

**FINE NEEDLE ASPIRATION CYTOLOGY AS A DIAGNOSTIC TOOL IN
LYMPHADENOPATHY FOR PEDIATRIC AGE GROUP –TERTIARY CARE CENTRE**

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ABSTRACT

Lymphadenopathy is a common and challenging clinical presentation, for the patient as well as clinician specially in the pediatric age. The etiology of lymphadenopathy may be varied ranging from reactive changes to malignancies and metastatic deposits. The aim of this study was to understand the role of FNAC in the diagnosing lymphadenopathy in pediatric population and to study the various cytomorphological patterns and their frequency in lymph node aspirates. Total 251 cases were considered for the study amongst which 25 cases were excluded based on the exclusion criteria. Standard FNA procedures were undertaken and the smears were stained with Leishman, Romanovsky Hematoxylin and Eosin, Papanicolaou stains and special stains were done where so ever needed. The study had a total of 117 males and 109 females. Majority of the lymph nodes measured 1 to 2 cm and were reported from cervical region (66.8%). Cytomorphological studies revealed reactive lymphadenitis (48.6%) as the predominant presentation followed by tubercular lymphadenitis (25.6%), acute suppurative lymphadenitis (8.8%) and neoplastic etiology (3.5%). FNAC is yet again proved as an important and rapid diagnostic modality for the clinical and etiologic work up in cases of lymphadenopathy. Reactive hyperplasia is the most common presentation in pediatric population but tuberculous lymphadenitis is one of the most common and frequent infectious etiologies diagnosed by FNAC.

KEYWORDS: FNAC, Lymphadenopathy, reactive hyperplasia, tuberculous lymphadenitis and lymphoma.

INTRODUCTION

Lymphadenopathy is a common clinical problem in pediatric age group with incidence varying from 38-45 %.¹ Evaluation of a child with lymphadenopathy is a common clinical scenario for the pediatrician.² Being a part of the reticuloendothelial system, they are the end points for lymphatics draining from the various parts of body. Also abundant phagocytic histiocytes, APCs and lymphoid population of cells provides as an ideal defense against pathogens. With this reference in background majority of children present with palpable cervical, axillary and inguinal lymph nodes.^[2,3,4] They are not considered clinically significant until the diameter exceeds 1 cm for cervical and axillary group of lymph nodes and more than 1.5 cm for inguinal lymph nodes. These are the superficial locations where lymph nodes are palpable while at other locations they can be seen with plain radiographs and ultrasonography.^[5]

Supraclavicular lymph nodes if palpable are always considered significant.^[4,6] Generalized lymphadenopathies is usually secondary to systemic infections and is characterized by enlargement of two or more noncontiguous lymph node regions.^[7] The differential diagnosis of lymphadenopathy is broad and should be considered as a systemic disease until proven otherwise. Through clinical examination and a detailed history helps in providing clues to underlying etiology. FNAC is adjunct diagnostic technique which is simple, safe, reliable, cost effective, easily available and with good diagnostic accuracy. The study was conducted to evaluate the diagnostic efficacy of FNAC in lymphadenopathy in pediatric age group and to study the various cytomorphological patterns and their frequency in lymph node aspirates.

MATERIALS AND METHODS

The study is a prospective study conducted over a period of 3 years in department of pathology at a tertiary care center. It included all pediatric patients aged from one month to eighteen years presenting with lymphadenopathy referred to our center. A detailed clinical history and a thorough clinical examination was performed. History of contacts with animals, immunization history, socioeconomic status and any relevant antibiotic therapy were recorded. Palpable lymph nodes were examined noting their size, site, number, mobility, presence of matting and any other local skin changes were noted. The procedure of FNAC was explained and FNA was attempted. Aspirated smears were stained with Leishman, Romanovsky Hematoxylin and Eosin, Papanicolaou stains and special stains where so ever needed. The lymph nodes measuring less than 1 cm were excluded as they were difficult to be sampled and deep seated and highly mobile lymph nodes no FNA was attempted as they were difficult to access.

