-defined cell borders, pale cytoplasm, vesicular nuclei with prominent nucleoli. Background showed lymphocytes.

The cases diagnosed as mixed malignant germ cell showed atypical cytology of any of the component tumor lineage. However, two cases (4.5%) were falsely labeled benign showing a few squames and keratin flakes giving impression of mature teratoma.

Immature teratoma on revealed discohesive and aggregates of neoplastic cells along with anucleatesquames. Four cases of immature teratoma were labeled as benign due to low cellularity showing

few anucleatesquames and keratin flakes only. (Figure 7)

Mucinous cystadenocarcinoma showed highly viscous mucinous background with aggregates of columnar mucin cells with high N:C ratio and nuclear pleomorphism. 2 cases of mucinous adenocarcinoma were labeled as benign on due to abundant mucin and mild nuclear atypia. (Figure 6)

Endometrioid carcinoma showed with cells having eosinophilic granular cytoplasm forming micro glandular pattern showing moderate to high nuclear pleomorphism and hyperchromatic nuclei giving 100% accuracy in these tumors (Table II).

There were 3 cases of undifferentiated tumors on histopathology labeled as malignant tumor on cytology. IC showed high N/C ratio, pleomorphism with abundant mitosis including atypical mitosis (Table II).

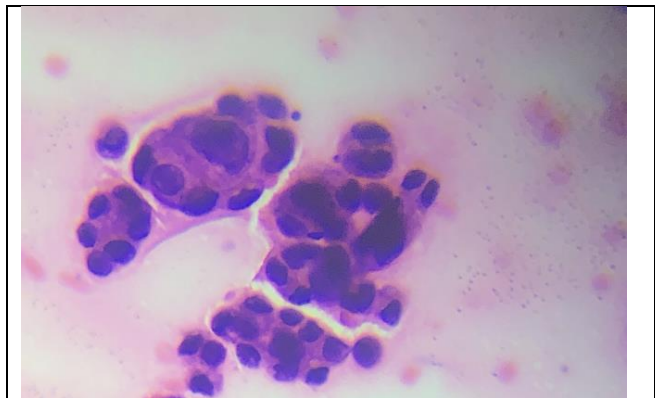


Figure 5. IC of Serous Cystadenocarcinoma Tumor- Positive correlation (H&E, x400)

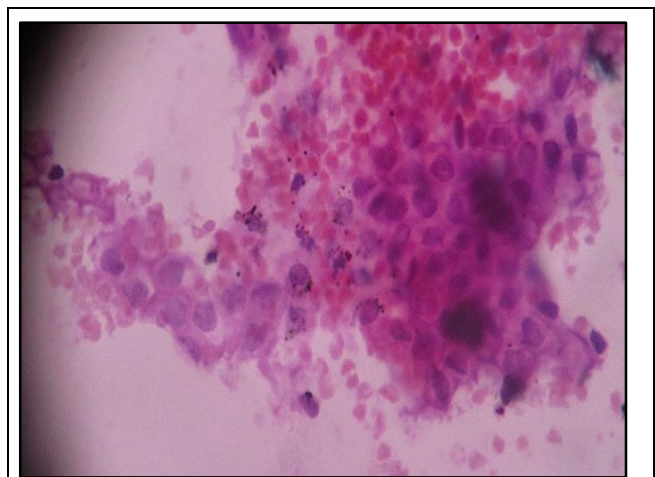


Figure 6. IC of Mucinous Cystadenocarcinoma- Negative correlation (H&E, x400)

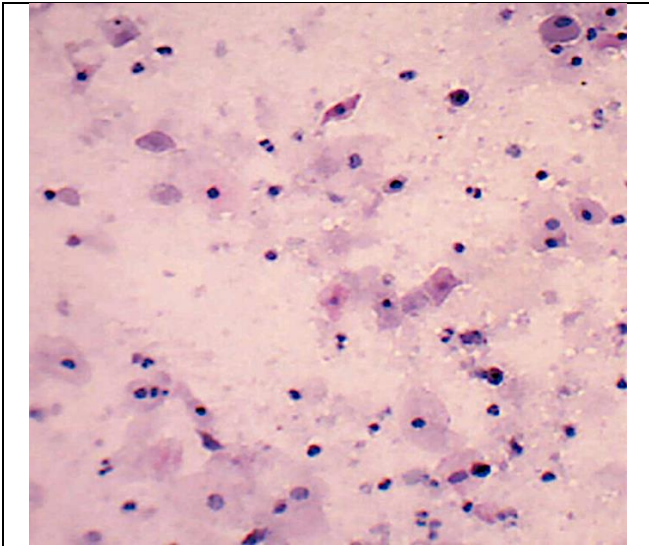


Figure 7. IC of Immature Teratoma- Negative correlation (H&E, x400)

