

FINE NEEDLE ASPIRATION CYTOLOGY- EFFECTIVENESS IN TUBERCULOSIS DIAGNOSIS

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ABSTRACT

Introduction- Tuberculosis is a specific infectious disease caused by bacteria belonging to the "Mycobacterium tuberculosis complex". It presents a great social and economic problem and is one of the major factors responsible for the high morbidity and mortality in India. The incidence of tuberculous cervical lymphadenopathy now accounts for two third of the extra pulmonary tuberculous lymphadenopathy. Most of these are supposed to be tuberculous in origin because of greater incidence of pulmonary tuberculosis in our country. At the same time there are other causes of lymph-adenopathy which are usually misdiagnosed as tuberculosis. It has been a common problem for both to clinicians as well as pathologist from to diagnose tuberculosis.

Methos and Materials- The present work is carried out in 100 clinically suspected cases of tuberculous cervical lymphadenitis attending E.N.T., Surgery, Paediatrics and Medicine Department of central India institute as an outdoor/indoor patient during the period of one year. Patients with enlarged cervical lymph nodes with a history suggestive of tuberculosis were included after taking an informed consent.

Results- Study was done on 100 clinically suspected cases of tuberculous cervical lymphadenitis, Tuberculosis was diagnosed in 57% cases by FNAC, smear and culture together, the maximum incidence of tuberculosis was observed in second and third decades, Females were more affected (64%) than males with the ratio of 1:2.3. By FNAC 42% accuracy was obtained, 30% cases were AFB smear positive in our study this rate of incidence is nearer to other authors. In our culture study, 57 cases were diagnosed as tuberculous and 4 cases as non-tuberculous cervical lymphadenitis. Culture positive was higher in granulomatous necrotic lesions. Sensitivity, specificity and predictive values of culture study were significantly higher than FNAC and smear. These methods of investigation needs considerable experience and confidence of a pathologist who perform the procedure for a better result. When culture was taken as Gold Standard, cytology was found to be more sensitive than smear.

Conclusions – from this study we concluded that Both FNAC and smear are quick, simple, less traumatic and cost-effective methods and used as a routine investigating procedure in OPD of urban and semi-urban hospitals. Simplicity of these techniques (FNAC & Smear) combined with early availability of results and good diagnostic accuracy warrants their clinical application. Missed cytological diagnosis and isolation of non-tuberculous mycobacteria justify culture studies on all suspected tuberculous lymphadenitis cases

INTRODUCTION

Tuberculosis is a specific infectious disease caused by bacteria belonging to the "Mycobacterium tuberculosis complex". The complex includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. fortuitum*, *M. kansasii* and *M. scrofulascium*. Tuberculosis is one of the commonest diseases remains a world-wide public health problem, even today. It presents a great social and economic problem and is one of the major factors responsible for the high morbidity and mortality in India.

Tuberculosis (TB) is a chronic granulomatous infection principally caused by *Mycobacterium TB* and less frequently by ingestion of *Mycobacterium bovis* infected unpasteurized cow's milk or by other atypical mycobacteria. TB is a large-scale health hitch with 8 million citizens infected yearly and 3 million people dying from diseases related to TB complications. The frequency of TB in underdeveloped nations is snowballing, and this is believed to coexist with poor hygiene environments and increased occurrence of acquired immunodeficiency syndrome. TB chiefly affects the pulmonary system besides involving extra-pulmonary locations comprising head and neck region. Extra pulmonary TB is rare occurring in 0.05-5% of

patients with TB. In this way, this disease rarely features in the differential diagnosis of head and neck lesions. Here, we report the case of a child patient diagnosed with submandibular TB lymphadenitis, which resolved completely after anti TB therapy

The disease is usually chronic with varying clinical manifestations. The disease primarily affects lungs and causes pulmonary tuberculosis. It can also affect intestine, meninges, bones and joints, lymph nodes, skin and other tissues of the body. Peripheral tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis, most commonly affects the cervical lymph node. (32) It is widely prevalent particularly in the malnourished, undernourished and debilitated children.

