Cytomorphological tissue reaction patterns in lymph node tuberculosis and their correlation with bacterial density

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ABSTRACT
Diagnosis of extra pulmonary lymph node tuberculosis is made by demonstration of different cyto-morphological tissue reaction patterns on FNA smears however definitive diagnosis is made by demonstration of AFB by culture or Ziehl Neelsen stained smears. This procedure is technically demanding and time consuming and is liable to fail on occasion for unexplained reasons.If identification of cytomorphological patterns could predict bacterial density, it would help to improve diagnostic accuracy and also serve as a control on the acid-fast staining procedure. Therefore this study was being undertaken to determine the correlation between bacterial density and cytomorphological patterns in lymph node tuberculosis.Methods- FNA was performed on 505 clinically suspected lymph node tuberculosis patients. May Grunwald Giemsa stained smears were used to analyse cytomorphological patterns and ZN stained smears for AFB detection. Bacterial density (BI) was calculated by utilizing Ridley's logarithmic scale.

Results- Seven distinct cytomorphological tissue reaction patterns were observed. Pattern 1 was predominantly exudative response comprising of neutrophils and mononuclear phagocytes and was the most common tissue reaction pattern 160/505 (31.6%) followed by pattern 4 i.e. epithelioid cell granulomas with necrosis 148/505 (29.3%). This study showed that multibacillary lesion (BI>1) was more often associated with pattern 1. Although the BI varied significantly across different cytomorphological tissue reaction patterns (p value=0.004), no trend was observed as both paucibacillary as well as multibacillary was seen with various proportions with all patterns.

Conclusion- Present study showed that multibacillary disease is more frequently associated with pattern 1 compare to pattern 4. However more studies are needed to establish a trend among different cytomorphological tissue reaction patterns.

1. Introduction

Tuberculosis still represents a threat and a challenge to humanity as it has done throughout history. It was declared a global emergency by the World Health Organization (WHO) in 1993 [1]. It is estimated that 95 per cent of the world’s tuberculosis cases and 98 per cent of the tuberculosis deaths occur in the developing world [2].

India has the highest burden of tuberculosis in the world and accounts for nearly one fifth (20%) of the global burden of the disease [3]. In India extra-pulmonary tuberculosis (EPTB) comprises 20% of all tuberculosis cases and it’s prevalence in the country varies between 8.3 – 13.1% in different districts [4]. Lymph node involvement is the commonest extra-pulmonary manifestation [5].

The diagnosis of tuberculosis, based on clinical features alone can be erroneous [6]. Soluble antigen fluorescent antibodies [6, 7] indirect haemagglutination, [6, 7] kaolin agglutination [8] and ELISA [9] are cumbersome and time consuming tests. Antibodies to mycobacterium tuberculosis are not definitive evidence of active tuberculosis because false positive results are seen in high prevalence areas [9].

Fine needle aspiration cytology (FNAC) provides an inexpensive, quick and safe alternative to histopathology [10]. Because of easy accessibility of lymph nodes by fine needle aspiration (FNA), the diagnosis of EPTB is made by demonstrating different cyto-morphological tissue reaction patterns on smear. However definitive diagnosis depends on demonstration of AFB by culture or smear. The morphological manifestations exhibit specific spectrum which is similar to other granulomatous inflammations; best exemplified by the Ridley-Jopling scale of leprosy [11]. Other examples of granulomatous inflammations manifested in a morphological spectrum are Cutaneous leishmaniasis, Cysticercosis, Lymphatic filariasis and other infections [12].
The morphological spectrum in tuberculosis is highly variable but has been broadly classified as [13]

1. Epithelioid granuloma without necrosis
2. Epithelioid granuloma with necrosis
3. Necrosis only

The rate of AFB positivity in cytological smears varies widely in different studies [10,14] and is found to be highest in purulent aspirates, followed by caseous and least often in aspirates with blood mixed particles [14].

AFB demonstration can be done either by conventional Ziehl-Neelsen (ZN) method or by using auramine and rhodamine separately or in combination. ZN stain is the special stain requisitioned most frequently in most of the laboratories. This procedure is technically demanding and time consuming, as has been shown by different studies [15]. Whatever the method adopted, it is liable to fail on occasion for unexplained reasons.

Granulomatous inflammation is a manifestation of many chronic inflammatory diseases like tuberculosis, sarcoid, leprosy, syphilis, and various mycosis. Yet in regions of high endemicity such as India and Sri Lanka [16], largely, granulomatous inflammations are considered to be of tuberculous origin unless proved otherwise. In this setting demonstration of AFB, although not diagnostic of infection by M. tuberculosis, improves the diagnostic accuracy.

