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Original Research Article

FINE NEEDLE ASPIRATION CYTOLOGY DIAGNOSIS OF LYMPHOPROLIFERATIVE DISORDERS AND THEIR CORRELATION WITH IMMUNOHISTOCHEMICAL STUDY OF CD3, CD20, CD15 AND CD30 ON CELL BLOCK PREPARATION.

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Abstract

Background: Lymphoma is used for lymphoid neoplasm that present as discrete tissue masses. They are divided into Hodgkin's lymphoma and non-Hodgkin's lymphoma. Fine needle aspiration cytology (FNAC) sometimes gives false negative interpretation in diagnosis of lymphoma and typing of lymphoma is not possible from FNAC. This can be overcome by cell block (CB) preparation. Histopathological examination is the gold standard in diagnosis of lymphoma and typing of lymphoma is also possible. Lymph node biopsy is associated with complications like lymphedema, allergic reaction to dye and nerve injury. Aim of this study is to correlate cytomorphology on FNAC smears with histomorphology and immunohistochemical findings on the cell block preparation in diagnosis of lymphoma. **Materials and Methods:** Hospital based calculation study was carried out in Department of pathology in collaboration with Department of Radiation oncology RIMS, Imphal. FNAC was performed on cases clinically suspicious of lymphoma and cell block preparation was also done. Cytomorphological, histo-morphological and immunohistochemical findings were done for all the cases and recorded and analyzed. **Results and Observation:** Sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the diagnosis of lymphoma were higher for the histo-morphological examination along with IHC study on the CB preparation compared to the FNAC study on the conventional smear preparation. **Conclusion:** Combined approach along with the IHC study on the CB preparation increases the accuracy in diagnosis of lymphoma and helps in typing of lymphoma as well especially in patients who are at risk for undergoing lymph node biopsy.
Keywords: Fine needle aspiration cytology, cell block, lymphoma

Introduction:

The term lymphoma is used for lymphoid neoplasms that present as discrete tissue masses. Within the large group of lymphomas, Hodgkin lymphoma (HL) is segregated from non-Hodgkin lymphoma (NHL). Two thirds of NHLs and virtually all Hodgkin lymphomas present as enlarged non-tender lymph nodes (often >2cm).[1] Hodgkin lymphoma (HL) accounts for a little less than 1% of all cancers globally. It has a bimodal incidence curve, occurs between 15 to 35 years of age or after 55 years of age. HL is classified nodular lymphocyte predominant Hodgkin's lymphoma and classical Hodgkin's lymphoma which is further sub-classified into nodular sclerosis, mixed cellularity, lymphocyte rich and lymphocyte depleted.[2] Lymphomas other than Hodgkin lymphomas comes under non Hodgkin lymphomas they arises from both T cells, B cells and NK cell origin. Most recent system of classification of non-Hodgkin lymphoma is 2016 updated World Health Organization classification.[1] Fine needle aspiration cytology (FNAC) is a simple, inexpensive, less traumatic, safe and fast diagnostic procedure to diagnose superficial lumps. It is widely used in the investigation of lymphadenopathy, and offers very quick preliminary diagnosis with minimal trauma to the patient. FNAC sometimes does not yield information for precise diagnosis and there is always the risk of false negative diagnosis and indeterminate diagnosis. In these cases, cell block (CB) preparation may be helpful. As morphology and immunophenotype are necessary for the diagnosis of most lymphoid neoplasms, FNAC alone is not sufficient, which can be overcome by cell block preparation.[3,4] The cell block technique has been in use for more than a century. Some of the most common techniques include inverted filter sedimentation, collodion bag, thrombin method, simple sedimentation, and milli pore filtration among others. The residual materials from fine needle aspiration are routinely processed into cell blocks, which are composed of random cells and tissue fragments., which could provide morphology and partial histological structures and can be sectioned for immuno-histochemical staining. Cytological features from the conventional smear along with the