The incidence of tuberculous cervical lymphadenopathy now accounts for two third of the extra pulmonary tuberculous lymphadenopathy. Most of these are supposed to be tuberculous in origin because of greater incidence of pulmonary tuberculosis in our country. At the same time there are other causes of lymphadenopathy which are usually misdiagnosed as tuberculosis.

Histopathological study requires considerable time and it may complicate after biopsy. Therefore, the need for less traumatic and some faster technique (AFB staining, FNAC) has been felt in this field and fine needle aspiration of cervical lymph nodes for cytology, AFB staining and culture could be a possible. (27,33). The Gold Standard for diagnosis of tuberculous lymphadenitis is the demonstration of mycobacteria in biopsy specimen by smear or culture. The sensitivity of these conventional methods is, however low when the specimen contains only a small number of organisms. Some studies demonstrated the accuracy of these conventional bacteriologic methods is less than 50% (9,31).

Fine needle aspiration avoids the physical and psychological trauma which occasionally encountered after biopsy, general anaesthesia, surgical operation and hospitalization. Fine needle aspiration being a simple outpatient procedure is well accepted by patients and has practically no complications. The efficacy of FNAC as a diagnostic procedure is already established and it has been found as effective as biopsy, particularly in cases of tubercular lymphadenitis. The aim of FNAC is to confirm the nature of lesion within 24 hours or less. Cyto-diagnosis by aspiration of cervical lymph nodes was begun by Guthrie in 1921.

The study is based on 100 patients with cervical lymphadenitis of different age group, rural as well as urban patients irrespective of their social standing and religion selected at random were included in the study

AIMS & OBJECTIVES-

This prospective cohort study done TO STUDY THE INCIDENCE, PRESENTATION AND DIAGNOSIS OF TUBERCULAR CERVICAL LYMPHADENITIS IN CENTRAL INDIA POPULATION for one-year periods in 100 patients. The aims are-

- To assess the diagnostic role of FNAC, Smear and culture of fine needle aspiration done on clinically suspected cases of tuberculous cervical lymphadenitis.
- To know different clinical presentation of tubercular cervical lymphadenitis.
- To know the most affected cervical lymph node group.
- To detect individuals and comparative sensitivity, specificity and predictive values of each diagnostic investigations.

MATERIAL AND METHOD

The present work is carried out in 100 clinically suspected cases of tuberculous cervical lymphadenitis attending E.N.T., Surgery, Paediatrics and Medicine Department of central India institute as an outdoor/indoor patient during the period of one year. Patients with enlarged cervical lymph node (s) with a history suggestive of tuberculosis were included after taking an informed consent. Relevant clinical details were recorded. Fine needle aspiration was performed aseptically.

Result:

On high power microscopic examination smear shows variable number of Epithelioid granuloma, Epithelioid cells, lymphocytes, macrophages, polymorphs and Langhan's giant cells against necrotic background.

Result:

Mycobacterium tuberculosis were stained bright red remaining field was stained blue. Acid fastness because of high content and variety of lipids, fatty acids and higher alcohol found in tubercle bacilli.

CULTURE OF MYCOBACTERIA:

Result: Mycobacterium tuberculosis form large, rough and orange yellow colonies whereas mycobacterium bovine produces small, smooth, flat, colourless and discrete growth.

CULTURE MEDIA:

Different types of culture medias are used for growth of mycobacterium tuberculosis. Lowenstein Jensen is most commonly used media.

Types:

- 1] Solid media (L-J media, Dorset egg media, petraghani media).
- 2] Blood media (Tarshish media).
- 3] Serum (Loffler's serum slope media, potato and Dubo's media).

Lowenstein-Jensen's media:

It is a solid media, most widely used for routine culture for M. tuberculosis and when the material is contaminated with other organisms.

It consists of coagulated Hen's egg, mineral salt solution, asparaginase and malachite green. Malachite green prevents growth of other bacteria.

BIOCHEMICAL REACTIONS:

Several biochemical tests have been described for the identification of mycobacterial species.

-Niacin Test; Human bacilli form Niacin when grown on an egg medium (L-J Medium). The human bacilli give a positive reaction, while the bovine type is negative.