If identification of cytomorphological patterns could predict bacterial density, it would help to improve diagnostic accuracy and also serve as a control on the acid-fast staining procedure. Therefore this study is being undertaken to determine the correlation between bacterial density and cytomorphological patterns in lymph node tuberculosis.

METHODS

Study setting

This was a hospital based prospective observational study conducted in the Department of Pathology at University College of Medical Sciences (UCMS) and Guru Teg Bahadur (GTB) hospital, Delhi.

Clearance from ethical committee of institution was obtained. Written informed consent was obtained from patients, or family members. The study was conducted over a period of one year from 2009 to 2010.

Sample Size

The study was carried out in aspirated material of lymph nodes from 505 patients having clinical diagnosis of tuberculous lymphadenitis. Out of which

All AFB positive cases regardless of cytomorphology were included. When AFB was not demonstrable, but the tissue reaction pattern showed granulomatous inflammation with necrosis was also included in this study.

All AFB negative cases with granulomatous inflammation but without necrosis, purulent exudates, acellular necrosis and patient’s on anti-tubercular therapy were excluded from the study.

Methodology

FNA was carried out using 20 ml disposable plastic syringe and 23 gauge needle. Air dried smears were stained by May Grunwald-Giemsa (MGG) for cytomorphological analysis. Modified Ziehl-Neelsen (ZN) staining was done for AFB detection.

Bacteriological index was calculated by applying Ridley’s logarithmic scale 17:

<table>
<thead>
<tr>
<th>Bacteriological Index (BI)</th>
<th>Relative Bacterial Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No bacilli in any of the 100 oil-immersion fields</td>
</tr>
<tr>
<td>1+</td>
<td>1-10 bacilli, on average, in 100 oil-immersion fields</td>
</tr>
<tr>
<td>2+</td>
<td>1-10 bacilli, on average, in 10 oil-immersion fields</td>
</tr>
<tr>
<td>3+</td>
<td>1-10 bacilli, on average, in each oil-immersion field</td>
</tr>
<tr>
<td>4+</td>
<td>10-100 bacilli, on average, in each oil-immersion field</td>
</tr>
<tr>
<td>5+</td>
<td>100-1000 bacilli, on average, in each oil-immersion field</td>
</tr>
<tr>
<td>6+</td>
<td>More than 1000 bacilli, on average, in each oil-immersion field</td>
</tr>
</tbody>
</table>

RESULTS AND OBSERVATIONS

Seven cytomorphological tissue reaction patterns were identified on MGG stained smears.

Pattern 1: (160 cases) predominantly exudative response comprising of neutrophils and mononuclear phagocytes (Fig. 1). This group was found to be the largest group. AFB positivity was found to be 90.6%, BI ranged from 1+ to 4+.

Pattern 2: (10 cases) predominantly reactive lymphoid cells with few scattered or clusters of histiocytes or focal necrosis (Fig. 2). AFB positivity was 50%. BI in this group ranged from 1+ to 2+.

Pattern 3: (114 cases) Epithelioid cell granulomas without necrosis (Fig. 3) AFB positivity was 22.8%, BI ranged from 1+ to 2+.

Pattern 4: (148 cases) Epithelioid cell granulomas with necrosis (Fig. 4). This group was the second largest group with AFB positivity 70.3% and BI ranged from 1+ to 3+.

Pattern 5: (39 cases) Predominantly acellular necrosis (Fig. 5). AFB positivity was 61.5% and BI ranged from 1+ to 3+.

Pattern 6: (22 cases) Pattern 4 or 5 with preserved stromal fragments containing blood vessels (Fig. 6). AFB positivity was highest (95.4%). BI ranged from 1+ to 2+.

Pattern 7: (12 cases) Histiocytic granuloma or foam cells (Fig. 7). All cases were HIV positive, and showed 91.7% AFB positivity. BI ranged from 3+ to 6+. In this group all cases except 1(AFB negative) were multibacillary.

Table 1 depicts correlation of cytomorphological tissue reaction patterns with bacterial density.

The data for table 2 are derived from table 1 and pertain to 380 patients which met the inclusion criteria of this study.

Of these 380 cases 336 i.e. (88.4%) were AFB positive. All 44 (11.6%) cases which were negative for AFB belong to pattern 4 i.e. granulomatous inflammation with necrosis.

