Research Article

Evaluation of the relevance of touch imprint cytology in the diagnosis of various neoplastic lesions

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ABSTRACT

Background: Imprint is a very simple and rapid technique for tissue diagnosis. Imprint is a touch preparation in which tissue is touched on a slide and it leaves behind its imprint in the form of cells on the glass slide. In present study we have correlated the cytological diagnosis by imprint with histological diagnosis and tried to evaluate the accuracy and usefulness of this.

Methods: The study was conducted in department of Pathology of Pt J.N.M. Medical College, Raipur (C.G) India. From neoplastic lesions imprint smears were prepared immediately after resection of surgical specimen. After preparation of imprint smears specimens were processed by routine histopathological processing. Final reports of both processes were compared to know accuracy of diagnosis by imprint cytology.

Results: Out of total 110 cases, 25 cases were benign and 85 cases were malignant. Out of the 25 benign cases, 14 (56%) were diagnosed correctly and correlated with histological diagnosis. while 11 (44%) cases were false negative. No false positive cases were there. Out of 85 malignant lesions 78 (91.76%) were diagnosed correctly and correlated on histopathology, while 7 (8.23%) were false negative. Overall diagnostic accuracy by imprint smear after histological correlation was 83.63% increasing to 91.76% for malignant lesions.

Conclusions: With an accuracy rate of 83.63% we can say that imprint cytology is a quick reliable simple and cost effective procedure.

Keywords: Imprint cytology, Neoplastic lesions, Diagnostic accuracy

INTRODUCTION

Rapid and accurate diagnosis is of paramount importance in the outcome of medical care. The imprint cytology is one of the upcoming methods that can be used in the diagnosis of malignant and benign lesions in shorter period though the histopathology remains the gold standard.1 A correct diagnosis helps in initiating the specific therapy in time, thus reducing morbidity and mortality. Histopathology is the universally accepted means of establishing a definitive pathological diagnosis, whereas use of cytology is controversial. Imprint is a very simple and rapid technique for tissue diagnosis. Imprint is a touch preparation in which tissue is touched on a slide and it leaves behind its imprint in the form of cells on the glass slide. In present study we have correlated the cytological diagnosis by imprint with histological diagnosis and tried to evaluate the accuracy and usefulness of this method.

Objectives of the study

The objective of the study was to assess accuracy of imprint cytology as diagnostic modality by correlation with histological diagnosis and to study the merits and pitfalls of the imprint smear technique as a diagnostic modality.
METHODS

Study area

The study was conducted in department of Pathology of Pt J.N.M. Medical college, Raipur (C.G) India.

Sample source

Patients with neoplastic lesions were referred to department of pathology; imprint smears were prepared immediately after resection of surgical specimen.

Sample Collection

Imprint smears were prepared from freshly resected unfixed specimens of neoplastic lesions by touching glass slides on the surface, with special focus on suspicious looking area. Before smear preparation blood was removed from the surface of tissue by touching gently on dry gauze piece. Smears were immediately fixed in 95% alcohol and stained with Papanicolaou’s stain. Air dried smears were stained with May-Grunwald-Giemsa. Reporting on imprint smears was done by a pathologist with good experience in cytology reporting. After preparation of imprint smears specimens were sliced and fixed in 40% formalin. After proper fixation samples were processed by routine histopathological processing and sections were stained by Hematoxylin and Eosin. Final reports of both processes were compared to know accuracy of diagnosis by imprint cytology.

RESULTS

Table 1: Accuracy of imprint cytology in various organs.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Diagnosed correctly</th>
<th>False negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Breast</td>
<td>30</td>
<td>83.33</td>
<td>6</td>
</tr>
<tr>
<td>FGT</td>
<td>46</td>
<td>86.79</td>
<td>7</td>
</tr>
<tr>
<td>MGT</td>
<td>1</td>
<td>50.00</td>
<td>1</td>
</tr>
<tr>
<td>GIT</td>
<td>3</td>
<td>75.00</td>
<td>1</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>5</td>
<td>71.43</td>
<td>2</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2</td>
<td>100.00</td>
<td>-</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>2</td>
<td>100.00</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>75.00</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>83.33</td>
<td>18</td>
</tr>
</tbody>
</table>

Present study included 110 subjects in which imprint smears were prepared from various organs (Table-1).

Table 2 and table 3 are showing correlation of imprint smear with histopathological diagnosis in various benign and malignant lesions.

Out of 36 breast lesions included in study, 10 were benign and 26 malignant. Among benign lesions there were 7 fibroadenomas and one case each of fibroadenosis, fibrocystic disease, and simple fibrosis. Imprint smears were unsatisfactory for latter three and one case of fibroadenoma, while six cases of fibroadenoma were correctly diagnosed by imprint smear.