-Nitrate Reduction Test: This is positive with M. tuberculosis and negative with M. bovis. This test is weakly positive with some atypical mycobacteria like M. kansasii and M. fortuitum.

-Catalase Test: Most atypical mycobacteria are strongly catalase positive while tubercle bacilli are weakly positive in comparison. e.g. M. kansasii, M. scrofulascium and M. fortuitum.

-Peroxidase Test Tubercles bacilli give positive peroxidase test but atypical mycobacteria are negative.

-Aryl Sulphatase Test: This is formed by atypical mycobacteria only. The organisms are grown in 0.001 M tripotassium phenolphthalein disulphate. 2N NaOH is added drop by drop to the culture. A pink colour indicates a positive reaction. e.g., M. fortuitum.

OBSERVATIONS=

From this study we observed that=

TABLE: I

AGE DISTRIBUTION OF THE PATIENTS WITH CERVICAL LYMPH

S. NO.	AGE [YEARS]	NO. OF NON-TUBERCULAR CERVICAL LYMPH NODES CASE	NO. OF TUBERCULAR CERVICAL LYMPH NODE CASES	TOTAL NO OF LYMPHADENOPATHY
1	1-10	4	7	11
2	11-20	10	23	33
3	21-30	7	19	26
4	31-40	12	4	16
5	41-50	6	1	7
6	51-60	3	2	5
7	61-70	1	1	2
TOTAL		43	57	100

- Most common affected age group are middle age group. 75% cases found in 11-40 years of age.

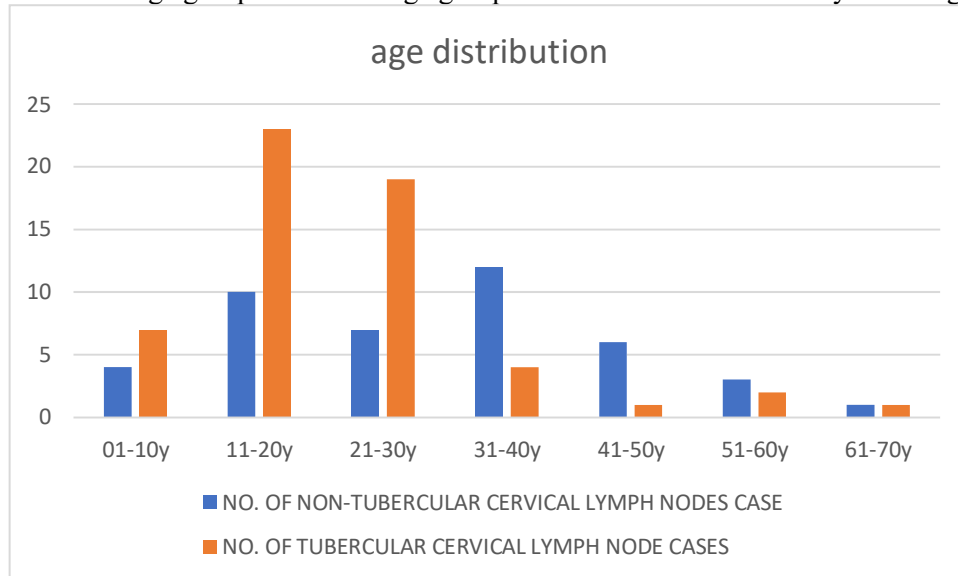


TABLE: II
SEX DISTRIBUTION OF PATIENT WITH CERVICA LN

S. NO	AGE	MALES	FEMALE	TOTAL
1	1-10	3	8	11
2	11-20	10	23	33
3	21-30	9	17	26
4	31-40	6	10	16
5	41-50	2	5	7
6	51-60	4	1	5
7	61-70	2	0	2
TOTAL		36	64	100

-Females are more affected than males.