Analysis of table 2 reveals that the AFB positivity in the different group varied from BI 1+ to BI 6+. Since more than 25% cells in the grid are blank, statistical analysis for test of significance would not have been possible. To overcome this problem it was decided to carry out subsequent statistical analysis by reclassifying the cases in two groups namely BI = 1+ (paucibacillary) and BI > 1+ (multibacillary) as in the leprosy model. Also the 11 cases belonging to pattern 7 were not
considered for further statistical analysis because their numbers were small 11/380 (2.9%) cases and to avoid the possibility of high BI due to HIV interfering with the analysis.

Table 3 shows the breakup of the remaining 325 AFB positive cases and their tissue reaction patterns.

**Fig 1: Pattern 1- Exudative response, MGGx400**

**Fig 2: Pattern 2-Necrosis with reactive lymphoid cells, MGGx400**

**Fig 3: Pattern 3-Granuloma in a reactive background, MGGx400**

**Fig 4: Pattern 4-Necrosis with epithelioid granuloma, MGGx400**

**Fig 5: Pattern 5-Acellular necrosis, MGGx400**

**Fig 6: Pattern 6-Necrosis with vascular fragments (arrow), MGGx200**

**Fig 7: Pattern 7-loose aggregates of histiocytes, MGGx400**
The high burden of tuberculosis in our hospital is reflected in the annual turnover of the cytopathology section. In the year 2010-11 the turnover of patients referred for FNAC was 10514. Of them 2243 (21.3%) were tuberculous cases. Therefore ZN stain is most frequently requisitioned stain after MGG stain in our laboratory.

In our cytopathology set up the smears are first screened by the residents prior to sign out by the faculty on the next day. A large proportion of time is spent in searching for AFB in ZN smears. An earlier study [15] has attempted to rationalize the laboratory procedure in order to achieve higher AFB pickup rates. It has been demonstrated that selection of smears for ZN staining on the basis of screening MGG slides for foci of necrosis improves the pickup rate of AFB from 42% to 92% [15].

The literature reports AFB positivity rates in patients with tuberculosis varying from 37.4% to 59.4% [10,14,18]. Highest AFB positivity has been found in smears associated with necrosis without granulomas followed by epithelioid cell granuloma with necrosis/abscess and epithelioid granuloma in reactive lymphoid background [10,18]. None of these studies attempted systematic analysis of tissue reaction patterns in relation to AFB positivity. There have been sporadic reports in the literature describing tissue reaction patterns; these patterns are thought to be limited to three main types [5,13].

**DISCUSSION**

| Table 2: CORRELATION OF AFB POSITIVITY WITH TISSUE REACTION PATTERNS IN 380 PATIENTS WITH LYMPH NODE TUBERCULOSIS:
| Cyto- 

- Table 1: CORRELATION OF AFB POSITIVITY WITH TISSUE REACTION PATTERNS IN 505 PATIENTS WITH LYMPH NODE TUBERCULOSIS:

| Table 3: CORRELATION OF PAUCIBACILLARY AND MULTIBACILLARY CASES WITH TISSUE REACTION PATTERNS IN 325 PATIENTS WITH LYMPH NODE TUBERCULOSIS:

| Cyto- 

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On the other hand another mycobacterial disease i.e. leprosy has fairly well defined spectrum of tissue reaction patterns ranging from epithelioid granuloma at one end to histiocytic granuloma at the other with the addition of necrosis being seen in lesions in nerve. Compiling these patterns in the literature we were able to identify seven tissue reaction patterns in our study.

To ensure the validity of frequency with which these tissue reaction patterns occurred, it was proposed to include only AFB positive cases and only those AFB negative cases in which granulomatous inflammation was accompanied by necrosis. The latter group was included because granulomatous inflammation with necrosis has a high probability of being tuberculosis in origin. Using this approach we are able to identify 325 cases in a period of approximately 14 weeks.

**Cytomorphological spectrum in lymph node tuberculosis:**

The commonest tissue reaction pattern (largest group) 145/336 was predominantly exudative response comprising of neutrophils and mononuclear phagocytes. BI in this pattern ranged from 1+ to 4+ (Table 2). Influx of polymorphs in more advanced lesions is believed to occur as a secondary complication of softening or liquefaction [19]. Liquefaction of the caseous center of tuberculosis is one of the most harmful host responses in tuberculosis. Like caseation, it is associated with delayed type of hypersensitivity (DTH). Liquefaction seems to be due to hydrolysis of the protein, lipid, and nucleic acid components of caseous tissue by the hydrolytic enzymes of macro phages and granulocytes [20] with a resultant exudative response. A gradual decrease in enzyme inhibitors within the caseous focus may also contribute to liquefaction. In adults a tuberculous lesion is usually well controlled until its caseous center liquefies. The liquefied caseum is an excellent culture medium for the tubercle bacillus along with a possible increase in blood supply. In this menstruum, the bacillus extensively multiplies extracellularly for the first time and attains a more fluid, pus like consistency. It is now that oxygen creeps in and AFB multiply [28]. Therefore, the numbers of organisms and AFB may also occur due to the focal sampling of the node or when necrosis is limited to a small region.

