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Case Report

Role of immunohistochemistry in clinicohistopathology-A rare case study

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ABSTRACT

Background : Disease affecting Lymph nodes form wide range of spectrum from simple to malignant pathology. Such cases are an important element to rule out malignancy in early stages. However Generalised Lymphedenopathy diagnosis become huge challenge. Hence, an insight into clinocohistopathological correlation with help of IHC markers becomes highly important to decide morphologic differential diagnosis.
Aims and Objectives: To study lymph node lesions with clinicohistopathological correlation with the help of IHC study.
Case Report: This is a combined observational and prospective study held in District hospital Vijayapur during August 2022 to September 2023. Patient details were taken with the questionnaires. During his first visit clinical examinations and investigations, CBC, PS study, FNAC of left lower cervical region, Urine routine, chest X-ray & USG of abdomen performed and patient was analyzed & diagnosed as Chronic appendicitis and treated. During patient's second visit, clinical examinations and investigations CBC, PS study, FNAC of left Axillary swelling, Urine routine, chest X-ray, USG of neck & abdomen, CT Neck & abdomen, USG guided FNAC of Liver, Bone marrow aspiration study, Biopsy of left lower Cervical lymph node for Histopathology study & IHC study performed. Case was studied using routine H&E, PAP stain slides of Biopsy material were evaluated by light microscopy. And for IHC, patient biopsy material referred to higher center, using specific monoclonal or polyclonal antibodies, paraffin sections were stained immunohistochemically (IHC) using a Peroxidase antiperoxidase (PAP) technique in referred higher center.
Result: Patient was diagnosed as B-cell Lymphoma as per WHO guidelines with the help of IHC markers.
Conclusion: From this study, concluded that IHC plays a significant role clinicopathology and helped in the definitive diagnosis and typing of tumours and appropriate treatment can be planned.

1. Introduction

Generalized Lymphedenopathy (GL) is a common symptom in all age groups patients coming to hospital. Differential diagnosis of GL is challenging. Lymphoblastic B-cell Lymphoma (LBL) is a rare, highly aggressive non-Hodgkin Lymphoma variant virtually indistinguishable from Acute Lymphoblastic Leukemia (ALL).[1] Lymphoblastic B-cell lymphoma (LBL) is a neoplasm of immature B-cells committed to the B-(B-LBL) or T-cell lineage (T-LBL) that accounts for approximately 2% of all Lymphomas.[2] Acute lymphoblastic leukemia (ALL)/lymphoblastic lymphoma (LBL) is a clonal hematopoietic stem cell disorder of B or T cell origin.[3] Acute Lymphoblastic B-cell leukemia (ALL) are malignant disorders of immature B or T cells that occur characteristically in children, usually under the age of 6 (75%).[4]

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Majority of malignant lesions of GL cases are under diagnosed, as a infective /reactive lymphadenitis due to lack of facility for the special investigations and high cost of IHC study. Within a short period, due to delay in diagnosis and irregular follow up, patient will land up in high morbidity and mortality. Hence, this study helped to gain the insight into proper diagnosis of GL to prevent patient from complications. Though IHC study expensive but it is gold standard, based on the recognition between an antigen and an antibody, IHC will pinpoint where in a tissue or cell a certain antigen is located, which will help to accurate diagnosis and typing of the tumour in tissue samples and hence will help in proper choice of treatment.

Though it helps in the accurate diagnosis of most of the tissue samples but IHC has its own limitations, as in many cases it poses a dilemma at the initial diagnostic level, to further separate the morphology that seems to be comparable in H & E staining, IHC may be performed as a sanctioned supplementary investigation of the tissue in addition to basic H&E staining.

2. Aims and Objectives

1. To evaluate the patient with GL.
2. To analyse reasons for under diagnosis of GL.

3. Case Report

This study was conducted by Pathologists, in District hospital Vijayapur from August 2022 to September 2023. Patient was 12 years male, presented to Pediatric & Surgery OPD in August 2022, with mild fever, nausea, vomiting associated with moderate, right lower abdomen pain since one month. He gave past history of multiple 2-3 tiny swellings on each side of the neck, mild loss of weight, and fatigue since three months, he went to local hospital took treatment, but symptoms persists, so he visited District hospital Vijayapur, for further treatment. On physical examination, Surgeon found 2-3, symmetrical swellings in upper, lower Cervical region, firm in consistency, largest swelling was measuring 1*1cms in left lower Cervical region. On palpation of abdomen, right Iliac and Umblical region tenderness was present, Patient advised for Complete blood cells count(CBC),Peripheral Smear(PS) study, Fine Needle Aspiration Cytology(FNAC) of left lower Cervical swelling, Chest X-ray, Ultrasonography(USG) abdomen and Urine routine.

