



Research Paper

Diagnostic efficacy of fine needle aspiration cytology in the evaluation of tuberculous lymphadenitis at the Diabetic Association Medical College, Faridpur, Bangladesh

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ABSTRACT: Tuberculous lymphadenitis (TL) is the most common presentation of extrapulmonary TB, the later being a global health problem nowadays. Fine needle aspiration cytology (FNAC) is a simple and important diagnostic tool in the evaluation of lymph node lesions. It is an inexpensive, easy to perform and the first choice investigation for patients in developing countries like Bangladesh. The prime aim of this study was to determine the sociodemographic features of 122 outdoor patients, characteristics and cytomorphological features of their TL, and the diagnostic accuracy of the FNAC. Purposively selected 122 male and female patients, who attended outpatient department of the Diabetic Association Medical College Hospital, Faridpur, Bangladesh, for diagnosis and treatment of their lymphadenopathy, were investigated. A standardized questionnaire was administered to obtain sociodemographic and clinical data. Three cytopathological patterns of the TL, viz., Pattern A- epithelioid granuloma (EG) without caseous necrosis (CN), Pattern B- EG with CN, and Pattern C- CN without EG, were correlated with Ziehl-Neelsen (ZN) staining, followed by acid fast bacilli (AFB) positivity tests. Chi-square tests were performed to correlate cytopathological patterns and AFB positivity. Finally, on correlating FNAC diagnoses with cytomorphological patterns, the sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of FNAC were estimated. Age groups of the TL patients ranged from 7 to 75 years, with an average of 32.49 ± 14.70 years, where the females were significantly higher in number than their male counterparts (male to female sex ratio= 1: 2.30). Irrespective of their gender, 56.55% of the patients had secondary level of education, 55.74% of them came from the rural areas and 59.02% respondents belonged to the low-income group. Small-sized (<3 cm) single-nodes, solid and cervical TL predominated, where fever, loss of appetite, weight loss and night sweat were common systemic features of the respondents. Majority of the TL belonged to Pattern B (58%), followed by Pattern C (24%) and Pattern A (18%). Overall, FNAC was found AFB positive for TL in 50.0% cases; of which Pattern A was 33.80%; Pattern B 77.27% and Pattern C 68.97%. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of FNAC were 92.31%, 73.33%, 66.67%, 94.29% and 80.28%, respectively for Pattern A. The corresponding values for Patterns B and C were 89.47%, 33.33%, 89.47%, 33.33% and 81.82%, and 86.96%, 66.67%, 90.91%, 57.14% and 82.76%, respectively. Diagnostic accuracies above 80% in all three cytomorphology patterns therefore indicated a quite satisfactory performance of FNAC in the diagnosis of TL in the patients under study. Because of its greater diagnostic efficacy, FNAC has gained a wide acceptance to both physicians and patients all over the world. The present diagnostic accuracy of FNAC provided an excellent example for the TL patients, who can avoid unnecessary surgery. It is therefore recommended that FNAC is a useful and reliable diagnostic tool that appears to be a safe and minimally invasive procedure, which provides preoperative information for appropriate management of the TL patients in the country.

KEYWORDS: Tuberculosis (TB), Fine needle aspiration cytology (FNAC), Tuberculous lymphadenitis (TL), Diagnostic accuracy, Sociodemographic variables.

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I. INTRODUCTION

Tuberculous lymphadenitis (TL), also known as swollen lymph nodes or lymphadenopathy, is among the most frequent presentations of tuberculosis (TB) that appears outside the lungs¹. It is a chronic, specific granulomatous inflammation of the lymph nodes with caseation necrosis, that tend to become confluent, replacing the lymphoid tissue and caused by infection with the bacterium *Mycobacterium tuberculosis* and related species. The disease manifests in different human populations at variable frequencies. In rural India, the prevalence of TL in children up to 14 years of age is nearly 4.4 cases per 1000². In the USA about 20% of patients with TB have extrapulmonary disease, where TL constitutes about 30-40% of cases³. While in Denmark, TL has been reported in 13.5% cases in patients with TB⁴. The incidence of TL has been investigated by a number of researchers in Bangladesh⁵⁻⁸. According to an estimate by WHO in 2018, however, the incidence of TB in Bangladesh was 221 per 100,000. The survey indicated that prevalence of the disease was higher among men than women, and in the urban compared to the rural areas⁹.