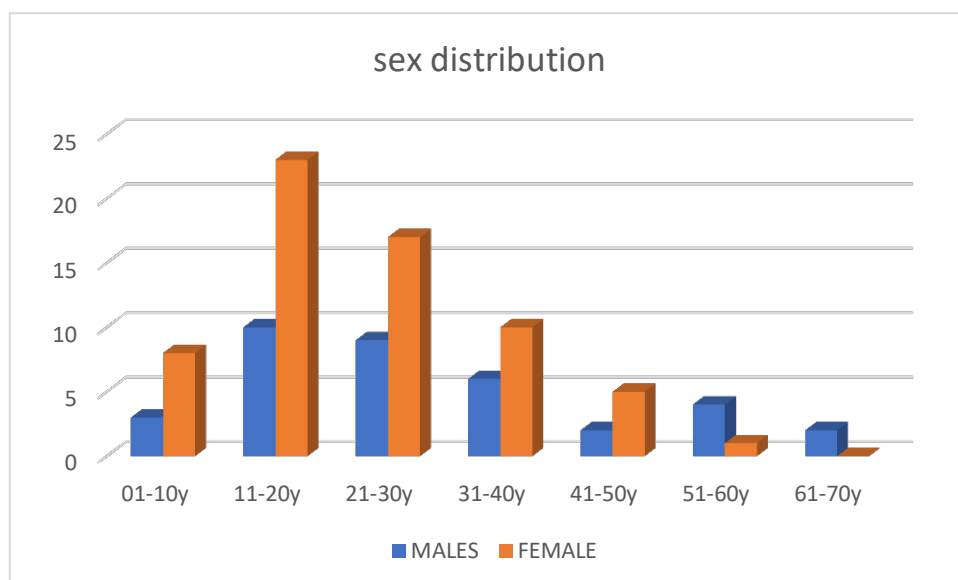


TABLE: III

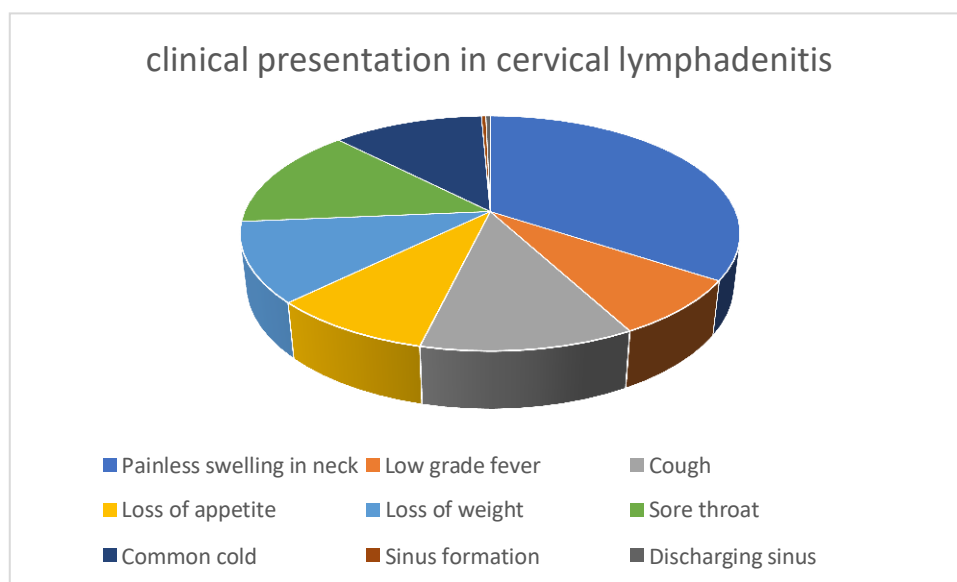
SEX DISTRIBUTION OF PATIENT WITH TUBERCULAR AND NON- TUBERCULAR CERVICAL LYMPHADENITIS

Cervical Lymphadenitis	MALE	PERCENTASE	FEMALE	PERCENTASE
Tubercular cervical lymphadenitis (57 cases)	17	29.82%	40	75.44%
Non- tubercular cervical lymphadenitis [43 cases]	19	44.19%	24	55.83%

TABLE: IV(A)

