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Histopathological Correlation of Lymph Nodes Imprints

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Abstract:
Introduction: *This study was done to evaluate the role of imprint smear cytology in various diseases affecting the lymph nodes. A correlation was done between the imprint smear cytology and histopathology to assess the accuracy and reliability of the imprint cytology in different lymph node lesions.* ***Methods****: From 52 cases of lymph node excision, imprint smears were taken and stained with PAP, H & E and MGG. The findings in imprints were compared with those of histopathology. With the help of sensitivity, specificity & kappa (k) score, the agreement between the two methods was determined.* ***Results****: Among the total 52 cases of imprint smears, 44 cases from breast carcinoma were reactive, among them 8 cases turned positive in histopathological section but negative in imprint. So, total reactive cases were 36.Out of the cases suspected to be Non-Hodgkin's lymphoma in imprint, 1 small cell lymphoma in imprint was finally diagnosed as mantle cell lymphoma in histology, & 1 large cell lymphoma in imprint was finally diagnosed as diffuse large B cell lymphoma in histology, 4 other cases were turned to be reactive in both imprint and HP. Hence the total reactive cases were 40.One (1) metastatic carcinoma from colon & 1 from gallbladder carcinoma were correctly diagnosed by imprint.* ***Interpretation and Conclusion****: Imprint smears showed almost perfect agreement in majority of the lesions. The technique was simple, rapid and can be used routinely as an adjunct to histopathology of lymph node lesions.*

Keywords: *Imprint smear, Lymph node, Non-Hodgking's lymphoma*

I. Introduction

The imprint cytology had been in use as a rapid and standard method for diagnosing lymph adenopathies since several years. Forkner was the first person to utilize imprint technique for cyto-diagnosis in lesions of excised lymph nodes.

In the later half of the 19th century various studies published on imprint cytology of lymph nodes had proved it to be an useful adjunct for histopathological diagnosis of inflammatory, granulomatous, lymphomatous and metastatic lesions of lymph nodes.

II. Materials & Methods

The material for this study was taken from 52 patients in R.G. Kar. Medical College who underwent lymph nodes excision biopsy. Clinical data collected in each case were: Age and sex, Signs and symptoms, Sites of lymph nodes involved, associated clinical findings, relevant investigation.

The excised lymph nodes were transported immediately to the laboratory. The gross features of the node were noted like: Size & Appearance of outer surface.

After describing the gross features, it was sliced into two halves. The sliced half was held gently either with forceps or with one hand so that the flat cut surface faced upwards; with the other hand, using grease free clean glass slides, four imprint smears were prepared. The lymph node was then fixed in neutral formalin and processed for histopathological examination. Wet fixed smears were stained with PAP and H & E. The air dried smear was stained by MGG stain. Special stains such as AFB stain, reticulin were also done in certain cases.

The stained smears were assessed for the following features:

1. Cellularity: Hypo-cellular - if number of lymphocytes < 50/smear
Moderately cellular - if number of lymphocytes = 50/smear
Hypercellular - if number of lymphocytes > 50/ smear

2. Distribution: Monotonous or Polymorphous

3. Cell Types: Reactive lymphoid cells**,** Tingible body macrophages**,** Atypical lymphophoid cells**,** Granuloma**,** Foreign cell.

According to the finding as noted the lesions were classified into a particular type of lesion .The accuracy of imprint diagnosis was determined by comparing with the corresponding histopathological diagnosis.

The diagnosis that agreed with the histopathological diagnosis was considered accurate and overall accuracy rate or sensitivity & specificity was calculated. The sensitivity for each condition of lymph nodes was calculated. The agreement between the imprint smear diagnosis and histopathological diagnosis was calculated using kappa score.

III. Results

In our study, out of 52 cases – 2 were metastatic and another 2 were lymphoma. Grossly the metastatic lymph nodes were enlarged in size. Outer surface was grey, cut surface chalky white in colour. The signs and symptoms of two metastatic diseases were nonspecific, mimicking chronic cholecystitis (pain, anorexia, elevated alkaline phosphatase). Upper abdominal pain and increased serum alkaline phosphatase level were the most common findings at presentation in case of gallbladder carcinoma metastatic (Fig 1 &2).[12]

Colorectal cancer was also asymptomatic for years; symptoms developed insidiously and frequently had been presented for months, associated with clinical attention by the appearance of fatigue, weakness, and iron deficiency anemia, occult bleeding, changes in bowel habit, or crampy left lower quadrant discomfort. Both the cases were associated with enlarged lymph nodes (Fig 3&4).[12]

Most patients presented with fatigue and lymphadenopathy and were found to have generalized disease involving the bone marrow, spleen, liver, and (often) the gastrointestinal tract, in mantle cell lymphoma.[12]

