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Pathology

TUBERCULOUS LYMPHADENITIS AND CYTOLOGICAL METHODS

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ABSTRACT The investigations remain the cornerstone in diagnosis and treatment of tuberculosis, the choice of which depends on socioeconomic strata in high burden countries. The use of molecular methods by western world has limited the usage of cytological methods(FNAC) for treatment monitoring only. An effort is made here to highlight the efficiency of cytologic methods in the evaluation of tuberculous lymph nodes. The burden of EPTB and the shift in trend over a period of time was also observed. The data was accessed from RNTCP registers for EPTB proportion and departmental register for the cytomorphologic pattern. The EPTB burden currently was 47% in our set-up with lymph nodes and pleura accounting for more than 50% of the cases, other sites in decreasing order of frequencies were abdominal, osteoarticular, CNS and others. The cytological study portrayed epithelioid granuloma without necrosis as the most common morphological pattern, a majority of them had blood-tinged aspirate with a paltry AFB positivity. The other two categories epithelioid granuloma with necrosis and necrosis without granuloma had cheesy and purulent aspirates with a significant AFB positivity rate. The modern molecular methods have not phased out the credibility of cytological (FNAC) methods for diagnosis of tuberculous lymphadenitis. Cytological methods still remains a sine qua non for diagnosis especially in areas where facilities for molecular tests are not available.

KEYWORDS : Extrapulmonary tuberculosis, FNAC, Aspirates, Cytomorphological patterns

INTRODUCTION

Tuberculosis an infectious disease by bacillus Mycobacterium tuberculosis is the leading cause of death from a single infectious agent, ranking above HIV/AIDS and is one of the top ten causes of death worldwide.[1] Tuberculosis a global health problem has existed since millennia.[1] The disease can affect any system in the body with a certain predilection for the lungs. Pulmonary tuberculosis represents the major chunk of the tuberculosis cases while extrapulmonary tuberculosis makes up for fifteen percent to twenty percent(15-20%) the representation which remains higher in HIV-infected people(50%).[2,3] The term extrapulmonary is applicable when tuberculosis affects organ other than the lungs. Lymph Nodes and pleura are the most common extrapulmonary sites. Tuberculous lymphadenitis accounts for nearly one-third of extra-pulmonary tuberculosis cases.[3]

The causative agent expelled into the atmosphere by sick pulmonary tuberculosis patients while coughing infects nearly one-quarter of the healthy population, however, it is known to cause disease relatively in a small proportion of infected individuals during their lifetime, 5-15% of 1.7 billion infected people.[1] Those with HIV and other risk factors such as under-nutrition, diabetes, smoking, and alcohol consumption are more prone to the disease when compared to their counterparts[1].

Despite a fall in the number of deaths and incidence rates globally and in India, the malady tuberculosis still continues to be a larger epidemic than previously estimated. The reflected fresh surveillance and survey data from India indicate an upward revision (2000-2015)[2] hinting that the previous estimates were low. India, Indonesia, China, Nigeria, Pakistan and South Africa accounted for 60% of new cases worldwide. India listed in the high burden countries accounts for more than one-quarter of the world's TB cases and deaths. India adds 2.8 million cases annually to the estimated 10.4 million new TB cases worldwide (90% adults; 65% male; 10% people living with HIV),[1] death due to TB was 435000(.4 million) in contrast to 1.8 million deaths worldwide (0.4 million deaths in HIV-positive people).The estimates for India are still considered as interim,[2] pending a national prevalence survey in 2017/18.

The clinically suspected tuberculous lymphadenitis cases are investigated for the evidence of disease. The diagnostic tests available are cytological, histopathological, culture-based and molecular methods performed on aspirates and excised specimen. Cytologic method (FNAC) is relatively simple, easy to perform, rapid in results, inexpensive cost wise and has a higher sensitivity and specificity rates of around eighty and ninety percent,[4] hence chosen as a first-line investigation for the diagnosis of TB lymphadenitis. Histopathological examination, on the other hand, is invasive and not cost-effective, culture-based methods are the gold standard but time-consuming while molecular techniques need expensive machines and good technical expertise.

The diagnosis of tuberculous lymphadenitis with FNAC is phasing out in western nations[1] on account of availability of molecular techniques. however, it still remains the investigation of choice in ill-equipped overpopulated areas where facilities for molecular techniques are not within reach. The cytologic criteria for the diagnosis of tuberculosis would be the presence of epithelioid granulomas, caseous or fibrinoid necrosis and multinucleated langhans giant cell.[4] The purpose(aim) of the study was to evaluate the efficiency of cytological methods in diagnosing tuberculous lymphadenitis, to analyze the EPTB burden with a note on a major shift of trend over time if any.