morphology and immunophenotype of cell blocks aid in the diagnosis and classification of lymphoma. Cell blocks maintain architecture which closely resembles that seen on surgical specimens.[5,6,7] Hematoxylin and eosin (H&E) stained sections of a high-quality CB can provide both cytologic and architectural detail to support the cytomorphologic findings on direct smears. A good CB can be very useful for IHC staining as well as cytochemical staining and molecular diagnostic studies such as fluorescence in situ hybridization, polymerase chain reaction, and next generation sequencing.[8] Immuno-histochemistry (IHC) is essential for the diagnosis and sub-typing of lymphoma. The basic IHC panel for suspected NHL includes antibodies against B cell (CD 20) and T cell (CD3) antigens and for suspected HL includes antibodies against CD 45RB, CD15 and CD30.[9] Histomorphology examination is the gold standard in diagnosis of lymphoma. As lymph-node biopsy is associated with complications like lymphedema, allergic reaction to dye and nerve injury, I am undertaking this type of study to find out the efficacy of histopathological and immunohistochemical study on cell block prepared from the aspirates in diagnosis and sub classification of lymphoma.

Objectives

1. To assess the cytomorphology of lymphoproliferative lesions on FNAC smears.
2. To assess the histomorphology and typing of lymphoma cases using immunohistochemical markers CD3, CD20, CD15 and CD30 on cell block prepared from the FNAC aspirates.
3. To correlate cytomorphology on FNAC smears with histomorphology and immunohistochemical findings on the cell block preparation in diagnosis of lymphoma.

Materials and Methods

Hospital based cross sectional study was carried out for two years starting from September 2018 to August 2020 in the Department of Pathology in collaboration with Department of Radiation Oncology, RIMS, Imphal. Patients attending Cytology OPD and Radiation Oncology Department OPD who are clinically suspicious of lymphoma were the study population. The sample size was calculated using formula $4pq/l^2$, where prevalence of lymphoproliferative disorders were taken as 4% according to the study conducted by Saha I et al[10] and allowable error was taken as 5. Sample size was found to be 96 and all the samples were selected purposively. FNAC was performed without anesthesia using 20 ml syringe with 22 G needle aspirator for cell block preparation. FNA specimens were collected in more than 2-5 needle insertions, followed by direct smear preparation. Material was also expelled from the fine needle aspiration to ethanol formaldehyde mixture in the ratio (1:9). After overnight fixation material was transferred to a 50ml conical centrifuge tube and centrifuged at 2400 rpm for five minutes to achieve a concentrated cell button. CB was then processed for regular histological sections. For FNA smears we used Giemsa stain and for cell block we used hematoxylin and eosin stain and monoclonal CD3, CD20, CD15 and CD30 for immunohistochemical staining. Cells were regarded as CD3 positive when immunoreactivity was observed in the cytoplasm in perinuclear position of malignant cell, CD20 when it was observed in the cell membrane of malignant cell.[11] Reed Steinberg cells of Hodgkin's lymphoma display a characteristic pattern of immunoreactivity with membranous staining combined with staining of golgi apparatus by CD15 and CD30.[12] The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of FNAC in diagnosis of lymphoproliferative disorders were calculated considering histopathological along with the immunohistochemical findings on cell block as standard. McNemar's test was applied and P value less than 0.05 were considered statistically significant. Ethical approval was obtained from the Research Ethics Board, RIMS, Imphal with reference number A/206/REB-Comm(SP)/RIMS/2015/441/59/2018, dated 30th Jan 2019. Informed written consent was taken from the participants for the study before recruitment. A code number was assigned and no names were taken to maintain confidentiality. Data collected were kept secured.

Results and Observation

FNAC of the lymph node were performed in 97 cases in the present study. In all the cases cell block preparations were also done along with immunohistochemical stains. All the findings were recorded and analyzed.

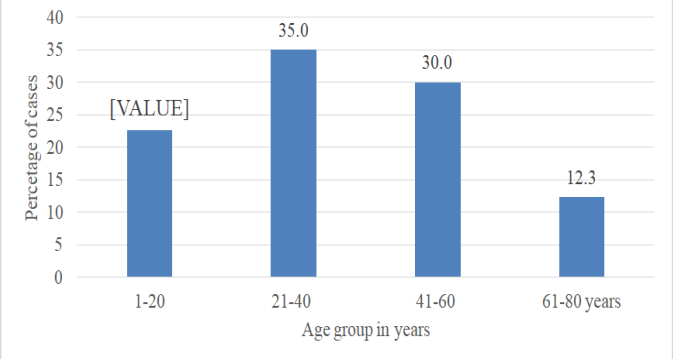

Fig 1: Percentage of cases in each age group (N= 97)

As shown in fig 1, the age range of the patient spans from 1-80 years old. The most common age group is 21-40 years (35%) followed by 41-60 years (30%).