Total case = 52Actual reactive = (44 – 8) + 4= 36 +4= 40(8 cases in metastatic carcinoma were +ve in histology but –ve in imprint.4 cases suspected of NHL turned to be reactive both in histology & imprint.). Imprint smears of reactive lymph nodes showed mixed population of cells representing the whole range of lymphocyte transformation, tingible body macrophages & associated with predominance of small lymphocytes in cases of reactive lymph nodes.[7]

Histological section of reactive nodes showed follicles with different size and shape, the margins of which were sharply defined, surrounded by mantle of small lymphocytes arranged circumferentially as an onion skin pattern and consisting of admixture of small, large lymphoid cells with irregular nuclei, numerous mitosis & tingible body macrophages(Fig & 6).[8]NHL = 2 (Both histology & imprint +ve)

In imprint small cell lymphoma showed pleomorphic small cells with coarse clumped chromatin, inconspicuous nucleoli, scanty cytoplasm and few prolymphocytes .[7]Histology showed lymphoid cells infiltrated as diffuse, vaguely nodular or nodula pattern where cells proliferated as broad mantles around residual reactive germinal centres with coalescence and extention into the interfollicular regions. Proliferative centres were absent butepitheloid histiocytes were seen.[9]

Imprint smear of DLBCL showed pleomorphic large cell population dominated by round nuclei, unevenly distributed nuclear chromatin, prominent usually single central nucleoli, occationally multiple nucleoli with moderate to abundant bluish cytoplasm.[7]

Histology of DLBCL showing lymphoid structure had been effaced by diffuse pattern of growth of large cells, which had round to oval nucleus that appeared vesicular due to margination of chromatin to the nuclear membrane, but large multilobated or cleaved nuclei Cells had moderately pale or basophilic cytoplasm and prominent centrally or eccentrally placed nucleoli.[10]

Metastatic carcinoma = 2 (Both histology & imprint +ve)

Imprint smear of mucinous metastatic colonic adenocarcinoma (Figure-6) showed discohesive three dimensional aggregates of tumor cells. Branching papillary fragments and microacinar areas might be present in the pool of mucin. Cell groups showed loss of polarity
with crowded disorderly arrangement.

Tumor cells had round, oval or cigar shaped nuclei, and many single cells. There was a prominent "dirty" tumor diathesis.[14] Histologically metastatic lymph node of colonic adenocarcinoma showed glands or irregular cluster of cells which were round to oval in shape, nuclei were uniform retained a basal location and surrounded by mucus. [9] Imprints of gallbladder adenocarcinoma (Figure - 4) showed loosely structured clusters of cancer cells with irregular large nuclei and very prominent nucleoli.[11] Histologically metastatic lymph node of gallbladder adenocarcinoma showed the cells which were arranged in nests, sheets, cords or tubular pattern and had irregular shaped hyperchromatic nuclei, scanty ill defined cytoplasm with inconspicuous nucleoli.[9]

IV. Discussion

Imprint smear study was simple, speedy and provided excellent cytomorphological details. The imprint smears were prepared from 52 freshly excised lymphnodes in ours study, we got 2 NHL and 2 metastatic deposit out of 52 total cases. There were no cases of Hodgkin's disease in the present study. The smears except for few were adequate when assessed by quality of stain, cellularity and dispersion of cells.[1,2,3]

In our study among two cases (4%) : one was small cell lymphoma in imprint which was finally diagnosed as Mantle zone lymphoma and other large cell lymphoma in imprint was identified as diffuse large B cell lymphoma. Only two cases were too small for any comments. More number of cases needed to be studied.

Agarwal et al[1], Patra et al [2], Ultman et al [4], Nagpal et al [3] etc. had observed the same type of pathology e. g. – lymphomas.

Agarwal et al[1] & Nagpal et al[3] observed an incidence 0f 10 to 14 %. The sensitivity in NHL among workers viz. Agarwal, Patra, Ultman and Nagpal ranged between 3-7%, which was comparable to the present study though the number of cases was low. Nagpal et al[3] found that smears had an even distribution of cells, with fewer distortions and good coloration of all cellular components providing maximal cytological details. The examination of smears in such cases revealed uniform results.

Suen et al[5] compared imprints and frozen sections of 198 lymph nodes. Their accuracy rate was 93.6%. They emphasized the usefulness of imprint smears over lesions missed by frozen sections.

Nagpal et al[3] also gave the importance of imprint smears compared to that of histopathological sections and commented that, lymph nodes being cellular organs, were poorly fixed, for which taking of thin sections became very difficult. Moreover there might be shrinkage of the cells.

In the present study histopathological sections were adequate for comments concerning histological types. But this investigation was invaluable to diagnose the various categories that could help in the further management.Of the 10 cases of metastatic lesions in lymph nodes only 2 cases were picked up by imprint smears. Here the accuracy rate of 2 metastatic deposit was 20%.