PRESENTING SYMPTOMS OF CASES OF CERVICAL LYMPHADENITIS

Symptoms	No. of cases	Percentage
Painless swelling in neck	100	100
Low grade fever	23	23
Cough	34	34
Loss of appetite	26	26
Loss of weight	32	32
Sore throat	41	41
Common cold	34	34
Sinus formation	1	1
Discharging sinus	1	1

**TABLE: IV (B)**

PRESENTING SYMPTOMS OF CASES OF TUBERCULAR CERVICAL LYMPHADENITIS

Symptoms	No. of cases	Percentage
Painless swelling in neck	57	100
Low grade fever	42	73.6
Cough	23	40.3

Loss of appetite	21	36.8
Loss of weight	34	59.6
Sore throat	11	19.3
Common cold	28	49.1

Most common presenting symptom of cervical lymphadenopathy is painless swelling in neck followed by sore throat while most common presenting features of tubercular cervical lymphadenitis is painless swelling over neck followed by low grade fever.

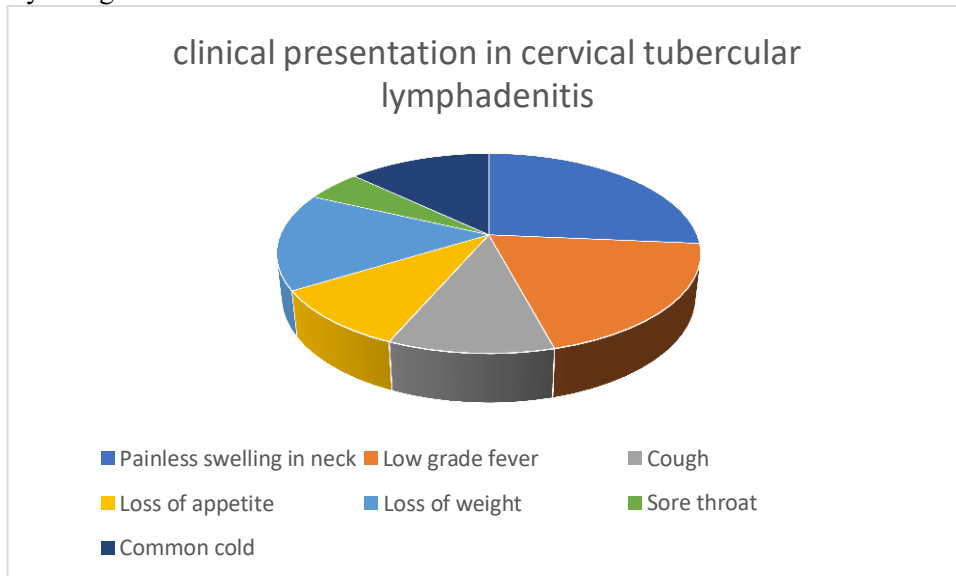


TABLE: V (A)

GROUP-WISE DISTRIBUTION OF CERVICAL LYMPH NODES

Total no. of cases of tubercular cervical lymphadenitis	Cervical group of lymph nodes involved	No. of cases
100	Upper deep cervical [post. Triangle]	51
	Submandibular	24
	Supraclavicular	17
	Submental	6
	Jugulodiagastric	2

TABLE: V (B)

GROUP-WISE DISTRIBUTION OF THE TUBERCULAR CERVICAL lymph nodes

Total no. of cases of tubercular cervical lymphadenitis	Cervical group of lymph nodes involved	No. of cases
57	Upper deep cervical [post. Triangle]	33
	Submandibular	10
	Supraclavicular	11
	Submental	3
	Jugulodiagastric	

-Most common lymph nodes group of neck involved is upper deep cervical followed by submandibular group in cervical lymphadenitis.

INVESTIGATIONS:

(A) FNAC: On the basis of cytology, cervical lymphadenitis can be divided into two groups, tubercular and non-tubercular.

TABLE: VI

CORRELATION OF MAJOR CYTOLOGIC FEATURES WITH PRESENCE OF VARIOUS CELLULAR CONSTITUENTS

Major cytological features	No. of cases	Lymphocytes	Giant cells	Macrophages
Epitheloid cells granuloma without necrosis	10	8	10	7
Epitheloid cells granuloma with necrosis	31	31	28	30
Necrosis without epitheloid cells granuloma	1	1	1	1
Total	42	40	39	38

The most common cytological features were the presence of Epitheloid cells granulomas, Langhan's giant cells, lymphocytes, macrophages and necrosis.