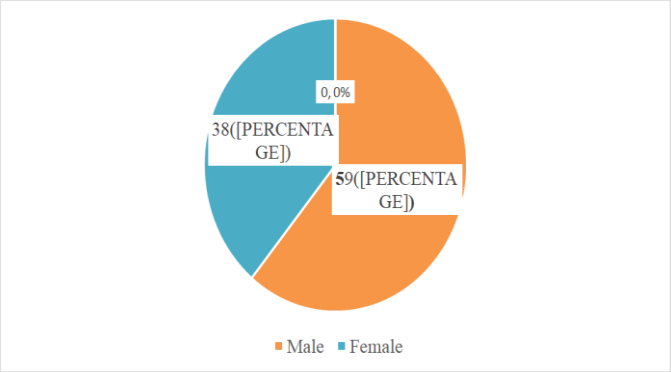

Fig 2: Sex wise distribution of cases (N= 97)

As shown in fig 2, 59(61%) cases were males and 38(39%) cases were females out of the 97 cases participated in the study.

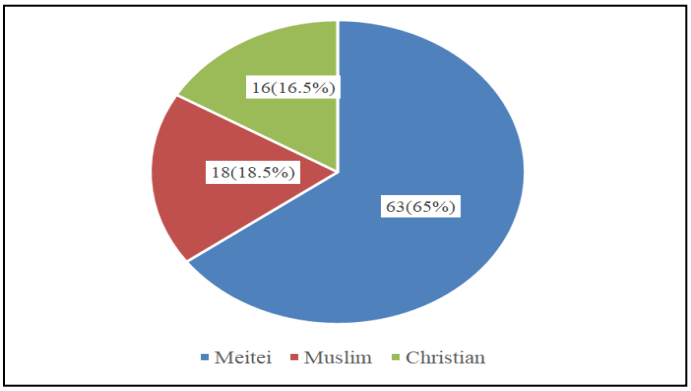

Fig 3: Religion wise distribution of cases (N= 97)

As shown in fig 3, out of the 97 cases participated in the study 63(65%) cases were from the Meitei religion, 18(18.5%) cases were from the Muslim religion and 16(16.5%) cases were from the Christian religion.

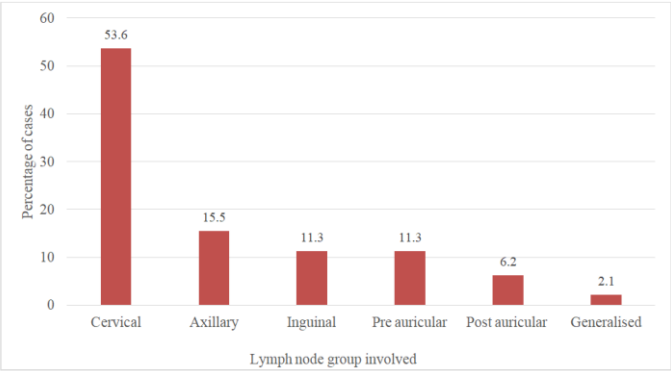

Fig 4: Percentage of cases in each involved lymph node group (N= 97)

As shown in fig 4, in maximum number of cases the involved lymph nodes were from the cervical region around 52(53.6%) cases followed by the axillary region around 15(15.5%) cases