The fourth common tissue reaction pattern was pattern 5 i.e. Acellular necrosis 24/336 (Fig. 5). BI in this group ranged from 1+ to 3+ (Table 2). No granuloma formation or epithelioid cells were noted in these cases. Macrophages cannot penetrate very far into the caseous center. Caseum is avascular, and adjacent blood vessels are thrombosed [27]. It has a moderately acidic pH, a relatively low oxygen tension, and usually a relatively high concentration of bacillary components that are toxic to the cells of the hypersensitive host. Thus, macrophages cannot eliminate the bacillus. In the tubercle bacilli within the caseous focus. Our study demonstrated areas of necrosis showed not only high positive rate but bacterial density was also high i.e. BI=1+ (Table 3).

The fifth common tissue reaction pattern we found was pattern 6 (21/336) i.e. Pattern 4 or 5 with preserved stromal fragments containing blood vessels. BI in this group ranged from 1+ to 2+ (Table 2). Mycobacterium is an obligate aerobe and hence the bacilli tend to localize close to preserved as well as degenerated or necrosed blood vessels in the necrotic areas [28]. At times the centre of granulomas may undergo liquefactive necrosis, acquiring a more fluid, pus like consistency. It is now that oxygen creeps in and AFB multiply [28]. Therefore, the numbers of organisms within the necrosis are variable.

The least common pattern observed was pattern 7 (11/336) in which loose histiocytic granulomas or foam cells were noted. BI ranged from 1+ to 6+ (table 2). In this group most patients 10/11 were HIV positive. In immuno-compromised persons (AIDS) there are qualitative and quantitative deficits in Th1 (CD4+) cells. Hence macrophages do not get activated. Non-activated macrophages release certain chemokines IL-1, TNF-a and lytic enzymes which contribute to local tissue destruction and caseous necrosis. In patients with persistent bacillary proliferation the bacilli takes place within macrophages [29] (Fig. 10). Normally in immuno-competent persons macrophage activation response takes place in response to T helper cell (Th-1) that activate macrophages to become bactericidal by release of IFN- and IL-2, which stimulate formation of phago-lysosome and nitric oxide synthesis. These activated macrophages differentiate into epithelioid histiocytes and effectively neutralizes tubercle bacilli [29].
Correlation of tissue reaction patterns with bacterial density:

The initial estimation of BI was done using 7 point (0 to 6+) Ridley jolping scale used for BI estimation in leprosy. The Ridley-jolping scale was chosen for this study because it is highly standardized and has been widely used for the estimation of BI in all mycobacterial disease. In contrast in tuberculosis several modifications [30] of BI are used ranging from subdividing cases into AFB positive and AFB negative through low and high AFB positivity to a 5 point scale (0 to 4+). Intention in choosing the Ridley-jolping scale was to achieve closer co-ordination of tissue reaction patterns with BI, should such coordination emerge. The correlation of AFB positivity with tissue reaction patterns in 380 patients with lymph node tuberculosis is shown in table 2.

Of 380 cases included in the study group only in two patterns, pattern 1 (Predominantly exudative response comprising of neutrophils and mononuclear phagocytes) and pattern 4 (Epithelioid cell granulomas with necrosis), the number were large enough for statistical comparison. It was found that multibacillary disease more frequently occur with pattern 1 (53.9%) compare to pattern 4 where it was only 23.4%. Although the BI varied significantly across different cytomorphological tissue reaction patterns (p value=0.004), no trend was observed as both paucibacillary as well as multibacillary was seen with various proportions with all patterns.

Conclusion

In conclusion seven distinct cytomorphological tissue reaction patterns were observed in AFB positive lymph node tuberculosis with a commonest being pattern 1 followed by pattern 4. Present study showed that multibacillary disease is more frequently associated with pattern 1 compare to pattern 4. However more studies are needed to establish a trend among different cytomorphological tissue reaction patterns.

Limitations of the study: Excluding AFB negative cases from the study could be a limiting factor. However this decision to include only AFB positive cases was taken to ensure maximal confidence in the diagnosis of tuberculosis.

Further studies are needed to identify tissue reaction patterns which may predict low or high rates of AFB positivity.

Acknowledgement:

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References
