Fine-Needle Aspiration Biopsies of Ovarian Masses: A Reliable Technique

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Key Words
Fine-needle aspiration · Cytological details · Ovarian masses · Diagnostic accuracy

Abstract
Objective: In gynecology, fine-needle aspiration (FNA) has an overall accuracy of 94.5% in differentiation between benign and malignant tumors. The purpose of this study was to determine reliable cytological criteria for categorizing ovarian masses into benign and malignant categories, their subtypes, and also to evaluate FNA accuracy in the diagnosis of ovarian tumors in relation to histopathological findings.

Study Design: A prospective study was performed on all patients with a preoperative diagnosis of ovarian tumor who were referred to our hospital between August 2013 and August 2015. During surgery, FNA was performed using an 18-gauge needle by a pathologist. Aspirated material was spread on clean glass slides and stained with Papanicolaou and Wright-Giemsa stains. The cytological findings and results were compared with the histological diagnosis.

Results: Of the 81 cases in this study, there was a discrepancy between the cytological and histological diagnosis in 9 cases. The overall cytological diagnostic accuracy in our study was 88.9% with a sensitivity and specificity of 78.1 and 95.5%, respectively. Conclusion: FNA of an ovarian mass is a minimally invasive procedure with acceptable diagnostic accuracy, especially when differentiating between benign and malignant lesions, and can be considered as a useful diagnostic modality for choosing an appropriate management course.

Introduction

Ovarian carcinoma accounts for the greatest number of deaths from malignancies of the female genital tract and is the fifth leading cause of cancer-related death in women in general \cite{1, 2}. Although most ovarian tumors occur in the elderly group, a certain percentage still affect females of reproductive age. On the other hand, since most radiological and clinical methods for the diagnosis of ovarian tumors have various pitfalls, even in certain benign conditions, clinicians prefer to remove the pelvic masses and send them to pathology for a definite diagnosis. Therefore, there is a need for more accurate preoperative diagnostic methods in order to save ovaries; one of these methods is fine-needle aspiration (FNA).

Being simple, rapid, safe and inexpensive, FNA is a popular diagnostic method worldwide \cite{3, 4}. In gynecology, FNA has an overall accuracy of 94.5% in the differ-
entiation between benign and malignant tumors [5]. Despite many controversial views regarding its safety, aspiration cytology has been accepted as an innocuous procedure that can be accomplished with minimal discomfort or complications [3, 4] and, in association with laparoscopy, aids in the management of ovarian cysts and masses.

Although FNA cannot be considered the first-hand diagnostic procedure for ovarian cancer in postmenopausal patients, it may be extremely helpful in young women, even during pregnancy, to safely differentiate functional and other benign ovarian cysts from malignant ones. During laparotomy for suspected unilateral diseases, FNA may provide sufficient data about the opposite ovary to allow that organ to remain in place, thus preserving its function in a young patient. It is also indicated for initial evaluation before treatment and may be beneficial in recurrent or metastatic tumors in premenopausal women [6].

However, as we mentioned earlier, most gynecologic oncologists hesitate to perform FNA from ovarian masses due to their concern about diagnostic accuracy and lack of information about the procedure efficacy and advantages in the literature. Therefore, the aim of this study was to determine reliable cytological criteria for categorizing ovarian masses into benign and malignant categories, their subtypes, and also to evaluate FNA accuracy in the diagnosis of ovarian tumors in relation to histopathological findings.

**Materials and Methods**

A prospective study was performed involving all patients with a preoperative diagnosis of ovarian tumor who were referred to Faghihi hospital (affiliated to Shiraz University of Medical Sciences) between August 2013 and August 2015 for surgery. During surgery (after the removal of the ovarian lesion by a gynecologist), FNA was performed using an 18-gauge needle by a pathologist. Aspirated material was spread onto clean glass slides. Half of the cytology slides were placed in Carnoy’s solution for fixation and Papanicolaou staining, and the other half were air dried for Wright-Giemsa staining. Afterwards, the tumor was fixed in neutral-buffered formalin and sent to the pathology department for routine permanent sectioning and hematoxylin and eosin (HE) staining.