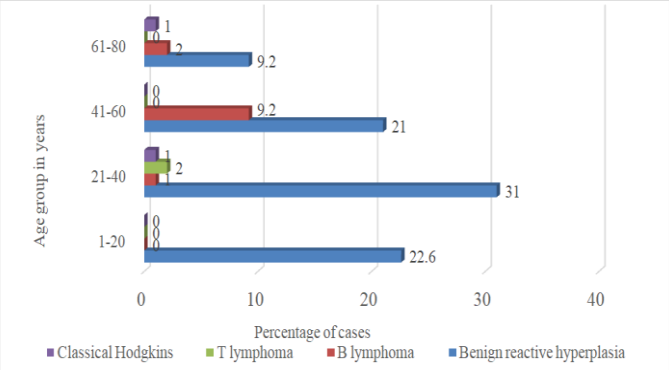

Fig 5: Age wise distribution of cases (N= 97)

As shown in fig 5, the most common diagnosis was benign reactive hyperplasia consisting of 81(83.5%) cases. Other cases include 12(12.2%) cases of B cell lymphoma (fig 6) , 2(2.0%) cases of T cell lymphoma and 2(2.0%) cases of classical Hodgkin lymphoma. The maximum number of B cell lymphoma was reported between the age group of 41-60 years (9 cases), 2 cases of T cell lymphoma was reported between the age group of 21-40 years and 2 cases of classical Hodgkin lymphoma shows bimodal age group distribution.