After studying all the clinical data and presentation the smears were examined under the microscope. Based on the cellularity they were categorized as high, moderate and low cellularity. Those smears which were hemorrhagic or with scanty cellularity were labelled as inadequate for opinion.

RESULTS

A total of 251 lymph node aspirates from pediatric age group presented to department of pathology in the duration of June 2007 to May 2010. Amongst these 25 cases were excluded as per the exclusion criteria and 10 cases though they didn't satisfy the inclusion criteria but were included in the study as they had adequate material to give a definite diagnosis. Therefore, the total no of cases included in the study were 226.

Total of 226 lymph nodes aspirates were obtained of which 117 were males and 109 females. The age range was from 3 months to 18 years. The maximum incidence was in the age group of 12 to 15 years for males and 16 to 18 years for females. A slight male preponderance was noted with male to female ratio of 1.07.

Table 1: Age and Sex Distribution

Age	Male	Female
0-3yrs	12	6
4-7yrs	21	7
8-11yrs	17	18
12-15yrs	44	30
16-18yrs	23	48
Total	117	109

The commonest group of lymph nodes aspirated were the cervical (151 cases), followed in decreasing order of frequency by the submandibular (31 cases), axillary (23), inguinal (12 cases), jugulodigastric (5 cases) and others (4 cases).

Table 2: Site of lymphadenopathy

Site	Number of cases
Cervical	151
Submandibular	31
Axillary	23
Inguinal	12
Jugulodigastric	5
Others	4

FNA was attempted in lymph nodes of varying sizes. The smallest lymph node measured 0.5 cm in greatest dimension and the largest measured 4cms in greatest dimension. Most of the lymph nodes (158 cases) ranged in size of 1 to 2 cm in greatest dimensions.

Table 3: Size of lymphadenopathy

Size of lymph node	Number of cases
< 1cm in greatest diameter	27
1-2 cm in greatest diameter	158
>2cm in greatest diameter	41

Cytomorphological evaluation of the aspirated material was done in total of 226 cases and no opinion could be made in 25 cases. Total of 110 cases were rendered the diagnosis of reactive hyperplasia 48%. Tuberculous etiology was identified in a total of 58 cases i.e. 25% and two cases of parasitic etiology were diagnosed amongst which one was identified as cysticercosis and other was diagnosed as filariasis. Acute necrotizing pathology was noted in 8.8% (20 cases) in which etiology could not be ascertained and two cases of chronic Sialadenitis were identified. Total seven cases of lymphomas were identified comprising of 0.8% (2 cases) of Hodgkin's lymphoma and 2.27% cases of Non-Hodgkin's lymphoma (5 cases). A single case of metastatic deposits was noted in which primary could not be identified and follow up unfortunately was not possible.

Table 4: FNAC diagnosis of lymphadenopathy in pediatric age group

FNAC diagnosis	Number of cases
Reactive hyperplasia	110
Tuberculosis	58
Acute suppurative lymphadenitis	20
Parasites	2
Chronic Sialadenitis	2
Hodgkin's disease	2
NHL	5
Metastasis	1

Total of 110 cases of reactive hyperplasia were identified in the study, amongst which a total of 79 cases were consistent with reactive hyperplasia. Twenty-five cases were suggestive of reactive hyperplasia and 6 cases were suspicious of reactive hyperplasia because smears were of low cell yield in which diagnosis was given but the degree of confidence of diagnosis was low. Follow up was done in 48% cases which showed decrease in size of lymph node post antibiotic treatment. Others underwent

and excision biopsy in which reactive hyperplasia was further confirmed and 3 cases were diagnosed with tubercular pathology.

Tubercular lymphadenitis was detected in 58 cases i.e. 25 %. Acid fast bacilli were noted in 20 cases (34%). Necrosis was noted 56% of cases and granulomas were noted in 63%. Giant cells were reported in five cases of tubercular lymphadenitis. Amongst the cases which presented with granuloma 44% presented with micro granulomas and 36% showed presence of macro granuloma. While seven cases showed presence of both micro and macro granulomas.