MATERIALS AND METHODS :

The present study by the cytology wing of Pathology department was undertaken at KVG Medical College & Hospital in Sullia, Dakshina Kannada district of Karnataka. The present study was a retrospective observational type.

The data from the RNTCP registers were considered for estimation of EPTB proportion, the total number of TB cases over the period of 4yrs (2014-2017) were documented, the cases were stratified according to the organs involved. The proportion of PTB and EPTB was calculated. The EPTB burden was observed for any shift in trend annually and as well as over a decade by comparing with the statistics of 2007-2009.

The data was gathered from the departmental registers. The clinically diagnosed and cytologically proven cases of tuberculous lymphadenitis(56 Cases) were considered for the study of morphological patterns, The stained smears (Papanicolaou, Hematoxylin/Eosin, and May Grunwald Giemsa) including those of Zeihl-Neelsen were accessed for review. The cases were stratified into three groups constructed in accordance with the patterns of Das[10] et al. Category I - Epithelioid granuloma without necrosis, Category II - Epithelioid granuloma with necrosis and Category III - Necrosis without epithelioid granuloma.

The gross nature of the aspirates were considered in parallel with the cytomorphological patterns. The cases were stratified according to the type of aspirates as blood tinged, cheesy or purulent. AFB positivity was noted.

RESULTS :

Fig 1. SHIFT IN TRENDS - EPTB PROPORTION

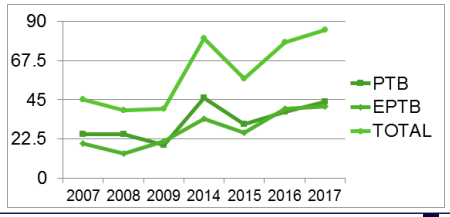

TABLE 1 . PTB AND EPTB BURDEN(2007-2009)					
Year	PTB& EPTB Cases	PTB Cases	%	EPTB Cases	%
2007	45	25	55.55	20	44.44
2008	39	25	64.10	14	35.89
2009	40	19	47.5	21	52.5
Total	124	69	55.64	55	44.35

TABLE 2 . PTB AND EPTB BURDEN(2014-2017)					
Year	PTB & EPTB Cases	PTB Cases	%	EPTB Cases	%
2014	80	46	57.5	34	42.5%
2015	57	31	54.3	26	45.7%
2016	78	38	48.7	40	51.3%
2017	85	44	51.8	41	48.2%
Total	300	159	53	141	47%

PTB - Pulmonary Tuberculosis , EPTB - Extrapulmonary Tuberculosis

TABLE 3 : TB DISTRIBUTION FORMS AND FREQUENCY			
	Anatomical Site	No of cases (n=300)	percentage (%)
PTB	Lungs	159	53%
EPTB	Lymph Nodes	42	14%
	Pleural Effusion	39	13%
	Abdomen	29	9.6%
	Osteoarticular	17	5.6%
	CNS	7	2.3%
	Others	7	2.3%

TABLE 4 : CYTOMORPHOLOGICAL PATTERNS IN TUBERCULOUS LYMPHADENITIS						
Patterns	Current study		Das et al		Hemalatha et al	
	Cases	%	Cases	%	Cases	%
I	29	52%	44	25%	29	19%
II	13	23%	68	39%	84	56%
III	14	25%	62	36%	34	23%

TABLE 4 : Cytomorphological Patterns : Category I - Epithelioid granulomas without necrosis - 29 cases (52%), **Category II -** Epithelioid granulomas with necrosis - 13 cases (23%), **Category III -** Necrosis without epithelioid granulomas - 14 cases (25%).

Age range : 6-62 yrs, Paediatric population - 5/56 cases, **Age groups -** Uniform scattering from second decade onwards. **Women -** 33/56 cases. **Lymph node groups -** Cervical > Axillary and Inguinal, Cervical - Upper deep cervical, submandibular and supraclavicular group.