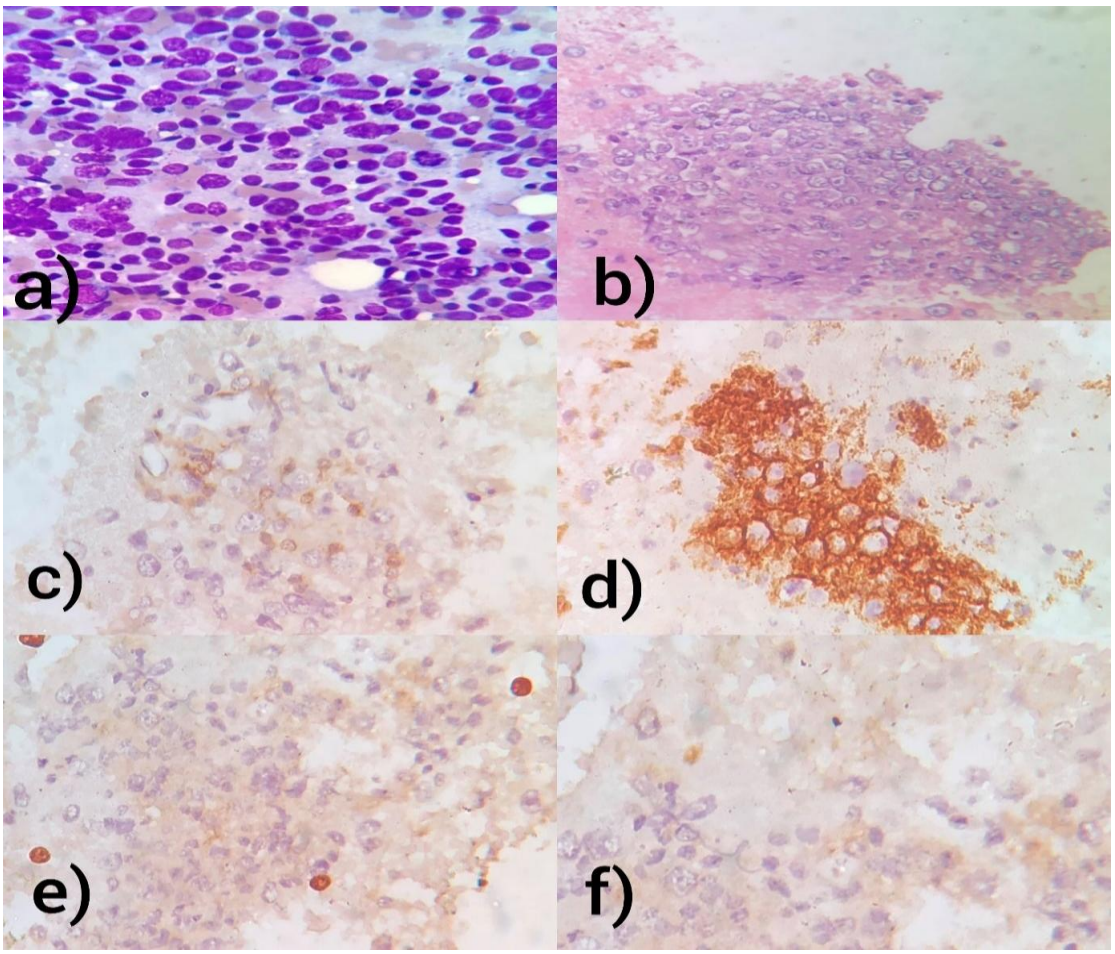

Fig 6: Photomicrograph of a B cell lymphoma case presenting with right level III cervical lymphadenopathy a) cytomorphology showing malignant lymphoid cells in dispersed singles [Giemsa,400X] b) histomorphology on cell block showing malignant lymphoid cells with irregular nuclear membrane, vesicular nuclei with some showing prominent nucleoli [H&E,400X] c) malignant lymphoid cells are CD3 negative [CD3,400X] d) malignant lymphoid cells are CD20 positive [CD20,400X] e) malignant lymphoid cells are CD15 negative [CD15,400X] f) malignant lymphoid cells are CD30 negative [CD30,400X]

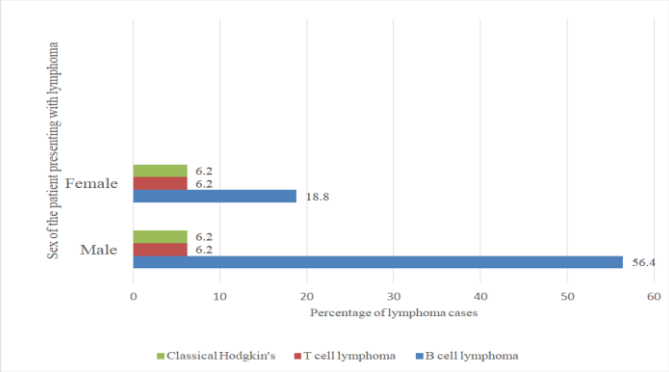

Fig 7: Distribution of various types of lymphoma between males and females (n= 16)

As shown in fig 7, of the 16 lymphoma cases 11(68.8%) cases were reported in males and 5(31.2%) cases were reported in females. B cell lymphoma is the most common lymphoma in both males and females.
As shown in table 1, of 97 cases the number of lymphoproliferative cases diagnosed by FNAC were 9(10.3%) cases out of which 1 case turned out to be false positive and out of 88(90.7%) NSRL cases diagnosed by FNAC 8(9.0%) cases were turned out to be false negative on comparison with the histopathological and IHC study on CB preparation.

Table 1: Histo-cyto correlation of the cases (N= 97)

HISTOMORPHOLOGICAL EXAMINATION ALONG WITH IHC STUDY ON CB				P value
FNAC	DIAGNOSIS	LYMPHOMA	BRH	0.039
	LYMPHOMA	08	01	
	NSRL	08	80	

BRH → benign reactive hyperplasia
NSRL→ nonspecific reactive lymphadenitis
Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated for FNAC diagnosis of lymphoproliferative disorders and were found out to be 50%, 98.7%, 88.8%, 90.9% and 90.7% respectively.

Sensitivity, specificity, PPV, NPV and accuracy for the diagnosis of lymphoma were higher for the histomorphological examination along with IHC study on the CB preparation compared to the FNAC study on the conventional smear preparation. Combined approach along with the IHC study on the CB preparation increases the accuracy in diagnosis of lymphoma and helps in typing of lymphoma as well.

The sensitivity of the two methods was compared using McNemar's test. A p value of 0.039 was observed and significant difference in the sensitivity was observed between the two methods in the diagnosis of lymphoma.