Table 5: Cytomorphological features of tuberculosis

Features	N=58	Percentage
Necrosis	33	56
Granuloma	36	63
AFB positivity	20	34
Giant cells	03	5
Granuloma with necrosis	14	24
Granuloma without necrosis	22	37
Necrosis without granuloma	16	27

Amongst 58 cases 33 cases (56%) could be followed up and were started on anti-tubercular treatment and showed clinical improvement. With the other 25 cases, 20 cases were lost for follow up as they had follow up referrals with other centers. The five cases had follow up with excision biopsy where the affirmative diagnosis of tuberculosis was given in 3 cases, 1 case was diagnosed as necrotizing granulomatous lesion with high suspicion of tubercular etiology and a single case was suspicious of tuberculosis with few ill formed granulomas.

Table 6: Types of granuloma

Type of granuloma	Number of cases	Percentage
Micro granuloma	16	44
Macro granuloma	13	36
Both	07	17

One percent case was diagnosed as chronic Sialadenitis (2 cases), both the lesions were identified in the pre auricular area and cytological features showed presence of acute inflammatory cells predominantly polymorphs and few histiocytes along with salivary gland acini.

There were two cases of parasitic etiology, amongst which one case showed teguments of cysticercosis from inguinal aspirates. The other case showed sheathed microfilaria from axillary lymph node aspirates along with high eosinophil counts. Unfortunately, both the cases were lost for follow up.

There were eight cases of malignancy amongst which 2 cases were those Hodgkin's Disease, one was a female and the other male. Both the cases on cytomorphological examination showed Reed Sternberg Cells. One of them went for histological examination and was confirmed as

lymphocytic predominant Hodgkin's Disease. NHL comprised of 60% of cases, i.e. a total of 5 cases. All these cases presented with generalized lymphadenopathy and symptoms of cough, fever, breathlessness and weight loss. There was a single case of cervical metastatic lymph node. She was a 15 years old girl, unfortunately we lost the case for further follow up and couldn't investigate the source of primary site.

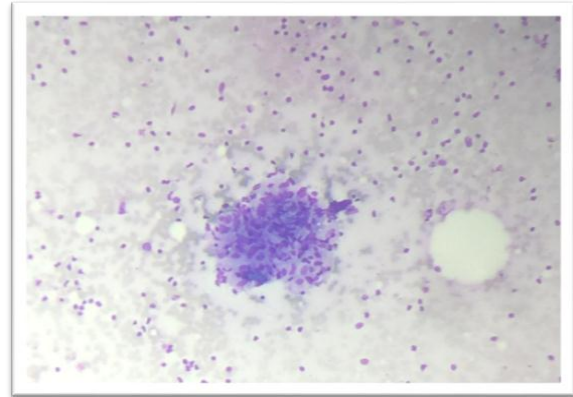


Figure 1: Smear showing macro granuloma in case of granulomatous lymphadenitis (Leishman x100)

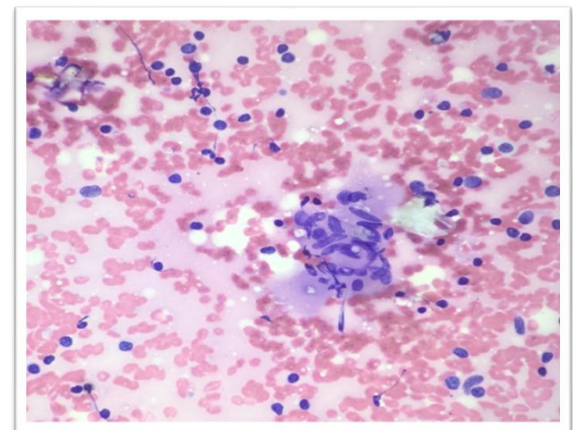


Figure 2: Smear showing a micro granuloma composed of epithelioid cells and lymphocytes (Leishman, x100)