In another study by Ultmann et a[l3] the accuracy for metastatic was found as 94%. They found that diagnosis of secondaries in a lymph node from tumour else where in the body did not present any diagnostic problem unless it was a sclerotic lesion. This was also observed in the present study. As the abnormal cancer cells were invariably present in the smears which were absolutely foreign to the lymphoid elements the detection did not cause any difficulty[3].

Wood WS et al[5] commented that hypocellularity and in adequate material were the most probable cause of false negative diagnosis in imprint. It occurred generally due to one of the following two reasons:

A. Insufficient cells - there was a dense fibrous stroma in some tumors, such as the linitis plastic type of gastric carcinoma, the breast carcinoma, the gall bladder carcinoma, some fibrous soft tissue tumors etc. In these cases the number of neoplastic cells transferred to the slide was insufficient to enlable the observer to make a correct diagnosis.

B. Interpretative errors – these occurred in cytological well differentiated tumors.

This present study showed that the detection of cancer cells were difficult in cases where there was extensive fibrosis, which was discernable in the histopathological sections of the particular lymph nodes. 8 cases failed to reveal metastatic deposit due to extensive fibrosis in lymph nodes.

Hasenburg et al[6], Tatomirovie Zet et al[l,2,3] reported a sensitivity rate of 90 to 93% and Jain et al[15] reported 100% sensitivity where they had included histopathology along with imprint for such comments. The present study showed 100% sensitivity when histopathology is included. The fact of extensive fibrosis was indeed a case of concern when only imprint smears were available for interpretation. The diagnostic sensitivity was hampered by the fact that small metastatic deposits confined to the sub capsular sinus and single cell metastatic was missed. However early micro metastases rarely produced significant lymph node enlargement and a lymph node if palpable was likely to contain enough tumor tissues to be detected.[7] Among the metastatic lesions seen in our study the incidences were of metastatic mucinous adenocarcinoma of colon & poorly differentiated adenocarcinoma of gall bladder.

The 52 samples of lymph nodes biopsies included in the study. During the period of study, lymph nodes were excised after clinical diagnosis of neoplastic lesions. Only 12 cases showed neoplastic changes, the rest showed reactive changes and thus acted as controls in the study. It was needless to point out that not all lymph nodes draining malignant lesions would show incidence of metastatic deposits. Many might show only reactive changes. Provision for these different stains in a country like ours should help in diagnosing the pathology in quite a few instances.

V. Conclusion

Imprint cytology is a type of applied cytology. The imprint smears reveal very subtle diagnostic changes in the various lymphoid cells and make it possible to arrive at a diagnosis in shorter period of time.

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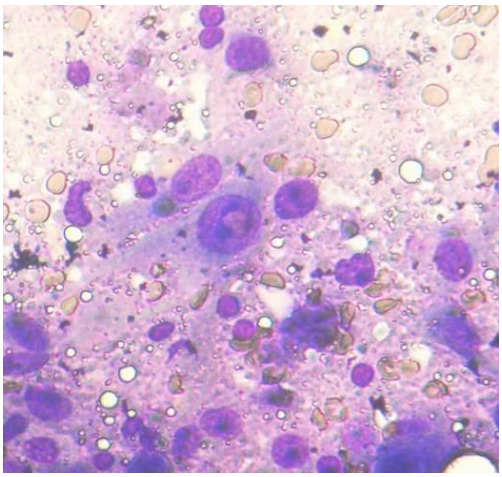

Figure – 1: Imprint Smear From Poorly Differentiated Adeno Carcinoma, Gall Bladder (MGGX400)

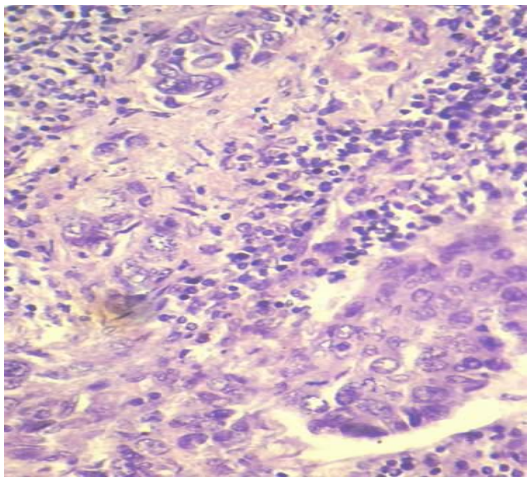

Figure – 2: H.P. Section From Poorly Differentiated Adeno Carcinoma. Gall Bladder (H&E X 400)