The epitheloid cell granulomas were present in 41 cases (97.6%), multinucleate giant cells were detected in 39 cases (92.8%) and macrophages were detected in 38 cases (90.47%). Appreciable lymphoid cells were noticed in 40 cases (95.2%).a

The number of cases associated with epithelial cell granuloma and necrosis were 31 (73.8%), without necrosis were 10 (23.8%) and only one case was associated with necrosis.

(B). SMEAR EXAMINATION (AFB STAINING):

Increase in AFB positivity was noticed in the presence of increasing degree of necrosis. AFB smear was positive in 65.85% of necrotic lesions and 5% of non- necrotic lesions. A lower rate of positivity was observed with presence of granulomatous features alone while necrosis was found to be associated with increasing AFB smear positivity (70.97%). The overall AFB smear positivity was 30% (30/100).

(C). CULTURE OF MYCOBACTERIA:

On Lowenstein Jensen's culture mycobacteria were isolated in 61 cases, of which 57 (93.4%) were identified as *M. tuberculosis* and 04 (6.5%) as non- tuberculous mycobacteria. On further speciation of NTM, two were as *M.kansassi*, one *M.scrofulascium* and one *M.fortuitum*. Culture positivity was higher in granulomatous necrotic lesions (87%). The minimum incubation time for isolation of *M. tuberculosis* was 21 days and the maximum was 42 days (mean 29 days).

TABLE: VII

COMPARISON OF CYTOLOGICAL AND MYCOBACTERIOLOGICAL FINDINGS

Cyodiagnosis	Cytological findings	Number	Smear positive	Culture positive	Tubercular mycobacteria	Non-tubercular mycobacteria
Suggestive of tuberculosis	Epitheloid cells granuloma without necrosis [group-1]	10	0	0	0	0
	Epitheloid cells granuloma with necrosis [group-2]	31	22	29	2	2
	Epitheloid cells granuloma with necrosis [group-3]	1	0	1	1	0
Suppurative	Necrosis with neutrophils	9	5	9	7	2
Non-specific lymphadenitis		36	3	18	18	0

	Insufficient aspirates	13	0	4	4	0
Total		100	30	61	57	4

TABLE: VIII

INDIVIDUAL POSITIVITY RATE OF INVESTIGATIONS

FNAC	42%
Smear	30%
Culture	61%

TABLE: IX

Species identification of mycobacteria

Species	Number	Growth rate	Types of growth	Niacin test	Catalase test	Nitrate reduction test	Aryl sulphatase test
M. tuberculosis	57	Slow	Rough	+	-	+	-
M. kansasii	2	Slow	Rough	-	+	+	-
M. scrofulaceum	1	Slow	Smooth	-	+	-	-
M. fortuitum	1	Rapid	Smooth filamentous/ rough filamentous	-	+	+	+

TABLE: X

TRUE & FALSE DATA OF INVESTIGATIONS

Investigation	True positive [a]	True negative [d]	False positive [b]	False negative [c]
Cytology	44	29	12	29
Smear	27	10	3	14
Culture	57	31	4	10

TABLE: XI

STATISTICAL COMPARISON OF FNAC, SMEAR AND CULTURE IN DIAGNOSIS TUBERCULOSIS

Tests	Sensitivity	Specificity	PPV	NPV
FNAC	60.27	70.73	78.56	50
Smear	65.85	76.91	90	41.6
Culture	85	88.57	93.4	75.6

DISCUSSION

Present study comprises 57 cases of tubercular lymphadenitis based on FNAC, Ziehl Neelsen's AFB stain and mycobacterial culture technique to assess the diagnostic value of these investigations.

Tuberculosis is still the commonest cause of lymphadenopathy in our country where as it is less in western countries because of:

-Tuberculosis of dairy has almost been eliminated.

-Pasteurisation of milk.