The demographic data, imaging studies and level of tumor markers (if available) of all the patients were recorded. All of the cytological and related histological (HE) slides were reviewed by a pathologist and recorded in a database. The interpretations of cytological slides were evaluated by a pathologist who was blinded to the histopathological diagnosis and gross findings. Specific cytological findings of each lesion, including cellularity, nuclear atypia, epithelial cluster morphology and presence of tumor diathesis, were described in detail. The results were analyzed for the amount of correlation between the cytological and histopathological diagnosis. The study was approved by the Medical Ethics Committee of Shiraz University of Medical Sciences.

**Results**

Out of the 90 cases included in this study, 9 were later omitted due to insufficient material (defined as an absence of any epithelial or mesenchymal cells or even the low number of macrophages). The age of the patients varied from 14 to 82 years with a mean of 44 years. Clinically, most of the patients presented with abdominal discomfort and pain. The distribution of different tumors according to their histological diagnosis is displayed in table 1.

In total, 49 out of 81 cases (60.5%) were benign and the remainder (39.5%) were malignant. Among all of the ovarian lesions, mucinous neoplasms were the most prevalent, accounting for 20% (21 out of 81) of all cases. Aspirates from benign mucinous cysts re-
revealed moderately cellular smears with honeycomb clusters and a blue/purplish mucinous background. In malignant mucinous tumors, the individual cells and clusters showed marked atypia and pleomorphism with signet ring-like vacuolated cells in a dirty mucinous background (fig. 1). In the second most prevalent group, i.e. serous tumors, the aspiration cytology showed mild cellularity with flat monomorphic clusters in the benign category and highly atypical hyperchromatic cells with mitosis and tumor diathesis in the malignant category (fig. 2). Endometrioid carcinoma showed moderately cellular smears consisting of a certain number of clusters and glandular formation with hyperchromatic and pleomorphic elongated nuclei, few mitotic figures and in 1 case hemosiderin-laden macrophages. In our only case of malignant Brenner tumor, aspiration cytology revealed cellular smears with many papillary clusters and highly atypical isolated cells in a necrotic background (fig. 3).

All of the endometriotic cysts revealed many hemosiderin-laden macrophages with few clusters of cuboidal cells in their aspiration. The other benign simple cysts showed very low cellular smears with a certain number of macrophages.

In the sex-cord stromal category we had 4 cases of granulosa cell tumor which demonstrated moderately cellular smears with small papillary and glandular-like