4. Observation and Results

CBC report: Hb- 12.4 gms%, WBC- 4130 cells/cumm. DC; Neutrophils- 80% Lymphocytes – 07%, Monocytes-8%, Eosinophils-4.9 Basophils-0.1%

PS study: Normocytic normochromic blood picture.(Figure 1)

FNAC of left lower Cervical Lymph node: Shows Polymorphicpopulation of Lymphoid cells, seen in Lymphocytic background, F/S/O-Reactive Lymphadenitis (Figure 2).

Urine routine: NAD.

Chest X-ray: NAD.

USG abdomen report: Chronic appendicitis.

4.1. Treatment

After one week under coverage of Antibiotics, Appendicectomy was done, patient was apparently recovered symptomatically and discharged, advised for regular follow up.

4.2. Follow up history

In July 2023, patient second time, presented to Surgery OPD with mild fever, night sweats, multiple symmetrical swellings in neck, Axilla and Inguinal regions, pain in Right side upper abdominal pain, with loss of appetite, loss of weight and fatigue. This time parents were noticed, patient weight was significantly reduced, number of swellings were increased, new swellings appeared in Axilla, Inguinal regions. On palpation there were multiple 5-7 swellings, symmetrically in Cervical (Upper, middle and lower), Axillary and Inguinal regions. Left lower Cervical swelling size was increased to 3*2 cms. Largest swelling was in left Axillary swelling measuring about 3*3 cms. All swellings were rubbery in consistency, mobile. Patient investigated in detail this time. CBC, PS study, Chest X-ray, Urine routine, FNAC left Axillary swelling, patient also subjected for USG of neck and abdomen, CT neck and abdomen, USG guided FNAC of Liver, Bone marrow aspiration(BMA) study, and Biopsy for Histopathological (HP) & IHC study.

4.3. Follow up Investigations reports

CBC reports: Hb- 10.0 gm%, WBC- 12800 cells/cumm. DC; Lymphocytes – 68%.

PS study: Atypical lymphocytes seen and reported as Microcytic hypochromic anemia with Lymphocytic Leukocytosis (Figure 3).

Chest X-ray report: NAD.

Urine routine: NAD

FNAC of left Axillary region: Lymphoblast like large round cells with little cytoplasm, mild nuclear pleomorphism, inconspicuous nucleoli and fine dispersed chromatin seen. Features suspicious of Blastic Lymphoma (Figure 4).

USG Neck and abdomen report: Shows Lymphadenopathy and Hepatomegaly. Liver shows well defined small nodular hypo echoic lesions, occasionally may be a target lesion, nonspecific diffuse infiltrative, reported as Suspicious of Metastasis in Liver, advised USG guided FNAC of Liver.

CT Neck: Shows multiple lymph nodes enlargement in multiple areas, Impression: Possibility of Lymphoma.

CT Abdomen: shows multiple homogeneous hypo attenuating hepatic lesions Suggestive of Hepatic Metastasis.

USG guided FNAC of Liver: Shows haphazardly arranged pleomorphic cells, with high N:C ratio and intra cytoplasmic vacuoles present, features suggestive of of Secondary deposits, advised biopsy and IHC study (Fig.5).

Bone marrow aspiration study from right Iliac region: shows distinct few Para trabecular areas infiltrates with occasionally large lymphoblast like cells, features suggestive of Metastatic deposits.

HistoPathological study of Biopsy from left lower cervical lymph node: Shows diffuse infiltration of lymphoblastic like large round cells, forms sheets and divide often, leading to effacement of lymph node. These cells having little cytoplasm, moderate nunclear pleomorphism and fine dispersed chromatin, architecture features suspicious of Blastic Lymphoma (Figure 6).

4.3.1. IHC study (Higher centre) report

1. B-cell lineage Tumour were shown positivity-LCA(+),CD20(+) (Figure 7, A&B)
2. T-cell lineage Tumour markers-: were shown negativity.CD5(-),CD2(-),CD4(-),CD8(-).(Figure 7 A,B,C,D).

IHC report: Blastic B-cell lymphoma.