Discussion

Imprint cytology was first described by Dudgeon and Patrick for rapid diagnosis of tumors.¹⁰ Cytological evaluation of ovarian neoplasms is exciting as well as challenging due to its diversity. Our overall sensitivity and specificity, 82.2% and 89.4%, respectively, were in accordance with the results of the above-mentioned studies. Ivan et al. showed the diagnostic accuracy of 84.6% in case of imprint cytology of ovarian masses.¹⁴ Taqdees et al. performed a study on imprint cytology of ovarian masses and showed positive predictive value of 91.6% and accuracy of 91%.¹⁵

The overall diagnostic accuracy proved by Azami et al. was greater for imprint cytology as comparison to frozen section, 80.6% and 67.7% respectively especially in case of mixed carcinomas.¹⁶ There are many advantages of IC over frozen section like IC is a simple, quick, and inexpensive method giving high quality preparations. Unlike frozen section, IC does not require special equipment or techniques and there is minimal chance of tissue loss during processing. Moreover, no freezing artifacts are seen in case of IC. In the studies, imprint cytology has been compared to frozen section and have shown equivalent diagnostic efficacy.¹⁷

In the present study accuracy in the benign lesions was more than the malignant lesions. Diagnostic accuracy for benign tumors by Rady et al. was also found to be high (87.80%). The cytology of benign lesions revealed aggregates of benign cells with no atypia and pleomorphism.¹⁸ The cases of serous and mucinous

cystadenomas diagnosed as positive on IC showed focal stratification and mild nuclear atypia. Melies et al. suggested extensive tissue sampling and radiological correlates for better interpretation of the results.¹⁹

All cases of Mature cystic Teratoma and Thecoma Fibroma were correctly diagnosed on IC giving accuracy of 100% similar to the findings by Cimic et al where the diagnostic accuracy of IC was high in case of primary ovarian tumors.²⁰

The borderline epithelial tumors were taken as Positive control as they share common morphological pattern with adenocarcinoma on histopathology and the only difference is stromal invasion.¹⁰ The tumor shows papillary architecture with branching stromal cores with cells showing variable cytological atypia. Diagnostic accuracy was 60% for these tumors on ICin. Our study as no complex branching or significant nuclear pleomorphism was seen. The findings are in contrast with Nomura et al where the study reported better interpretation results as the tumors cytology showed marked atypia.²¹

Diagnostic accuracy for malignant lesions was found to be 84.2%. The false negative results were due to low cellularity and lack of significant atypia. Similar to our study, Melies et al reported 82.2% accuracy for malignant tumors.¹⁹ Abe et al reported similar diagnostic difficulty for immature teratomas reporting 54.5% efficacy in these cases and high efficiency for germ cell tumors.²²

Ivan et al compared cytology and frozen section in a study for on the table diagnosis of lesions of ovary and revealed the accuracy was 100% for benign and 84.6% for malignant tumors.¹⁴ Positive predictive value was high in our study (92.7%) as compared to the negative predictive value (75%) in contrast to Rasta et al where negative predictive value was more (75.9%).

The limitations we experienced in the study were insufficient cellularity in few cases and interpretation errors especially in cases of epithelial tumors. As we cannot comment upon definitive stromal invasion, histopathology is essential to assess the presence or absence of stromal invasion and for the subtyping of a tumor with low malignant potential.²³ Facing similar difficulty, P.Chacko et al and Vaidya et al suggested scraping of the slides for maximizing the cell yield.^{24,25}

Conclusions

Imprint cytology is a method showing excellent capability to produce immediate diagnosis in intraoperative consultation of ovarian masses. We are persuaded by the efficacy of Imprint Cytology and endorse its utility in ovarian tumors. Especially in developing countries like Pakistan, when frozen sections are unavailable, this low-cost technique can help surgeons and histopathologists make quick diagnosis regarding the nature of the ovarian mass and is especially helpful in young females who are potential candidates for conservative surgery to preserve fertility.

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