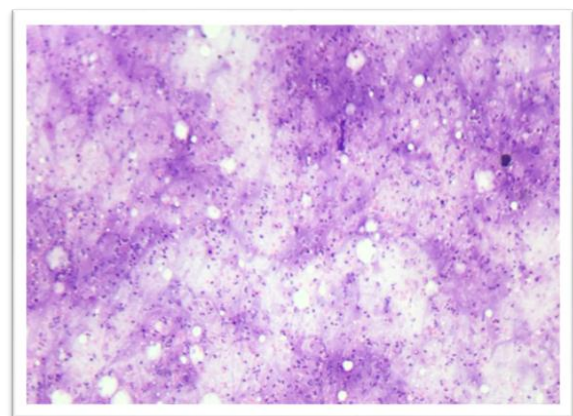


Figure 3: Case of tuberculous lymphadenitis showing caseous necrosis and granulomas (Leishman, x100)

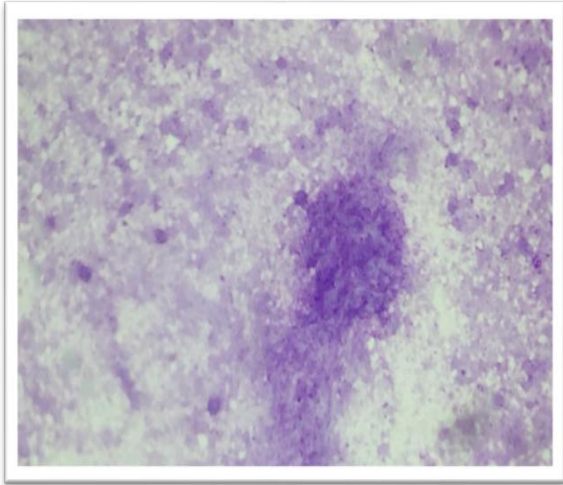


Figure 4: Tuberculous lymphadenitis showing caseous necrosis, granuloma formation and degenerating cells in the background (Leishman, x 400)

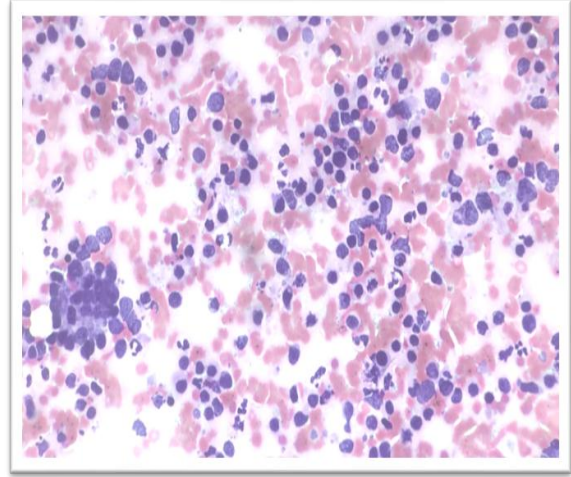


Figure 7: Case of Non Hodgkin lymphoma showing mono-morphous population of atypical lymphoid cells (Leishman, x400)

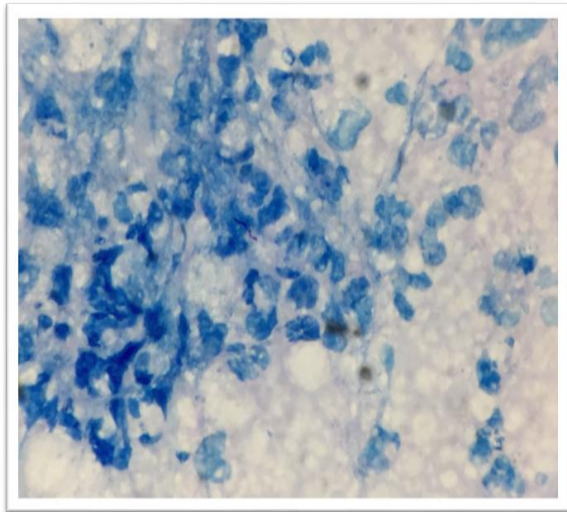


Figure 5: Acid fast bacilli in a case of tuberculous lymphadenitis (ZN, oil immersion)