4.3.2. Final diagnosis: B- cell Lymphoblastic Lymphoma

Treatment: Started with Intensive Chemotherapy. Patient took one month irregular treatment, he denied the regular treatment due to intolerance and side effects. Patient was died after one month of treatment, due to respiratory distress, as a complications of tumour metastasis.

Shows diffuse proliferation of Lymhoblastic like round, large cells that form sheets and divide often. These cells have little cytoplasm, mild nuclear pleomorphism, and fine dispersedchromatin.Figure 6

5. Discussion

The results of the present study and their comparison with the results obtained by different previous studies are discussed here. In our study, initially, in patient first visit, case was diagnosed as benign lesion i.e. reactive lymphadenitis, laterin second visit, with detail evaluation and following further investigations, was diagnosed as malignant lesion, i.e. LBL Lymphoblastic Lymphoma. Sometimes reactive /benign lesions look like that of malignant lesions, most often is like poorly or not at all differentiated malignant tumour.

In patient first visit, symptoms and investigations were in favorable to reactive lymphadenitis, might be reactive

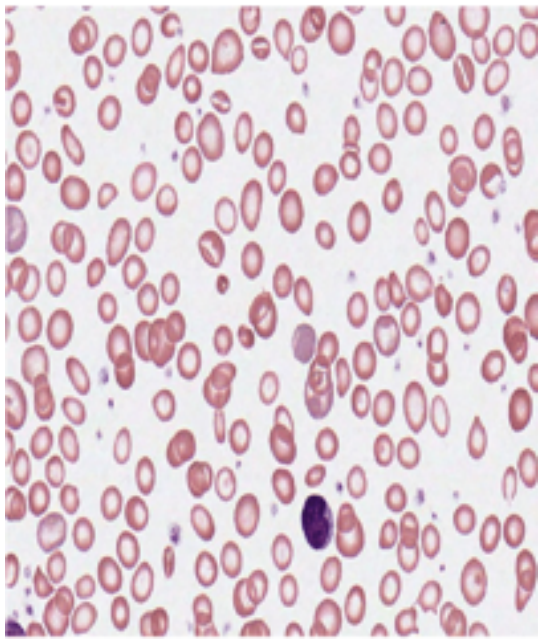

Figure 1: PS study: Normocytic normochromic blood picture. Leishman's stain (40X)

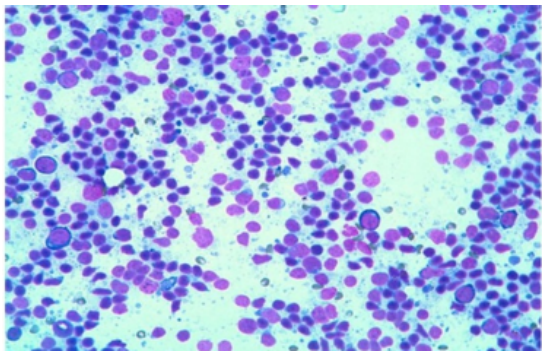

Figure 2: FNAC of left lower Cervical swelling: Reactive lymphadenitis PAP (40X)

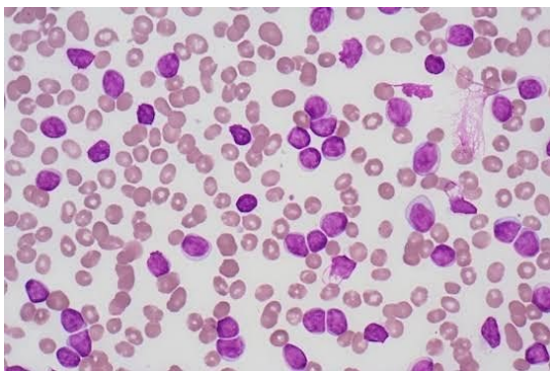

Figure 3: PS study: Microcytic hypochromic anemia with lymphocytic leucocytosis. (Shows many Atypical lymphocytes). Leishman's stain (100X)

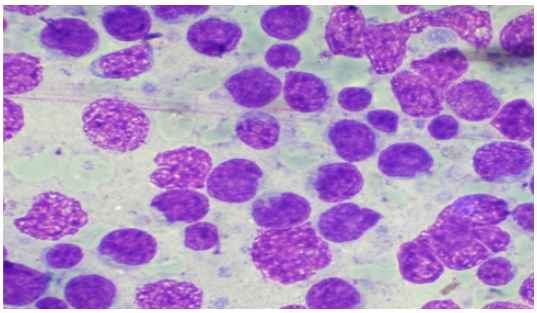

Figure 4: FNACof left Axillary swelling: Suspiciousof Blastic Lymphoma. MGG (100X).(Lymphoblast like round, large cells with little cytoplasm, mild nuclear pleomorphism, inconspicuous nucleoliand fine dispersed chromatin).