Table 2: Correlation of imprint cytology with histopathological diagnosis in various benign lesions.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Diagnosed correctly</th>
<th>False negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Breast</td>
<td>6</td>
<td>60.00</td>
<td>4</td>
</tr>
<tr>
<td>FGT</td>
<td>6</td>
<td>54.54</td>
<td>5</td>
</tr>
<tr>
<td>MGT</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2</td>
<td>100.00</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>56.00</td>
<td>11</td>
</tr>
</tbody>
</table>

Malignant lesions constituted 25 infiltrating duct carcinoma and one cystosarcoma phylloids with borderline malignancy. By imprint cytology 24 cases were correctly diagnosed and 2 smears were unsatisfactory. Out of total 36 breast lesions, 30 (83.33%) were diagnosed correctly and 6 (16.66%) were unsatisfactory in imprint smear.

Figure 1: Imprint cytology of Fibroadenoma.

Figure 2: Imprint cytology of Warthin’s Tumour.

Among 53 lesions of female genital tract included in study, 38 were from cervix (37 cases of cervical carcinoma, and one case of cervical leiomyoma), and 8 were from uterus (leiomyoma), and 7 from ovary (2 cases each of mucinous cystadenoma and dysergerminoma and
one case each of serous cystadenocarcinoma, mucinous cystadenocarcinoma and mixed Mullerian tumour.

Table 3: Correlation of imprint cytology with histopathological diagnosis in various malignant lesions.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Diagnosed correctly</th>
<th>False negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Breast</td>
<td>24</td>
<td>92.30</td>
<td>2</td>
</tr>
<tr>
<td>FGT</td>
<td>40</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>GIT</td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Soft tissue</td>
<td>5</td>
<td>83.33</td>
<td>1</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>75</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>91.76</td>
<td>8</td>
</tr>
</tbody>
</table>

Among cervical lesions 35 carcinomas were correctly diagnosed while 2 carcinomas and one leiomyoma were unsatisfactory on imprint smear. Out of 8 uterine leiomyomas, 4 were correctly diagnosed and 4 were unsatisfactory on imprint smear. In ovary out of 2 benign cystadenomas both were correctly diagnosed. Among malignant cases one serous cystadenocarcinoma, two dysgerminomas, one mucinous cystadenocarcinoma and one mixed Mullerian tumour were correctly diagnosed by imprint smear. Out of 53 cases of female genital tract 46 (86.79%) were diagnosed correctly and 7 (13.20%) were unsatisfactory.

Figure 3: Imprint cytology of malignant fibrous histiocytoma.

From male genital tract one case of correctly diagnosed testicular embryonal carcinoma was there. While in one case of nodular hyperplasia imprint smears were unsatisfactory. Out of 2 cases from male genital tract 1 (50%) was correctly diagnosed and 1 (50%) was unsatisfactory.

From gastrointestinal tract 3 cases of adenocarcinoma colon and one case of non-Hodgkin’s lymphoma were there in the study. Out of 3 cases of adenocarcinoma colon two were diagnosed correctly and one was unsatisfactory. One case of non-Hodgkin’s lymphoma was diagnosed correctly on imprint smear. Out of 4 cases of gastrointestinal tract, 3 (75%) were correctly diagnosed and 1 (25%) was unsatisfactory.

Table 4: Diagnostic accuracy of imprint cytology in benign and malignant lesions.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Diagnosed correctly</th>
<th>False negative</th>
<th>False positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Benign</td>
<td>14</td>
<td>56</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>78</td>
<td>91.76</td>
<td>7</td>
<td>8.23</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>83.63</td>
<td>18</td>
<td>16.36</td>
</tr>
</tbody>
</table>

Out of 7 cases of soft tissue lesions included in study, 3 cases of fibroadenoma, one case each of malignant fibrous histiocytoma and embryonal rhabdomyosarcoma were diagnosed correctly, while one case each of myxoid liposarcoma and neurilemmoma were unsatisfactory. Out of 7 cases, 5 (71.42%) were diagnosed correctly and 2 (28.57%) were unsatisfactory in imprint smears.

Figure 4: Imprint cytology of rhabdomyosarcoma.

From thyroid gland and salivary gland each 2 cases were included. From thyroid gland lesions follicular adenoma and nodular hyperplasia thyroid. From salivary gland lesions were warthims tumour and acinic cell carcinoma. All 4 were correctly diagnosed on imprint smear with 100% correlation.

From kidney 2 cases of wilm's tumour and 2 cases of renal cell carcinoma were included in study, out of which 2 cases of renal cell carcinoma and one case of wilm's tumour were diagnosed correctly on imprint smear. So out of 4 kidney lesions, 3 (75%) were diagnosed correctly and 1 (25%) was unsatisfactory in imprint smear.

Out of total 110 cases, 25 cases were benign and 85 cases were malignant. Out of the 25 benign cases, 14 (56%) were diagnosed correctly and correlated with histological diagnosis, while 11 (44%) cases were false negative. No false positive cases were there. Out of 85 malignant lesions 78 (91.76%) were diagnosed correctly and correlated on histopathology, while 7 (8.23%) were false negative. Diagnostic accuracy by imprint smear after histological correlation was 83.63%, 18 (16.36%) were false negative (Table-4).
DISCUSSION

In present study accuracy of imprint was 83.63% which was similar to the study of Rohit Sharma et al & Singh et al with 84% accuracy.\(^1\)\(^2\) We observed that sectioning the tissue before preparation of imprint smear along with removal of blood and tissue fluid from the surface by touching gently on dry gauze piece increased chances of getting a good imprint smear. Dudgeon and Barret, Tribe et al, Pilar & Rubenston and Amarjeet singh et al suggested some points to improve the accuracy especially.\(^3\)\(^6\)

1. The tissue surface to be imprinted should be flat and there should be no portion of fat protruding from the edges as these tend to smudge the imprint.