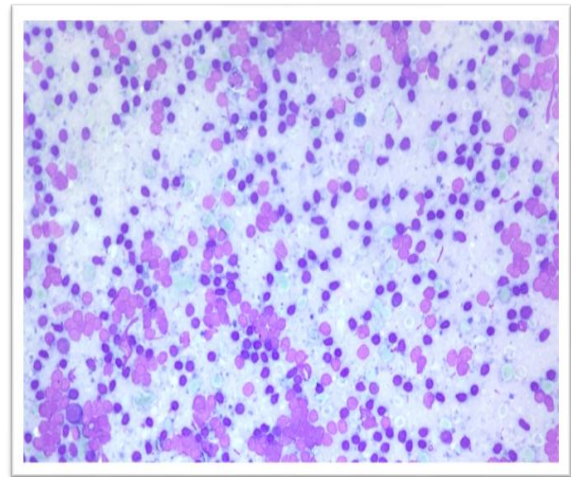


Figure 8: Reactive lymphadenitis showing polymorphous population of lymphocytes (Leishman, x100)

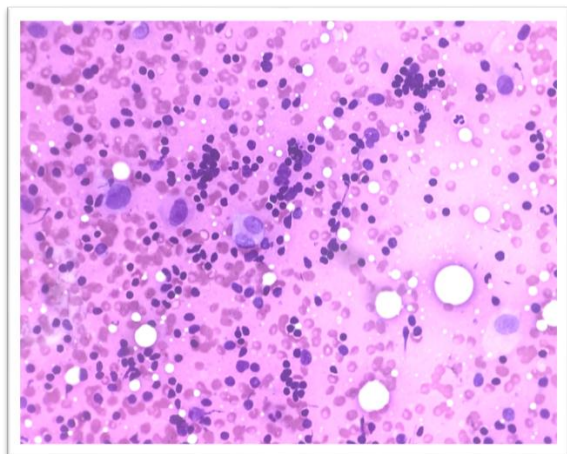


Figure 6: Hodgkin's lymphoma smear showing RS cells and polymorphic population of cells against haemorrhagic background (Leishman, x 100)

DISCUSSION

Lymph node enlargement is a common clinical presentation in our set up. The causes for lymphadenopathy can be varied etiologies and majority of times are self-limiting. In a developing country like ours, tuberculosis, suppurative skin infections and airway tract infections are few of the major causes for localized lymphadenopathy. It has been documented that any significant lymph node enlargement not subsiding for more than two weeks needs to be further investigated.^[8] As compared to adults, frequency of lymph node enlargement in children is more due to benign self-limiting diseases and so the number of diagnostic and therapeutic surgical interventions are also low.

In the past few decades FNAC has become a popular and initial diagnostic tool for evaluation of lymphadenopathy and a guide for further management. During the current study an attempt was made to study the various cytomorphological profiles of lymph node lesions in

children and to correlate the same with the clinical presentations.

In the current study reactive hyperplasia was the commonest lesion 48.6% (110/226), followed by tuberculous lymphadenitis 25.6% (58/226) acute suppurative lesions 8.8% (20/226). Other studies showing similar findings were Hag I A *et al.*^[9], Annam V, Kulkarni MH and Puranik RB^[10] and lake MA and Oski FA.^[11] Number of cases of Lymphomas recorded in the current study were very few, similar findings were found in the study conducted by Lochan *et al.*^[12]