Fine needle aspiration cytology (FNAC) is a simple, quick and inexpensive method of making a diagnosis that is used to sample superficial outgrowths like those found in the neck and is usually performed in the outpatient clinics¹⁰⁻¹². It causes minimal trauma to the patients and carries virtually no risk of complications. It is particularly relevant in developing countries like Bangladesh where facilities for surgical biopsy are insufficient⁸. FNAC therefore plays an important role in diagnosing TL which avoids unnecessary surgical intervention¹³⁻¹⁵. Apart from diagnosing TL, FNAC is also used frequently for testing breast lumps¹⁶⁻¹⁷, granulomatous inflammation¹⁸, thyroid swellings¹⁹ and liver lesions²⁰.

In recent years, FNAC followed by cytopathological patterns of the lymph node tuberculosis have been utilized to diagnose extrapulmonary TB in patients in Bangladesh⁸, India¹², Nepal²¹, and China²². Here we report: (i) the sociodemographic features of some selected outdoor patients with lymphadenopathy who visited Diabetic Association Medical College (DAMC), Faridpur, during a period of six months from March to September 2022, (ii) the characteristics of the swollen lymph nodes, (iii) the distribution and cytomorphological features of the lymphadenopathy and (iv) the diagnostic accuracy of the used FNAC.

II. METHODOLOGY

Location and study period: This descriptive and cross-sectional survey was conducted at the Department of Pathology, DAMC, Faridpur, Bangladesh, during a period of nine months from January to September 2022. Institutional ethical clearance was obtained before the commencement of the study. Further analyses of the experimental data were conducted at the Genetics & Molecular Biology Laboratory, University of Rajshahi, Bangladesh during October and December 2022.

Sample size and inclusion criteria: One hundred and twenty two purposively selected patients, both males and females of different age groups, who attended outpatient department of the hospital for diagnosis and treatment of TL were investigated. Patients who refused to be included in the study were excluded. Acellular smears with crushed morphology or poorly stained slides and extensive neutrophilic infiltration seen on microscopy were also excluded. Informed consent of the patients was taken and, in case of minors, consent of parents or guardians was obtained before FNAC procedure (detailed below). Standardized questionnaire was administered to obtain sociodemographic and clinical data.

Sociodemographic variables: The age of the respondents was subdivided into eight groups from 0-9 to 7-79 years. Gender, literacy, residential and economic status of the patients along with their history of exposure to TB was noted. For describing economic status, the patients were arbitrarily divided into low-income (BDT <15,000 per month), middle-income (BDT 15,000-<1,00,000 per month) and high-income (BDT ≥1,00,000 per month) groups.

TL characteristics: Detailed clinical history and meticulous physical examination including the size, types, texture and positions of the TL were taken into account. Moreover, systemic features of the patients such as fever, weight loss, loss of appetite, night sweat as well as asymptomatic characters without constitutional symptoms of TB, if any, were recorded. Positions of the TL were determined as described by the American Academy of Otolaryngology Head and Neck Surgery²³.