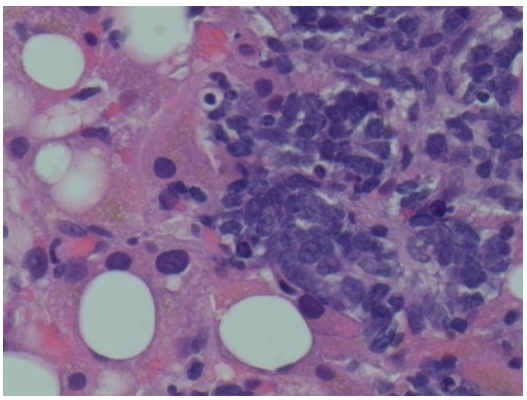

Figure 5: USG guided FNAC of Liver: Shows features of secondary deposits.H&E(40X)(Haphazardly arranged pleomorphic cells, with high N:C ratio and intracytoplasmic vacuoles present).

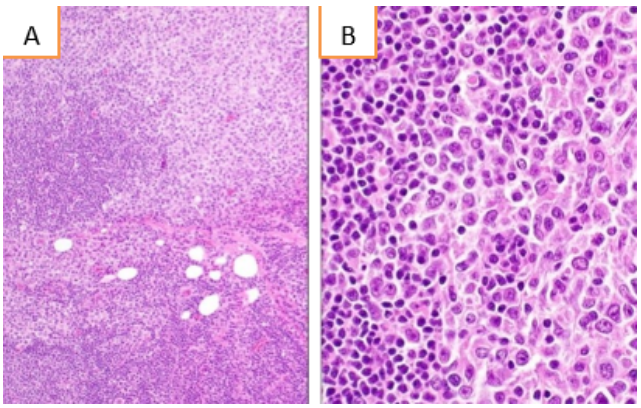

Figure 6: HPR of Biopsy of Left lower Cervical lymph node: Lymphoma; **A:** H&E(10X); **B:** H&E(40X)(Shows diffuse proliferation of Lymhoblastic like roundlarge cells that form sheetsand divide often and have little cytoplasm, mild nuclear pleomorphism and fine dispersed chromatin).

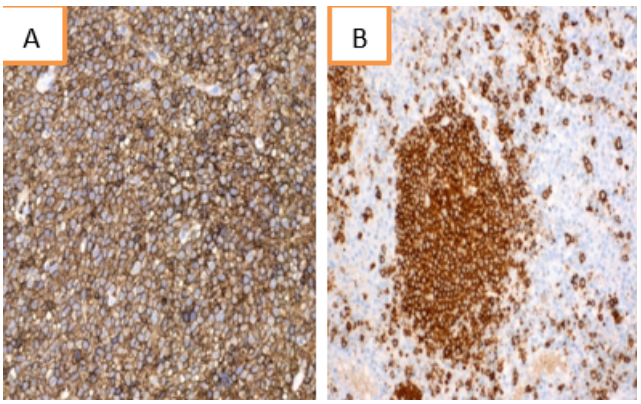

Figure 7: IHC report: B cell lineage Tumour markers- **A:** LCA(+), **B:** CD20(+)were shown positivity IHC(40X).