In the current study, maximum number of patients were in age group of 12 -15 years in males (19.4%) and 16-18 years in females (21.2%). These findings were comparable with other studies, but were in contrast to the study conducted by Singh *et al.* in which maximum number of patients were in age group of 5 to 10 years (48.59%) followed by 10 to 16 years of age group (35.34%)^[12,13,14] Similar findings were concluded in the studies conducted by Ponder TB, Smith D and Ranzy I.^[15] The lymphoid growth reaches a peak at the age of 4 to 8 years. So with the ongoing antigenic stimuli the lymphoid growth may get a spurt in the process and can exceed the normal limits. This could be the reason for maximum number of cases in this age group.^[14]

In our study the incidence reported in males were more (51.7%) than females (48.23%) with male: female ratio of 1.07. The male predominance was again noted in the age group of 12 to 15 years and for females in age range of 16 to 18 years. Similar results were obtained by Mitra S Ray and Mitra P K (1.3:1)^[16] and Moore *et al* (3:1).^[17] The male preponderance in the present study was similar in these studies. This could be due to the prevailing customs of providing more attention to male children in Indian society rather than an increased biological susceptibility in boys. The study done by Ahmed *et al.* showed reverse ratio 0.47:1 with predominance of females in the study group.^[18]

The cervical group of lymph nodes were most commonly seen in our study as compared with other studies.^[12,13,14] In study conducted by Ellison *et al.* he studied 100 children with generalized lymphadenopathy and observed swelling in the neck as most common presenting symptom (52% of cases).^[19] This is attributable to rich lymphatic supply in the neck region.^[16] In the current study reactive hyperplasia was the major presentation, which can be attributed as a specific or non-specific response to disease process, also the easy accessibility of cervical lymph nodes for examination and evaluation could be one of the other causes of predominance of cervical lymph nodes.

Study conducted by M Pradeep Reddy *et al* for the purpose of study of lymph node they considered lymph node swelling measuring 1cms in cervical and axillary areas as significant and more than 1.5 cm in inguinal area

and more than 0.5 cm for any other site¹³ In the study most of the lymph nodes measured 1-2 cm. Total of twenty-seven lymph nodes measured less than 1cm in greatest dimension. Of these 20 cases were noted in cervical area, 5 in axillary and 2 in inguinal region. Amongst the twenty-seven cases of cervical area, 15 cases showed florid reactive hyperplasia, 5 cases showed granulomatous inflammation amongst which three were Z/N positive. In another study conducted by Bedi *et al.*^[20] and Ahmed *et al*^[21] they have suggested that smaller lymph nodes have less chance of being tuberculous and had experienced that lymph nodes up to 1 cm diameter are indicative of reactive process. However, these findings were not accountable as a limiting factor in our study.

Cytomorphological pattern obtained in this study predominantly were non-specific reactive hyperplasia (48.6%) followed by tubercular pathology (25.6%), acute suppurative lymphadenitis (8.8%) as compared to neoplastic causes which mainly comprised of lymphoma. Similar results were seen in studies done by J Buchino *et al.*^[8], Singh *et al.*^[14], Mitra *et al.*^[16] and Annam V, Kulkarni MH and Puranik RB.^[10] Reactive hyperplasia is the most common finding in pediatric age groups and in our study 110 /226 were diagnosed with the same. The usual cytomorphological findings noted in reactive hyperplasia comprises of polymorphous population of lymphocytes with histiocytes and occasional polymorphs. Reticulum cells at times are hyperplastic and may be bi-nucleated, hence presenting with diagnostic difficulty with Hodgkin's lymphoma. Moreover, FNA samples only a focal area of lymph node and so the possibility of entirely missing the concurrent pathology are high, resulting in false positive and false negative diagnosis. Considering this a clinical and physical examination with detailed clinical history should always be taken in view with cytological examination of lymph nodes presenting with reactive hyperplasia.