### Table 2. Cytological and corresponding histological diagnosis in concordant cases (n = 72)

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>n</th>
<th>Cytological diagnosis</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional cyst</td>
<td>4</td>
<td>Benign cyst</td>
<td>TN</td>
</tr>
<tr>
<td>Leiomyoma</td>
<td>3</td>
<td>Negative for malignancy</td>
<td>TN</td>
</tr>
<tr>
<td>Endometriotic cyst</td>
<td>3</td>
<td>Endometriotic cyst</td>
<td>TN</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>1</td>
<td>Negative for malignancy</td>
<td>TN</td>
</tr>
<tr>
<td>Serous cystadenoma/cystadenofibroma</td>
<td>11</td>
<td>Benign serous cyst</td>
<td>TN</td>
</tr>
<tr>
<td>Mucinous cystadenoma</td>
<td>12</td>
<td>Benign mucinous cyst</td>
<td>TN</td>
</tr>
<tr>
<td>Borderline mucinous tumor</td>
<td>2</td>
<td>Suspicious for borderline mucinous tumor</td>
<td>TP</td>
</tr>
<tr>
<td>Thecoma/fibroma</td>
<td>6</td>
<td>Benign spindle cell tumor</td>
<td>TN</td>
</tr>
<tr>
<td>Granulosa cell tumor</td>
<td>3</td>
<td>Sex-cord stromal tumor</td>
<td>TP</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>1</td>
<td>Positive for malignancy</td>
<td>TP</td>
</tr>
<tr>
<td>Transitional cell carcinoma</td>
<td>1</td>
<td>Malignant epithelial tumor</td>
<td>TP</td>
</tr>
<tr>
<td>Dermoid cyst</td>
<td>7</td>
<td>Dermoid cyst/negative for malignancy</td>
<td>TN</td>
</tr>
<tr>
<td>Dysgerminoma</td>
<td>3</td>
<td>Dysgerminoma</td>
<td>TP</td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td>3</td>
<td>Malignant epithelial tumor</td>
<td>TP</td>
</tr>
<tr>
<td>Papillary serous cystadenocarcinoma</td>
<td>5</td>
<td>Malignant epithelial tumor</td>
<td>TP</td>
</tr>
<tr>
<td>Endometrioid carcinoma</td>
<td>4</td>
<td>Malignant epithelial tumor</td>
<td>TP</td>
</tr>
<tr>
<td>Leydig cell tumor</td>
<td>1</td>
<td>Leydig cell tumor</td>
<td>TP</td>
</tr>
<tr>
<td>Metastatic tumor</td>
<td>2</td>
<td>Malignant epithelial tumor</td>
<td>TP</td>
</tr>
</tbody>
</table>

TN = True negative; TP = true positive.

### Table 3. Cytological and corresponding histological diagnosis in discordant cases (n = 9)

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>n</th>
<th>Cytological diagnosis</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulosa cell tumor</td>
<td>1</td>
<td>Negative for malignancy</td>
<td>FN</td>
</tr>
<tr>
<td>Immature teratoma</td>
<td>2</td>
<td>Benign lesion suggestive of teratoma</td>
<td>FN</td>
</tr>
<tr>
<td>Borderline mucinous tumor</td>
<td>4</td>
<td>Benign mucinous cyst</td>
<td>FN</td>
</tr>
<tr>
<td>Tubo-ovarian abscess</td>
<td>1</td>
<td>Positive for malignancy</td>
<td>FP</td>
</tr>
<tr>
<td>Hemorrhagic corpus luteal cyst</td>
<td>1</td>
<td>Positive for malignancy</td>
<td>FP</td>
</tr>
</tbody>
</table>

FN = False negative; FP = false positive.
structures and also many isolated oval cells with grooves. Occasionally, Call-Exner bodies could also be seen (fig. 4). No atypical mitosis was found in these 4 tumors. One interesting finding in one of our sex-cord stromal tumors was the presence of numerous mast cells (fig. 5). The sex-cord stromal nature of the tumor was confirmed by an IHC study (showing positivity for inhibin marker) and was finally signed out as suggestive of a granulosa cell tumor. Mast cell infiltration has been reported in rare cases of sex-cord stromal tumors (thecofibroma and sclerosing stromal tumor), so its presence may be a clue to the sex-cord stromal origin of the ovarian mass. In our 3 leiomyoma and thecofibroma cases, aspiration cytology showed low cellular smears with only few tissue fragments and some isolated bland-looking spindle cells.

The germ cell group consisted of teratoma and dysgerminoma. Needle aspiration of all dermoid cysts yielded multiple squames with few sebaceous gland-like structures in a sebum-like creamy background (which we termed ‘powdery’; fig. 6). In our 3 dysgerminoma cases, cytological findings showed cellular smears of many atypical round cells with vesicular nuclei and prominent nucleoli in a background of small mature lymphocytes (fig. 7). Metastatic tumors also revealed cellular smears with clusters of highly atypical cells in a dirty background.