2. Sometimes the first imprint contains excess tissue fluid and blood and it was found that subsequent imprints give better cytological results.

3. The ease with which any tumor gets imprinted varies considerably. In order to obtain imprint smears of one cell thickness, the amount of pressure applied at the time of imprinting varies. Benign looking lesions usually require more pressure in order to obtain sufficient cells for diagnosis while malignant tumors get imprinted more easily.

4. Malignant tissue imprints were more cellular than those of benign looking lesions.

We also observed that accuracy of imprint smears increases by these methods.

In present study we observed that accuracy was more for malignant (91.76%) as compared to benign lesions (16.36%). The imprint smears from benign lesions were found to be hypo cellular and required more pressure while imprint from malignant lesions were hypercellular and required less pressure. Similar observation were made by other workers like Dudgeon and Barret, Tribe et al, Pilar and Rubenston, Solanki et al, Suen et al and Helpap et al.\(^7\)\(^9\) In benign condition cells appears in group and do not separate readily so the accuracy in benign lesions was low than malignant lesions.

Mitotic figures were less in number in imprint than corresponding paraffin section of malignant lesion. Kjellegren et al, Tribe et al and Singh et al noticed the same and hypothesized that cells in mitosis tend to rupture during imprinting.\(^5\)\(^8\)\(^10\)

Present study had 18 (16.36%) of false negative cases. Suen et al considered that false negative reports may be due to one of the following reasons - (a) Interpretative errors in well differentiated tumor (b) Insufficient cells, lack of clarity of cellular structure and indefinite character of tumor cells.\(^5\) In the present study insufficient material was the main cause behind the false negativity.

Cellularity was slightly low in Papanicolaou’s stained smear than May-Grunwald-Giemsa stained because of loss of cells during wet fixation but cellular features and nuclear details were good in Papanicolaou’s stained smear for diagnosis. Contrary to this finding, Tribe and Auzermanian in their study on imprints did not find the Papanicolaou’s stain helpful for detail morphological study, both of them preferred the May-Grunwald-Giemsa.\(^5\) In the present study we found smears stained by both stains contain good cellularity and cellular details, so we recommended that both stains are useful. Staining quality also depends upon the thickness of the smear, thick smears stained dark. For good diagnostic accuracy smear should be only of one cell thickness.

In spite of giving definite diagnosis and having high success rate biopsy diagnosis is dependent upon processing techniques and long-time involved in these techniques. For any lesion surgeons want to have definitive diagnosis before surgery as it helps in planning for surgery and patient counselling. Sometimes this planning is not possible because either fine needle aspiration cytology/biopsy is not performed for fear of needle tract metastasis/rupture of capsule or report is inconclusive.\(^11\) In such scenario imprint smears can prove useful as they will avoid a two stage procedure of initial biopsy and then surgical excision. Their utility is also highlighted when during surgical exploration for a benign lesion surgeons encounter a suspicious lesion and wants to quickly rule out malignancy, as this can avoid a repeat operative procedure by change of intraoperative surgical plan.

Various authors have compared imprint to frozen section and found that imprint cytology is a quick, reliable, simple and cost effective procedure which can be used intraoperatively. Imprint method has the advantage of excellent preservation of cellular details, no tissue loss, no freezing artifacts.\(^12\)\(^13\) No requirement of any specialized instruments and less time required to make diagnosis makes it useful as an intra-operative diagnostic procedure in less equipped centres without facilities of frozen section.\(^14\) Processing and reporting on very small tissue fragments is difficult on frozen section but they provide sufficient cells for imprint cytology and tissue is saved for permanent histopathology sectioning.\(^15\)

There are some drawbacks regarding the imprint cytology. It is not reliable for providing the information on the depth of infiltration of tumor, although it might provide information on the original site of tumor histogenesis and the histological pattern. That information is based on spatial inter relationships between cells and three dimensional shape of individual cells, in situ carcinoma for example cannot be diagnosed by imprint smear. Another disadvantage is that certain percentage of misdiagnosis may occur. Well-differentiated tumors and tumors with a dense fibrous stroma cannot be diagnosed by imprint cytology method.\(^8\)
CONCLUSION

With an accuracy rate of 83.63% we can say that imprint cytology is a quick reliable simple and cost effective procedure which can be used intraoperatively. Imprint cytology does not alter the quality of biopsy specimen. No requirement of any specialized instruments and less time required to make diagnosis makes it useful as an intra-operative diagnostic procedure in less equipped centers without facilities of frozen section. In spite of drawbacks it can be concluded that imprint is a simple, fast, easy and reliable technique for the diagnosis of malignant tumors. However it is not a substitute of conventional histopathology but can be complementary to it.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES


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