One of the oldest disease known to mankind is Tuberculosis, true incidence and prevalence of which has never been established. An estimated 1 million cases of TB occur in children world-wide amongst which 75% occur in the 22 high burden zone countries which includes India. According to the World Health Organization (WHO) in 2007 smear positive TB in children in less than 14 years accounted to 0.6-3.6% of reported cases.^[22] Superficial tuberculous lymphadenitis is one of the most common forms of extra pulmonary tuberculosis. Tubercular lymphadenitis results from lymphatic spread from the primary focus and usually involves the cervical nodes. Even when the other investigations like montoux and chest X ray are negative, FNAC of the lymph node are definitely helpful. Children with tuberculosis act as mediators of continuing transmission in the population, hence early diagnosis with help of least interventional modes helps in prevention of further transmission and spread of disease.

Although a definite confirmatory diagnosis can only be made after demonstration of AFB or culture inoculation.^[23] In the current study, aspirates were not subjected to culture. Although 58.6% showed AFB positivity on Z/N staining. In order to confirm the causative agent of tuberculosis culture and other ancillary tests are mandatory, but it is presumed that in children most of the cases are caused by *Mycobacterium tuberculosis*. However, in exceptional cases if there is co-existent infection with HIV then detection of causative agent with either culture or DNA hybridization techniques is mandatory.

Many studies have been done in an attempt to study the cytomorphological patterns of tuberculosis and many categorization of tubercular patterns with AFB correlation have been done. In our set up *Mycobacterium Tuberculosis* is common in comparison to other granulomatous diseases, so the presence of granulomas in the lymph node aspirates are highly suggestive of tuberculosis. In the current study a total of 63% of cases showed granulomas amongst which, 24% showed presence of both caseous necrosis and granuloma and 37% were considered having tubercular etiology on grounds of high suspicion with presence of granulomas alone without any associated caseous necrosis. Combination of both micro and macro granulomas were noted and seven cases showed presence of both. Presence of caseous necrosis is hallmark in diagnosing tuberculosis as it is more sensitive and specific to tuberculosis. In the current study 56% showed necrosis, comprising of caseous necrosis with polymorphs and histiocytes. Twenty cases showed presence of AFB. The presence of polymorphs in the background are due to the immune response of the host to the antigen of tubercle bacilli. Overall ZN positivity was low 34% and that can be explained as its defined by the clinical stage and immunological status of the patient and a minimum 5 to 10000 bacilli/ml of the aspirate are required to get a positive result on ZN stain^[25], the detection of AFB was more in the smears containing necromantic material alone than in those with granuloma. Needle aspiration may not be positive if it's done at the normal part of lymph node where AFB or caseation necrosis is absent. AFB staining is a must as its mostly positive in cases where cytology showed inflammatory exudates only with occasional giant cells, which may suggest TB but will not be strongly reported as TB lymphadenitis in cytology, but AFB load is high in these smears. This has also been found in other studies.^[10,12,16,20]

As the prevalence of *Mycobacterium tuberculosis* has decreased, diseases due to nontuberculous mycobacterium have assumed a greater importance, and a common occurrence of disseminated *M. Avium* complex disease in AIDS patients have stimulated interest in these organisms. The common form of disease is chronic pulmonary disease resembling tuberculosis, benign cervical lymphadenopathy in children, skin and soft tissue infections and disseminated disease in

immunocompromised persons. Non tubercular mycobacterium commonly presents in children at one to five years of age as infection of submandibular, submaxillary, cervical or pre auricular lymph nodes. In the absence of immunodeficiency infections lymphadenitis is the most common manifestation of NTM in children. It rarely affects adults. NTM cultures are positive in 50-82% excised nodes. About 12% of culture proven mycobacterial cervical lymphadenitis in children is due to mycobacterium tuberculosis. The others are due to mycobacterium avium complex and mycobacterium Scrofulaceum. In contrast to this in adults more than 90% of the culture proven mycobacterial lymphadenitis is due to *Mycobacterium tuberculosis*.