Fig. 1. a Cytological slide showing a bland-looking honeycomb cluster in a benign mucinous cyst. Papanicolaou stain. ×400. b–d Cytology of a malignant mucinous cystadenocarcinoma, showing nuclear atypia and pleomorphism. Papanicolaou stain. ×400.
Of the 81 cases in this study, there was a discrepancy between the cytological and histological diagnosis in 9 cases (table 2). Most of this category (4 out of 9) belonged to mucinous neoplasms, in which we reported a benign mucinous tumor in cytology that became borderline in histology. Two of the cases we reported as positive for malignancy in cytology proved to be a tubo-ovarian abscess (fig. 8) and hemorrhagic corpus luteal cyst on the histology slides. Also, for 2 immature histological teratomas we reported dermoid cysts on cytology due to the lack of any immature component in all the submitted slides. Furthermore, in at least one of the granulosa cell tumors we reported a false negative finding for malignancy according to the cytological analysis. The reason for this mistake was the low cellularity of the smear with very bland-looking nuclei.

Overall, in most of the cases we could diagnose the exact subtype of the tumor by cytological findings, especially in the tumors of the surface epithelial category. All cytologically diagnosed dermoid cysts correlated well
with histopathology. Also, out of 11 cases reported as benign serous cystadenoma, 10 had a concordant histological diagnosis, and the remaining case happened to be a benign mucinous cyst. On the other hand, all of the metastatic tumors and most of the thecofibromas could only be reported as positive or negative for malignancy, respectively, with no further details given.

Imaging findings of 76 patients were available, in which there was a discrepancy between the radiological diagnosis with both the cytological and histological diagnosis in 14 cases. Most of the cases in this category belonged to ovarian masses of mucinous type, which showed septation in their radiology, and also to the hemorrhagic simple cysts with increased internal echo and complexity.

The serum level of surface epithelial tumor markers such as CA125 and CA15–3 were measured in our patients prior to surgery (in 70 out of 81). The CA125 serum level results correlated very well with their cytological diagnosis.

Fig. 4. Histology and cytology of a granulosa cell tumor. a HE stain. ×250. b Cytology slides showing moderately cellular smears with mostly isolated cells and small nuclei and Call-Exner bodies. Papanicolaou stain. ×250.

Fig. 5. Histology and cytology of a sex-cord stromal tumor. a HE stain. ×250. b Small isolated round to spindle cells are seen with abundant mast cells. Wright stain. ×400.
Discussion

The hesitation of gynecologists to perform FNA on pelvic masses prior to their surgical excision still persist mainly due to two beliefs: first, because of concern about its safety and probable complications, and second, due to major doubts about its diagnostic accuracy. The overall cytological diagnostic accuracy in our study was 88.9% with a sensitivity and specificity of 78.1 and 95.5%, respectively. This is comparable to the studies of Ganjei et al. [5] and Khan et al. [7], in which the diagnostic sensitivity of aspiration cytology was also low, leading to the conclusion that one should not rely on cytological findings alone, but should also consider radiological findings and tumor markers of the patient in order to make a final diagnosis. If we had considered the radiological findings and tumor marker levels in our study, the sensitivity would have improved to 89.2%. In the study by Ganjei et al. [5], the overall diagnostic accuracy was improved from 63 to 69% when the cyst size was taken into consideration.

In a study conducted by Uguz et al. [8], the overall sensitivity and specificity was 95.1 and 90.4%, respectively. The high sensitivity in their study can be explained by the presence of a high number of malignant tumors.

The surface epithelial group (either benign or malignant) in our study showed an excellent cytological and pathological correlation, as was also seen in the studies by Sood et al. [9] and Gupta et al. [10]. We had no false positive results in this category, leading to a positive predictive value of 100% in tumors in the surface epithelial category. It is necessary to mention here that in the subgroup of serous tumors, in addition to the absence of false positivity, no false negative results were detected. This leads
us to the belief that FNA can be of high diagnostic value in this specific category.