Discussion

FNA of lymph nodes is particularly useful for the diagnosis of lymphoma. A primary diagnosis of malignant lymphoma based on cytological morphology however is not adequate. One of the constraints of the FNA smear is the limited availability of material for adjuvant diagnostic investigations. The CB technique overcomes these limitations of FNA smears in diagnosis of lymphoma. Cell Blocks are very useful not only for visualizing tissue architecture but also for performing additional immunohistochemical studies to further classify lymphomas.[4]

Size of the aspiration needle used for making CB in this study was 22 G which is smaller in size compared to the 21 G needle that was used for CB preparation in the study by Zhang S et al[4]. Our concern was whether patients could tolerate excessive bleeding without anaesthesia. In our study we found out that the quick insertion of the needle several times when a small amount of bleeding appears may allow more specimens to reach the shank and the tube, thus ensuring the collection of sufficient amount of sample for CB preparation. Zhang S et a[4] also used the same technique for preparation of CB from the LN aspirates in their study and concluded that the results were optimal with the usage of this technique.

In this study it was observed that the predominant lesion encountered from the LN aspirate was BRH (83.5%) which is similar to the study conducted by Duraiswami R et al[13] (49.8%), however the percentage of cases were higher in our study whereas the studies conducted by Zhang S et al[4] and Roy A et al[14] where lymphoma was the predominant lesion encountered from the lymph node aspirate.

The occurrence of NHL was higher in males compared to females in this study which is in concordance with the study by Roy A et al[14] , Naresh KN et al[15], Kalyan K et al[16] and Padhi S et al[17].

The occurrence of lymphoma encountered in this study was 16.5% which is relatively low compared to the study conducted by Zhang S et al[4] and Basnet S et al[18] in whose study the occurrence of lymphoma was 54.8% and 33.3% respectively and is higher than the study conducted by Duraiswami R et al[13] in whose study the occurrence of lymphoma was around 1.6%.

In this study B cell lymphoma constitutes the highest percentage of lymphoma cases reported around 75% which is higher than the study conducted by Paul T et al[19] in whose study the percentage of B cell lymphoma was reported around 61.5% and is lower than the study conducted by Sharma M et al[20] in whose study the percentage of B cell lymphoma constituted around 89.3%.

T cell lymphoma in this study constituted around 12.5% which is in concordance with the study conducted by Sharma M et al[20] in whose study the percentage of T cell lymphoma reported around 10.7%.

The male gender (60.8%) was predominant among the patients who underwent FNA evaluation of lymph nodes in this study while in the study conducted by Duraiswami R et al[13] female was predominant (60.7%) and is in concordant to the study conducted by Sharma M et al[20] were male gender (61%) was predominant.

In this study the maximum number of lymph node aspirates were from the cervical region which is in concordance to the study conducted by Gayathri M et al[21].

The peak incidence of B cell lymphoma in this study was in the range of 41-60 years which is similar with the study conducted by Sharma M et al[20] and Vallabhajosyula S et al[22] in whose study the median age was around 55.5% .

The sensitivity, specificity, PPV and NPV for histomorphological examination along with IHC study on CB preparation was higher than that of FNAC in diagnosis of lymphoma in the current study which is in concordance with the study conducted by Zhang S et al[4] where the accuracy for the diagnosis of lymphoma from CB was higher than that of FNAC smears.

Goswami FJ et al[23], Thapar M et al[24] and Matreja SS et al[25] in their study concluded that the diagnostic accuracy of CB was higher than the CS preparation which is in concordance with the current study.

In our study significant difference in sensitivity, specificity, PPV and NPV were observed between the histopathological examination along with IHC study on CB preparation and FNAC study on CS in diagnosis of lymphoma and IHC study on CB preparation helps in increasing the diagnostic accuracy and typing of lymphoma.

We were unable to further subclassify B cell and T cell NHL from the cell block preparation due to non-availability of extended diagnostic markers and lack of more specific architectural details in our cell block preparation. Further molecular study to identify specific mutations could not be done as molecular studies are not available in our institute.

CONCLUSION

Worldwide the primary lymphoproliferative disorders are responsible for significant morbidity and mortality, hence the early diagnosis and typing of lymphoma helps in the treatment of the patients. Many at times, some cases may be difficult to diagnose morphologically on conventional smear preparation. In such scenarios the help of histopathological examination on CB and IHC study on CB can be taken, which helps in diagnosing and in typing of lymphoma. Hence the combined approach along with the IHC study on CB increases the diagnostic accuracy and in typing of lymphoma.

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