Acute suppurative inflammation may occur due to various causes ranging from non-specific to secondary infection, benign and malignant causes. In a study conducted by Somaiah G et al they attempted to study cervical lymph node enlargement with clinical and laboratory correlation. There patients were mostly in the age group of 4 to 8 years which incidentally is the peak age for lymphatic growth too. In majority of the patients the presenting symptom was cervical swelling followed by fever and cough. Similar findings were concluded by Ellison et al,^[23] they found common findings like tonsillitis, otitis media, impetigo and oro-dental infections. On culture examinations they isolated staphylococcus aureus, H influenza and beta hemolytic streptococci and the aspirates taken from these lymph nodes on culture showed similar results.^[26] Neck swellings are the most common presentation and their association with fever should be indicative of antibiotic therapy and follow up of the cases should be done. Also Z/N screening should be considered in all the cases of suppurative lymphadenitis as immune mediated response variation may give false positive or negative results.

In the study 35 % cases were of neoplastic etiology. Amongst which 2 cases were of Hodgkin's Lymphoma and 5 cases were of Non-Hodgkin's Lymphoma and as single case of metastatic deposits was detected in the group. Among the malignancies NHL is the most common lymph node malignancy in children.^[27] The application of FNAC in the diagnosis of lymphoma is still controversial particularly in low grade NHL.^[11] FNA cytology can be particularly useful as a minimally invasive method to obtain samples of fresh cells for cytogenic analysis. In contrast FNAC plays a greater role in management of Hodgkin's Disease as compared to NHL as it helps in the primary diagnosis, staging and monitoring the recurrence of disease.^[28] FNAC smears in Hodgkin's Disease are composed of Reed Sternberg Cells and their variants in an appropriate background of inflammatory cells. In lymphocytic predominance the classic RS cells are rare and the smears contain many epithelioid cells, histiocytes and L&H variant of R S cells, this may be wrongly diagnosed as TB if careful scan of other abnormal cells is not done.^[28]

FNA is more accepted for the diagnosis of Hodgkin's disease than for NHL and it may be the diagnostic procedure in high risk surgical candidates and in patients where masses are inaccessible. The cases of Hodgkin's lymphoma and NHL were few in this study to draw conclusions about diagnostic accuracy as no biopsy was done in the cases. Single case of metastatic lymph node was noted and unfortunately it could not be investigated further as the primary site was not known and the patient was not there for follow up. Other studies like those of Lake MA and Oski F,^[11] Annam V, Kulkarni MH and Purnik RB,^[10] Singh et al^[14] and Ponder TB, Smith D and Ramzy I^[15] considering lymphadenopathies in children had similar findings.

Single case of cyst cercus was noted with histiocytic infiltrate in inguinal lymph node. Filariasis lymphadenitis was noted along with eosinophils in the axillary lymph node. Unfortunately, no follow up was possible in both the cases. Two cases of chronic sialadenitis were encountered in the study in the submandibular region.

Total number of inadequate smears in the study were 5.9%. Considering the difficulty and co-operation needed by the child and the parent the inadequate smears are less in the study. Other studies conducted by Ahmad et al (8%),^[18] Annam V, Kulkarni MH, Puranik RB (3.57%) and Schwartz MR and Ramzy I (10.88%)^[15] showed similar incidence of unsatisfactory samples for diagnosis.

CONCLUSION

FNAC is safe, reliable and less time consuming OPD procedure which can be used as an initial diagnostic tool in young patients with clinical suspicious lymphadenitis. Early diagnosis with help of FNA especially in children assists in more rational planning of treatment. Even if a lymph node measures less than 1 cm it's still worthy of giving an attempt for FNA if the child is co-operative. To conclude reactive hyperplasia is the most common cause of lymphadenopathy in the pediatric population. This was followed by tuberculosis and acute necrotizing Lymphadenitis. Z/N stain should be performed in all cases of tuberculosis and acute necrotizing lymphadenitis. In case of strong suspicion of tuberculosis, AFB needs to be thoroughly screened even in the hypo cellular areas. The diagnostic ease of Hodgkin's Lymphoma on FNA can be used for early diagnosis and cure of the same. If the lymph node aspirates are inadequate / non-specific and the lymph node persists for 2-3 weeks of treatment and excision biopsy should definitely be considered.

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