In the sex-cord stromal group, granulosa cell tumor was reliably diagnosed on cytological slides. The cytological findings in our granulosa cell tumors shared many similarities with a study by Ali et al. [11], who also mentioned some more details about spindle-shaped hyperchromatic stroma cells within cellular clusters. In 2 cases of thecofibroma and leiomyoma, the smears were hypocellular, containing rare tissue fragments and bland-looking spindle cells. Both of them were cytodiagnosed as negative for malignancy but definite subtyping could not be conducted.

The germ cell tumor category consisted mainly of teratomas and also 3 cases of dysgerminoma, each of which showed a good correlation between cytology and histology. Our results were in concordance with those of Sood et al. [9] and Gupta et al. [10].

Fig. 7. Histology and cytology of a dysgerminoma. a HE stain. ×250. b Aspiration cytology showing isolated atypical round cells with vesicular nuclei and prominent nucleoli in a small lymphocytic background. Papanicolaou stain. ×400.

Fig. 8. Histology and cytology of a tubo-ovarian abscess. a HE stain. ×250. b Cytology smear showing a cluster with atypical hyperchromatic nuclei. Papanicolaou stain. ×250.
We had 9 cases with discordant cytology and histopathology results (table 2). In the false negative category, 4 cases that were cytodiagnosed as benign mucinous cyst were revealed to be borderline mucinous tumor by histology. All of them had minimal cellular atypia and the diagnosis of borderline mucinous tumors was based on complex architectural changes, which were not evident on the cytological slides.

Athanassiadou and Grapsa [12] showed that although FNA is the most accurate diagnostic method in cytopathology, its value in the diagnosis of borderline lesions is limited, mainly because of its inability to establish the absence of stromal invasion. The same conclusion was made in our study too, although the presence of tumor diathesis may favor carcinoma over borderline lesions in cases with marked nuclear atypia.

On the other hand, we had two false positive cytological diagnoses with a subsequent histopathological diagnosis of tubo-ovarian abscess and hemorrhagic corpus luteal cyst. Both of these benign conditions may show regenerative changes, including an increase in nuclear size, mild pleomorphism and hyperchromasia that could be a pitfall in a cytological diagnosis. The radiological findings of these 2 cases were a lobulated enlarged mass suggestive of a malignant process in the tubo-ovarian abscess, and a benign cyst with high internal echo in the hemorrhagic corpus luteum case.

In 2 patients with a diagnosis of immature teratoma, the immature neuroepithelial component was not evident on the cytological slides. Aspiration from different parts of the tumor in a multidirectional pattern may be helpful to capture these foci in cytological smears, although these immature components may be limited to small foci of the tumor requiring the examination of multiple representative histological sections.

Among the 28 malignant cases of this study, 25 (92.6%) could be diagnosed correctly based on cytological studies. This indicates that this method has a good positive predictive value in the diagnosis of malignant ovarian tumors [5, 8, 13, 14]. The surface epithelial tumor category was the most frequent malignancy in our study. They were cytologically diagnosed as malignant in 100% of the cases, but a definite subtyping was not applicable in all patients due to high-grade morphology and a lack of differentiation.

In the present study, because our goal was to provide more details about cytological findings, 9 cases were excluded due to insufficient material. However, when we followed the pathology results of these cases, 8 happened to be benign cysts. Therefore, as was also mentioned in the study by Ray et al. [6], these cases should not be labeled as insufficient. Although this statement may be true, it is not applicable to every hypocellular case because aspiration of the cyst fluid in tumors with a solid and cystic component may result in false negative cytology. In this situation, clinical data and radiological findings may be of great help for a correct cytodiagnosis.

In conclusion, FNA of an ovarian mass is a minimally invasive procedure with an acceptable diagnostic accuracy, especially regarding differentiating benign from malignant lesions, and can be considered as a useful diagnostic modality for choosing the appropriate course of management.

Acknowledgements

The authors would like to thank Shiraz University of Medical Sciences, Shiraz, Iran, the Center for Development of Clinical Research of Nemazee Hospital, and Dr. Nasrin Shokrpour for editorial assistance.

Disclosure Statement

The authors have no conflicts of interest to declare.
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