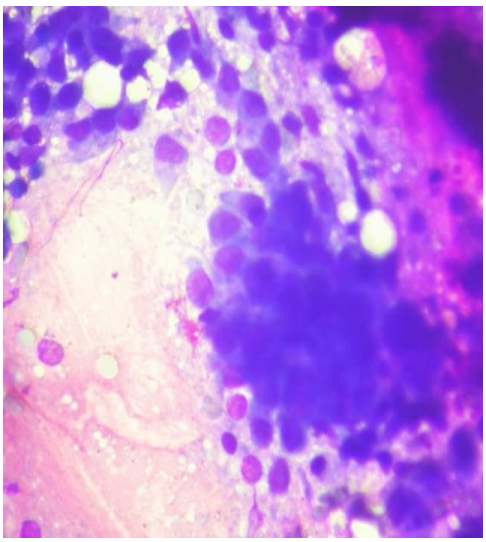

Figure – 3: Imprint Smear In Metastatic Mucinous Adeno Carcinoma Colon (MGGX 400)

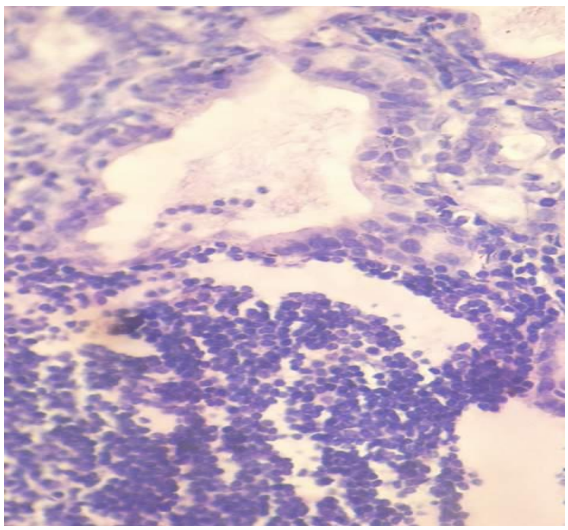

Figure – 4: H.P. Section From Metastatic Mucinous Adeno Carcinoma Colon (H&E X 400)

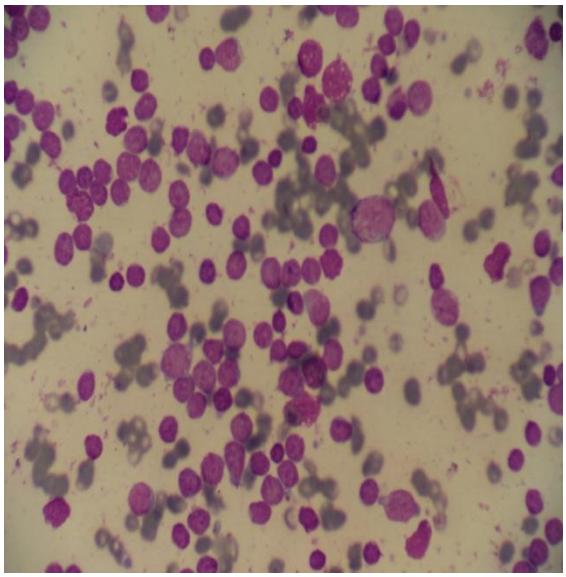

Figure – 5: Imprint Smear Of SLL (H & E X 400)
Cervical Lymph Node

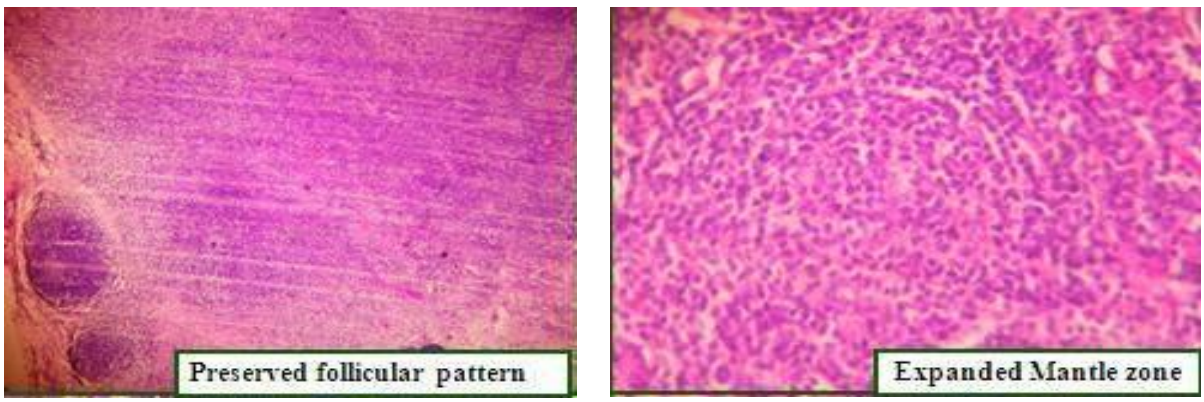

Figure – 6: H.P. Section Showing Mantel Cell Lymphoma (H&EX400)