TABLE 5. ASPIRATES AND AFB POSITIVITY				
Aspirate Type	Aspirate Numbers		AFB Positivity	
	Cases	%	Cases	%
Blood- Tinged	30	53.57	6	20
Cheesy	17	30.35	12	70.5
Purulent	9	16.07	6	66.6
	56	100	24	42.85

TABLE 5 : Aspirate type and AFB Positivity : Blood-tinged - 30/56 cases (54%), AFB positivity 6/30cases(20%). **Cheesy -** 17/56 cases(30%), AFB positivity - 12/17cases(70.5%), **Purulent -** 09/56 cases(16%), AFB positivity - 6/9 cases(67%)

DISCUSSION :

EPTB BURDEN

Extrapulmonary tuberculosis(EPTB) proportion in the current study was 47% as indicated in table 2, tuberculous lymphadenitis(TBLN) the most common form of EPTB together with pleura accounted for more than fifty percent of EPTB cases. Other organs involved in decreasing order of frequency were abdomen, osteoarticular, CNS and others(Table 3). Berg[5] et al in their study at health facilities in Ethiopia have reported a higher than average incidence of extrapulmonary tuberculosis (EPTB) ranging from 10-50% in different regions while Sunnetcioglu[6] et al demonstrated the burden of extrapulmonary tuberculosis (EPTB) to be 49.4 %. The reason for a high percentage in these geographical areas remain unclear and appears multifactorial.

A number of studies have indicated a shift in trend over the period of time[7,2] on account of factors such as better notification and HIV infection. The EPTB proportion in our set up at 2007-2009 was 44.35%(Table 1) and 2014-2017 was 47%(Table 2) with only a minimum increment. There were no cases of HIV infection. This shift in the trend over a period of time (10 yrs) was statistically significant, $P < 0.05$ but the annual shift in trend (2007-2009 & 2014-2017) was not, $P > 0.05$(Fig 1). The study in Korean peninsula reported EPTB proportion of 14% in 2007 but incremented to more than 20% in 2013.[8] The genuine proportion of EPTB appears to be much higher. Adolescent age, female gender, Asian and African ethnicity, and human immunodeficiency virus (HIV) infection are some of the independent risk factors.[8]

CYTOMORPHOLOGICAL PATTERNS

Has the advent of molecular techniques really phased out the cytological methods rendering them useful only for the purpose of treatment monitoring or do cytological methods still have a role in diagnosing tuberculous lymphadenitis? the answer would be both a yes and no depending on the socioeconomic strata of the society. Now, let's switch on to the cytological methods for evaluation of morphological patterns. Even before delving into the details, it would be worthwhile to have an idea of the existing literature. The available literature speaks on several different cytomorphological patterns of tuberculous lymphadenitis, Afrose[9] et al have documented seven patterns in lymph node aspirates. Pattern one of Afrose had predominantly an exudative response, Pattern two comprised predominantly of reactive lymphoid cells with few scattered or clusters of histiocytes or focal necrosis. The third, fourth and fifth pattern was akin to that of Das[10] et al, Pattern six had components of four or five along with stromal fragments and pattern seven comprised of histiocytic granuloma or foam cells

The three simplified morphological patterns appreciated in the current study were designed in accordance to the patterns of Das[10] et al. Category I (Granuloma without necrosis) was the commonest pattern followed by other categories, granuloma with necrosis and predominantly necrotic types accounting for 52%, 23%, and 25% respectively. The comparative study as indicated in Table 4. The category I had the least AFB positivity, and the aspirates were blood-tinged in comparison to category II and III which had cheesy and purulent aspirates with a significant AFB positivity rate.

The ease with which the test can be done, the cost-effectiveness, the necessity of least Sophisticated instrument makes the cytological (FNAC) techniques a popular, reliable and vital investigation methodology that is made available to a common man at all under-resourced geographic areas. Clinical examination together with cytological methods would be the ultimate care a patient might need and get in an overpopulated nation with a high TB burden. We insist and recommend cytological (FNAC) methods for diagnosis and culture techniques for confirmation with molecular tests as an additional investigation in case of its availability.

ASPIRATES AND AFB POSITIVITY :

The detection of M. tuberculosis with Ziehl-Neelsen stain allow a quick diagnosis, bacterial load of 5000 and 10,000 bacilli/ml are an absolute necessity for them to be detected by these stains.This explains the higher rate of positivity for biopsy (sensitivity>70-80%) in comparison to biological fluids (5-20%).[12] Nearly 30-50% of EPTB may be smear-negative.[12] Lymph node aspirates tend to occupy a slot between biopsy and biological fluid and can be expected to have a positivity rate of around 40-70%.