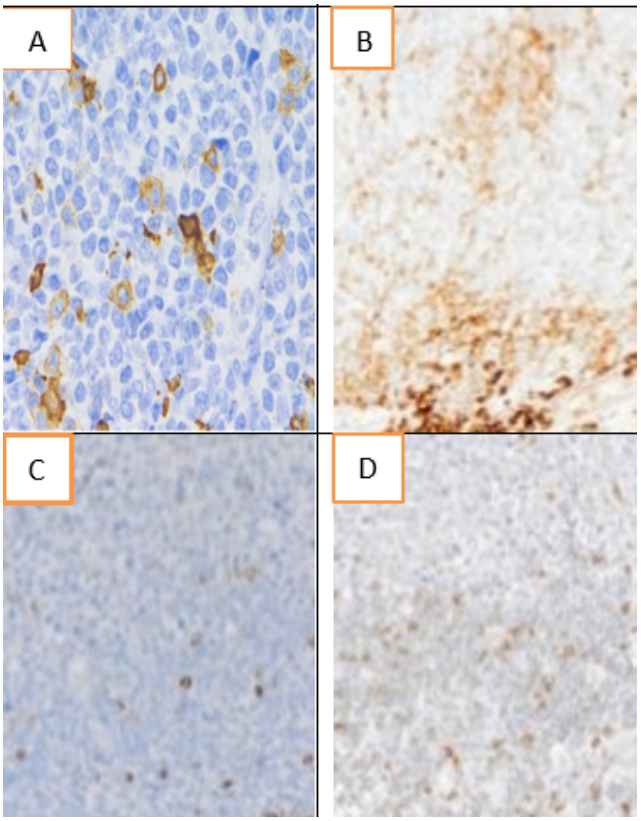

Figure 8: IHC report: Tcell lineage Tumour markers- **A:** CD5(-). **B:** CD2 (-). **C:** CD4 (-), **D:** CD8 (-), were shown negativity. IHC(40X)

response to underlying Appendicular infection, In second time patient visit, clinical presentation was different, raising suspicious of malignancy and proper detail evaluation of patient was done and finally diagnosed as B-cell Lymphoblastic Lymphoma.

Morphological findings of Lymphoblastic like cells was not visible in patient first visit, investigations reports. In second visit, clinical symptoms were raising suspicious of malignancy, recommended detail investigations for patients, hence pathologists worked out, performed routine and special investigations like biopsy study for HP and IHC testing, where the first results were equivocal.

In patient first visit, FNAC of left lower Cervical swelling, in first time, shown reactive lymphadenitis, in second visit, biopsy from same swelling, with HP and IHC testing confirmed accurate diagnosis of B-cell Lymphoblastic Lymphoma.

IHC markers were used to confirm a diagnosis of Blastic B cell Lymphoma. While IHC's ability to provide a definitive diagnosis was crucial, as in our case we are equipped with limited tests due to financial restraints, low economic status of patient is also a restraining factor in conducting IHC studies the patient's irregular follow up, lack of awareness, unwillingness to undergo a biopsy and the expensive expense of the test in his second visit both slowed down the process of diagnosis. Or might be in his first visit, malignancy process might have been not started or might be in beginning and undetectable stage of malignancy, which was masked with reactive response and that reactive response might have been correlated to underlying Chronic Appendicular infection.

Training, knowledge, and sensitization of authorized signatories and clinicians towards interpretational abilities should be considered as preventative measures to reduce the number of discordant instances. Through one-year comparison of HP and IHC diagnosis, we were able to greatly enhance our laboratory services in terms of performance assessment, patient care, and overall quality. As our case was rare entity, as similar study conducted by Kline KAF, et al,[5] rare malignancies developing from lymphocyte precursor cells, LBL, and ALL have historically been viewed as different manifestations of the same disease process. Similar to our case, in study,[2]in tissue sections, LBL is generally characterized by a diffuse or, as in lymph nodes and less commonly, para cortical pattern.

Appropriate therapy can only be administered once a correct histologic diagnosis of cancer and classification of the tumour type has been made. Modern diagnostic tools allow for precise subtyping of malignant tumours. IHC is a very important tool in accurate typing of tumours and assessing their true malignant potentials. In the current study, patient first visit investigation reports, in favour of reactive lymphadenitis, in patient's second visit, symptoms and investigations reports were suggestive of Lymhoma, which was might have been masked with different combined presentation and investigation reports during in his first visit, again IHC played important role in confirming diagnosis and typing the tumour during his second visit.

Similar to the research conducted by Neval Zkaya et al.[6] diffuse LBL, the most common diagnosis, were frequently misdiagnosed as classical Hodgkin lymphoma, but in our study, initially first time visit, patient was diagnosed as reactive lymphadenitis and in his second time visit was diagnosed as LBL. Where as in our case LBL was diagnosed as reactive lympedenitis, after IHC study, with tumour markers helped to arrive as LBL diagnosis. According to study,[6] there were 29 samples that were first diagnosed as lymphoma but were later deemed to be benign/reactive, 20 of which were found to be Reactive follicular hyperplasia. Our study was similar to the research conducted by M. J. Matasar et al.[7] whereby in both 2001 and 2006, a single case with a reported diagnostic of low-grade Follicular Lymphoma was considered to reflect benign pathology (follicular hyperplasia or gradual transformation of germinal centres).Where as in our study benign pathology(reactive lympocytosis) gradual transformation to Lymphoma?. Similar study was conducted by Ramya Potti et al.[8] proved that HP and IHC study of lymph nodal biopsies, definitely help in accurate diagnosis of Lymph node lesions.