-The wide spread removal of tonsils which removes the portals of entry of tubercle bacilli and also reduces the pyogenic infections to be spread via haematogenous route (Lester C. W. 1948).

The usual age at which the disease clinically manifests as found out in the present study was highest in second and third decades (shown in table I).

Comparison with other authors is shown in table XI.

Authors	Year	No. of cases	average age of incidence
Lester C. W.	1948	72	15 years
J.A. Ross	1953	51	27 years
Trivedi & Basu Malik	1953	78	11-20 years
R.K. Narang <i>et al.</i>		60	21-30 years
Natraj G. <i>et al.</i>	2002	250	21-30 years

Thus, the findings of our study are in accordance with observation of other. In the present study, only 6 cases were found in below 10 years of age. The low incidence could be because of treatment without prior investigations. But, Patra *et al* (1983) and J.P. Singh *et al* (1989) reported highest incidence of tubercular lymphadenitis in below 10 years of age.

In the present study the sex incidence was found to be more in females than in males. The male to female ratio of tubercular lymphadenitis was found to be 1:2.3. Male to female ratio reported by some authors, e.g., Natraj G. *et al.* 2002 (1:1.3), Sunarto Reksoprawipro (1:2.14), R.K. Narang *et al.* (4:5).

In our study incidence of tubercular cervical lymphadenitis in rural and urban areas were 61.4% and 38.6% respectively. Higher incidence in rural area also reported by Radhika S. *et al.* (1993), Gutpa S.K. *et al.* (1993), Patra *et al.* (1993), Natraj G. *et al.* (1982).

In our study most of the patient reported most commonly with painless swelling in neck, low grade fever, cough, loss of appetite & weight. Gupta S.K. *et al.* and Radika *et al.* (1993), Tarun Dua *et al.* (1996), Natraj G. *et al.* (2002).

In the study, cases diagnosed as tubercular cervical lymphadenitis involved upper deep cervical lymph nodes (57.89%), supraclavicular lymph nodes (19.3%), Submandibular lymph nodes (17.54%) and submental lymph nodes (5.26%).

Tubercular lymphadenopathy frequently occurs in the neck (57%) and in supraclavicular area (26%) involving 1-3 nodes (Polesky *et al.*, 2005). The posterior triangle lymph node was involved in 59.4% cases, anterior triangle lymph node in 21.9% cases and more than one triangle in 18.75% cases (Mervyn Deitel, Toronto).

Cytodiagnosis of tuberculous lymphadenitis is usually based upon demonstration of epitheloid cells and Langhan's giant cells in smear (Koss L.G., 1979). However, epitheloid granuloma can be seen in nontuberculous lesions and occasionally in malignancies (Christ and Feltes Kennedy, 1982).

Presence of epitheloid cells is the first step in establishing a diagnosis while morphological, microbiological and clinical features can be of additional help (Lucas, 1955). Even in the absence of epitheloid cells and giant cells, necrotic material is proved to be useful as it yields the highest positivity of acid-fast bacilli (Rajwanshi *et al.*, 1987).

We have made an attempt to evaluate the role of various cell types in tuberculous lymphadenitis assessing their presence or absence in different smear pattern (epitheloid cells with or without giant cells and with or without necrosis) and by correlating these with smear and culture positivity.

The cytological features which are specific for tubercular lymphadenitis are caseous necrosis, epitheloid cells and multinucleated giant cells. In places where mycobacterial infections are prevalent and other granulomatous diseases are uncommon, diagnosis of tuberculosis can be made confidently when the above features are present. In the present study epitheloid cells, multinucleated giant cells, lymphocytes and macrophages were in 97.6%, 92.8%, 95.2% & 90.47% in cytology of aspirates from tuberculous lymph nodes and abscesses respectively. FNAC have an important role in diagnosis of tuberculosis of lymph nodes.

In our study 42% cases were diagnosed as tubercular lymphadenitis by FNAC. This percent of accuracy is quite nearer to the various authors e.g. Rajwanshi *et al.* (1987) 46.6%), Metre and Jayram (1987) 49.8%), Radhika *et al.* (1989), 23.58%, Vibha Talwar *et al.* (1990) 54%, Natraj G. *et al.* (2002) 53.20%.