The rate of AFB positivity varies with the type of aspirates. Aspirates vary with the course of the disease and are either blood tinged, cheesy or purulent[13] with progression from initial to an advanced state. The lymph nodes are firm, enlarged and discrete with nonspecific hyperplasia in the beginning stages and undergo various transformations in the course of a disease process. The nodes become rubbery, fixed and matted due to periadenitis a little later. Areas of central softening (caseation) and abscess formation is the eventual outcome, an indication of advanced stages.[14] Bacterial load increases

with advancing stages. This explains the paltry positivity rate detected in the category of granuloma without necrosis in the present study.

Most of the aspirates in our study were blood tinged with many cheesy and few purulent ones. The cheesy aspirates had maximum AFB positivity rate in our study (70.5%) with an overall average of 42.8%(Table 5), of course, granulomas were best defined in blood tinged aspirates when compared to cheesy and purulent ones. Metre et al demonstrated 56.4% AFB positivity, with purulent aspirates having the maximum value (66%). One of the reasons for the low positivity rate of 42.8 % in our set up could be due to the fact that majority of the cases were early in the course of the disease process when bacterial load would be low.

CONCLUSION :

The availability of modern valuable tools like Quanti FERON-TB Gold In-Tube (QFT-GIT) and Xpert® MTB/RIF assay for the diagnosis of tuberculosis has not phased out the credibility of cytological (FNAC) methods as a tool in the evaluation of tuberculous lymphadenitis. Cytological methods still remains a sine qua non for diagnosis especially in areas where facilities for molecular tests are not available. Epithelioid granuloma without necrosis, the most frequent morphological pattern with despicable AFB positivity had blood tinged aspirates. The cheesy and purulent aspirates had significant AFB positivity.

EPTB accounts for 47% of the tuberculosis cases, Tuberculous lymphadenitis the most common form of EPTB was very closely followed by pleural tuberculosis, the shift in trend over a period of time was statistically significant.

References

1. Global Tuberculosis Report WHO 2017
2. Global Tuberculosis Report WHO 2016
3. Sharma SK, Mohan A, Extrapulmonary tuberculosis. Indian J Med Res 120, October 2004, pp 316-353
4. Miliauskas J. Lymph nodes, Chapter 5. Orell and Sterrett's Fine Needle Aspiration Cytology, 5th edition, Churchill Livingstone, 2012, Pages 77–117
5. Berg et al. Investigation of the high rates of extrapulmonary tuberculosis in Ethiopia reveals no single driving factor and minimal evidence for zoonotic transmission of Mycobacterium bovis infection BMC Infectious Diseases (2015) 15:112
6. Sonnetcioglu A, Sonnetcioglu M et al . Comparative analysis of pulmonary and extrapulmonary tuberculosis of 411 cases. Annals of Clinical Microbiology and Antimicrobials 2015 14 : 34
7. Dasgupta S, Chakrabarti S, Sarkar S. EPTB -Shifting trend of tubercular lymphadenitis over a decade - A study from eastern region of India. Biomedical journal 40 (2017), 284 -289
8. Lee JY, Diagnosis and Treatment of Extrapulmonary Tuberculosis, Tuberc Respir Dis 2015;78:47-55
9. Afrose R, Singh N, Bhatia A, Arora VK. Cytomorphological tissue reaction patterns in lymph node tuberculosis and their correlation with bacterial density.Annals of Tropical Medicine and Public Health. Year:2014 | Volume:7 | Issue:6 | Page:255-262.
10. Das DK, Pant JN, Chachra KL, Murthy NS, Satyanarayan L, Thankamma TC, et al Tuberculous lymphadenitis: Correlation of cellular components and necrosis in lymph-node aspirate with A.F.B. positivity and bacillary count. Indian J Pathol Microbiol. 1990;33:1–10
11. Hemalatha A, Udaya Kumar M, Harendra Kumar ML. Fine needle aspiration cytology of lymph nodes: A mirror in the diagnosis of spectrum of lymph node lesions. J Clin Biomed Sci 2011;1:164-72
12. M Ramirez-Lapausa, A Menendez-Saldana, A Noguerado-Asensio. Extrapulmonary tuberculosis: an overview: Rev Esp Sanid Penit 2015; 17: 3-11
13. Metre MS, Jayaram G. Acid-fast bacilli in aspiration smears from tuberculous lymph nodes. An analysis of 255 cases. Acta Cytol 1987;31:17-9.
14. Jones PG, Campbell PE. tuberculous lymphadenitis in childhood: the signifcance of anonymous mycobacteria. Br J surg. 1962;50:302- 314.