FNAC procedure: Patients fulfilling the inclusion and exclusion criteria were selected for FNAC. This was done using a 22 gauge needle and 10 ml disposable syringe for each prick per patient. No local anesthesia was used, and the needle was inserted into the palpable swollen lymph nodes, either once or twice depending upon the size of the TL. Cellular materials were aspirated into the syringe and expelled onto slides. Then thin smears were prepared by gentle friction of two slides. All the smears were wet fixed in 95% ethyl alcohol for at least 30 minutes, and the air dried smears were processed for haematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN)

stains, followed by acid fast bacilli (AFB) positivity tests. Slides were examined under the light microscope by an expert histopathologist. The finding of ZN staining was labeled as positive for AFB when pink-coloured, beaded and rod-shaped microbes were found.

Statistical analysis: Descriptive statistics of all experimental data were analyzed by SPSS (version 20.0) for Windows. All the parameters were expressed as frequencies and percentages. In addition, age of the patients was also presented as mean and standard deviation. The summarized data were presented in the form of tables and figures. On correlating the FNAC diagnoses with cytomorphological patterns, the sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of FNAC were estimated, where culture was taken as the gold standard^{8,19,24}. Pearson's 2×2 chi-square tests were performed to correlate cytomorphological patterns and AFB tests⁸, where $P < 0.05$ was considered as significant.

III. RESULTS AND DISCUSSION

Sociodemographic features

Table 1 shows some sociodemographic features of the respondent patients who were classified into eight age groups ranging from 7 to 75 years, with an average of 32.49 ± 14.70 years, where the number of females was significantly higher than that of their male counterparts (male to female sex ratio = 1: 2.30; $\chi^2 = 18.885$, $P < 0.001$ at 1 df). The highest number of both males and females belonged to 20-29 age group followed by 30-39 and 40-49 age groups in females but 30-39 and 10-19 age groups in males. Irrespective of their gender, however, more than half (56.55%) of the patients had secondary level of education, followed by primary (26.23%), illiteracy (9.84%) and graduate or above (7.38%). Majority of them came from the rural areas (55.74%) in comparison with the semi-urban (23.77%) or urban residence (20.49%). The highest number of the respondents belonged to the low-income group (59.02%) than the middle-income (26.23%) and high-income (14.75%) groups. Compared to a much higher proportion of the patients with the history of no exposure to TB (93.44%), only a small fraction of them (6.56%) had experienced an exposure to the disease.

Table 1 Distribution of the patients by their sociodemographic features (n=122, Diabetic Association Medical College, Faridpur, Bangladesh)

| Sociodemographic features | | | No. patients | Percentages |
|---|-------------------|---------|--------------|-------------|
| Age groups (yrs) | Males | Females | | |
| 0 – 9 | 1 | 3 | 4 | 3.28 |
| 10 – 19 | 5 | 12 | 17 | 13.93 |
| 20 – 29 | 17 | 22 | 39 | 31.97 |
| 30 – 39 | 6 | 20 | 26 | 21.13 |
| 40 – 49 | 3 | 12 | 15 | 12.30 |
| 50 – 59 | 4 | 8 | 12 | 9.84 |
| 60 – 69 | 1 | 7 | 8 | 6.56 |
| 70 – 79 | 0 | 1 | 1 | 0.82 |
| <i>Total</i> | 37 | 85 | 122 | 100.00 |
| Range: 7-75; average 32.49 SD 14.70 yrs | | | | |
| Gender | | | | |
| Male | | | 37 | 30.33 |
| Female | $\chi^2 = 18.885$ | df=1 | 85 | 69.67 |
| <i>Total</i> | | | 122 | 100.00 |
| Literacy status | | | | |
| Illiterate | | | 12 | 9.84 |
| Primary | | | 32 | 26.23 |
| Secondary | | | 69 | 56.55 |
| Graduate and above | | | 9 | 7.38 |
| <i>Total</i> | | | 122 | 100.00 |
| Residential status | | | | |

| | | |
|---|-------------------------------|--------|
| Rural | 68 | 55.74 |
| Semi-urban | 29 | 23.77 |
| Urban | 25 | 20.49 |
| <i>Total</i> | $\chi^2= 25.885$ df=2 P<0.001 | 122 |
| Economic status | | |
| Low-income group (BDT <15,000 per month) | 72 | 59.02 |
| Middle-income group (BDT 15,000-<1,00,000) | 32 | 26.23 |
| High-income group (BDT \geq 1,00,000) | 18 | 14.75 |
| <i>Total</i> | $\chi^2= 38.623$ df=2 P<0.001 | 122 |
| History of exposure to TB | | |
| Yes | 8 | 6.56 |
| No | 114 | 93.44 |
| <i>Total</i> | 122 | 100.00 |