In the present study, the rate of AFB positivity was 30%, which is nearer in accuracy with various authors e.g., Lucas (1955) 18%, Krishnaswamy (1975) 25%, Lau *et al.* (1988) 53%, Vibha Talwar *et al.* (1990) 40%, Gupta S.K. *et al.* (1993) 25%, Radhika S. *et al.* (1993) 45%, Tarun Dua *et al.* (1996) 27.11%, Natraj *et al.* (2002) 49.4%.

The overall smear positivity in various reports range from 18% to 53%. In comparison, the overall smear positivity in the present study at 30% was on lower side. This may be due to the fact that we had screened the smears after acid fast stain and not fluorescent stain.

Each lymph node aspirate samples were inoculated on two slants of L.J. medium at 37°C in presence of CO₂ for at least eight weeks. Each culture was examined every day for the first week and then weekly thereafter. All the positive cultures were first confirmed for their acid fastness and subsequently identified by using the following criteria: Rate of growth, Type of growth, Niacin test, Catalase test, Nitrate reduction test and Aryl sulphatase test. In the present study there is strong suggestion that the three cytological groups differed from each other.

There is increase in culture positivity from zero percent in those with granuloma alone to 93.5% when necrosis was associated with granuloma and to 100% when necrosis alone was seen (Tarun Dua *et al.*, 1996; Natraj G. *et al.*, 2002). Similar opinion has also been put forward by others probably due to the fact that the central necrotic portion of tubercle contains more bacilli.

In our study, mycobacteria were isolated in 61 cases of which 57 (93.44%) were identified as *M.tuberculosis* and 04 (6.56%) were non-tuberculous mycobacteria.

The prevalence of NTM in our study was 6.56% but *M.tuberculosis* still appears as the most common causative agent of lymphadenitis. Finding also seen in other studies done on NTM in India e.g Ramnathan *et al.* (1999) isolated with a rate of 5.26%; (2002) 3.85% and Vibha Talwar *et al.* (1990) 21%.

Prevalence of mycobacterium tuberculosis in the study of various authors are: Natraj G. *et al.* (2002) 50%, Polesky *et al.* (2005) 62%, Vibha Talwar *et al.* (1990) 30%.

SUMMARY & CONCLUSION

From this study we concluded that Both FNAC and smear are quick, simple, less traumatic and cost-effective methods and used as a routine investigating procedure in OPD of urban and semi-urban hospitals. Simplicity of these techniques (FNAC & Smear) combined with early availability of results and good diagnostic accuracy warrants their clinical application. Missed cytological diagnosis and isolation of non-tuberculous mycobacteria justify culture studies on all suspected tuberculous lymphadenitis cases.

Study was done on 100 clinically suspected cases of tuberculous cervical lymphadenitis. Tuberculosis was diagnosed in 57% cases by FNAC, smear and culture together. The maximum incidence of tuberculosis was observed in second and third decades. Females were more affected (64%) than males with the ratio of 1:2.3. By FNAC 42% accuracy was obtained, which is comparable to the accuracy found by the other authors. 30% cases were AFB smear positive in our study this rate of incidence is nearer to other authors. AFB smear was more positive in necrotic lesions. Both FNAC and smear are quick, simple, less traumatic and cost-effective methods. Both FNAC and smear can be used as a routine investigating procedure in OPD of urban and semi-urban hospitals. Simplicity of these techniques (FNAC & Smear) combined with early availability of results and good diagnostic accuracy warrants their clinical application. 57 cases were diagnosed as tuberculous and 4 cases as non-tuberculous cervical lymphadenitis. Culture positive was higher in granulomatous necrotic lesions. Sensitivity, specificity and predictive values of culture study was significantly higher than FNAC and smear. These methods of investigation need considerable experience and confidence of a pathologist who perform the procedure for a better result. When culture was taken as Gold Standard, cytology was found to be

more sensitive than smear. Missed cytological diagnosis and isolation of non-tuberculous mycobacteria justify culture studies on all suspected tuberculous lymphadenitis cases.

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