Similar study.[9] B-lineage Lymphoblastic Lymphoma is a clinicopathologic entity distinct from other histologically similar aggressive Lymphomas with blastic morphology. Similar to our study, the study[10]concluded, complexity and spectrum of cases presented in review highlight the importance of clinicopathologic correlation and the value of ancillary studies in the classification and workup of patients with B-ALL/LBL. In study conducted by Jia-Yu Ling. Et al.[11]mature lymphoid system malignancies didn't express early antigens, such as CD34 and TdT, but expressed myeloid associated antigens, especially CD13 and CD33, but in our study lymphoid associated antigens were expressed. In study our study LCA and CD20 lymphoid associated antigens were expressed, where as in[12]the World Health Organization (WHO) suggested immunophenotype for pre-T ALL/LBL typically includes the expression of TdT, CD3, and CD7, while CD2, CD3, CD4, CD5, CD8, and CD10 are variably expressed. In Pratibha Iyengar et al. study[13] a case study, 46 yrs female with increasing abdominal girth, investigation revealed bilateral ovarian tumors but no evidence of systemic disease. A diagnosis of precursor B-cell Lymphoblastic lymphoma was established by histologic examination, IHC staining, and molecular analysis, in our case, patient presented with multiple neck swellings, diagnosis of Lymphoblastic B-cell Lymphoblastic lymphoma was established by histologic examination, IHC staining. In M Ozdemirli et al. study[14] a limited panel of antibodies can lead to an erroneous

diagnosis, B-lymphoblastic lymphoma may be negative for CD45 and CD20 but positive for CD99 and even for keratin, mimicking Ewing's sarcoma, in our study wider antigenic profile of both positive and negative markers and well choosed antibodies helped accurate diagnosis. Correct diagnosis is extremely important because LBL usually is curable in the pediatric age group with appropriate therapy.

In study conducted by Gao Y, et al.,[15] the enlarged superficial lymph nodes showed no obvious change in size, Immunohistochemistry revealed the following: cluster of differentiation (CD) 3 (+), CD5 (+), CD7 (+), transmission disequilibrium test (TDT) (+), myeloperoxidase (MPO) (-), and lysozyme (Lys) (-). The lymph node morphology and immunohistochemical results indicated T-LBL, but in our study final diagnosis was B-LBL, as LCA (+), CD20 (+) and CD3 (-), CD5 (-), CD7 (-). In Margit Schraders et.al study,[16] in the WHO classification scheme, pediatric LBL is considered to be the same disease entity as pediatric acute lymphoblastic leukemia (ALL). However, it is unclear whether the genetic basis of pediatric LBL development is similar to that of pediatric ALL, similarly from our study, we felt that genomic study also required for better classification of Tumours of lymphoblastic origin. In study,[16] the authors believe that the distinction of Lymphocytic B-cell Lymphoma from its histologic mimics, Lymphocytic T -cell Lymphoma and BVMCL, has important clinical implications, but in our study IHC helped to identify the LBL.

6. Conclusion

The present study has significantly highlighted and created awareness about the role of IHC in Histopathology. Generalised Lymhedenopathy is a common symptom, occupying higher number OPD cases. It has made us understand that sometimes findings may be muffled and leading to wrong diagnosis and a pathologist has to be extra cautious while making morphological diagnosis. One can learn from mistakes committed earlier by maintaining a record of such cases. Additional biopsy and IHC testing were recommended for patients where the first results were equivocal.

It is also important to remember that morphology alone cannot suffice for exact typing and grading of some lesions. Some cases must undergo IHC analysis for this. In modern diagnostic tools IHC plays definitive diagnosis, classification made to characterize and type the tumour. It is best to use an wider antigenic profile of both positive and negative markers, which may be achieved by a panel method consisting of well choosen antibodies. It was also noted that a wider and complete panel of IHC is required for arriving at a conclusive diagnosis.

IHC is crucial for accurately classifying tumours, hence IHC must be made a routine part of Histopathology. Also, IHC can detect some of the diagnostic histopathology errors and it t is a positive way of creating insight among pathologists that sometimes morphological diagnosis may differ from the actual pathology and making them believe that IHC study should be an integral part of Histopathology.

7. Source of Funding

None.

8. Conflict of Interest

None.

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