Characteristic features of the TL

The morphology and sites of the TL and the systemic features of the experimental patients are presented in Table 2. The size of the swollen lymph nodes differed significantly among the respondents ($\chi^2= 86.770$; P<0.001 at 2df), most of them were of <3 cm (73.77%) followed by 3-6 cm (15.57%) and >6 cm (10.66%). Only a small proportion of them were of multiple type (8.2%) in comparison with the majority of single nodes (91.80%). As regards their texture, the lymph nodes were either solid (69.88%), abscess (20.48%) or of discharging sinus (9.64%). Cervical TL constituted the highest (n= 100, 81.96%), then axillary (7, 5.74%), supraclavicular (6, 4.92%), inguinal and submandibular (4 each, 3.28%) while the submental TL (1, 0.82%) was least in number. The present results revealed the following systemic features of the respondents: fever (85.25%) > loss of appetite (59.02%) > weight loss (54.92%) > night sweat (50.82%) > asymptomatic (45.90%), where some of the patients experienced more than one systemic feature.

Table 2 Morphological features of the TL (n=122, Diabetic Association Medical College, Faridpur, Bangladesh)

| Morphological features of the TL | | No. patients | Percentages |
|----------------------------------|-------------------------------|--------------|-------------|
| Size | | | |
| <3 cm | $\chi^2= 86.770$ df=2 P<0.001 | 90 | 73.77 |
| 3-6 cm | | 19 | 15.57 |
| >6 cm | | 13 | 10.66 |
| <i>Total</i> | | 122 | 100.00 |
| Types | | | |
| Single | | 112 | 91.80 |
| Multiple | | 10 | 8.20 |
| <i>Total</i> | | 122 | 100.00 |
| Texture | | | |
| Solid | $\chi^2= 53.131$ df=2 P<0.001 | 77 | 69.88 |
| Abscess | | 32 | 20.48 |
| Discharging sinus | | 13 | 9.64 |
| <i>Total</i> | | 122 | 100.00 |

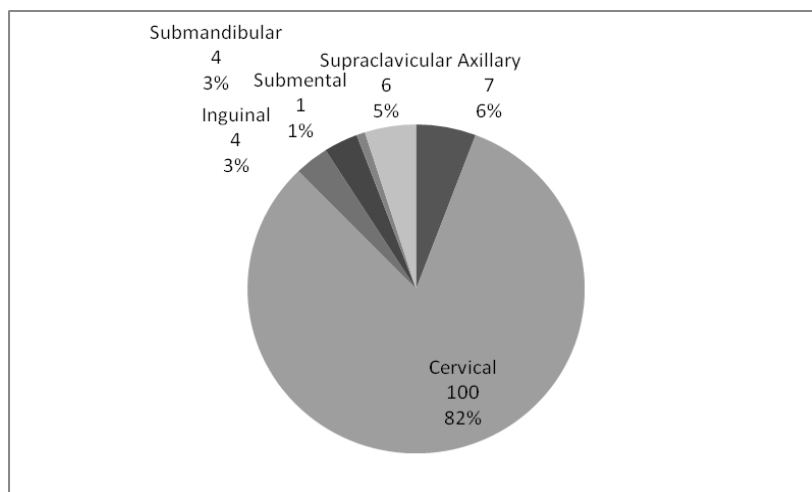


Fig. 1 Pie diagram showing distribution of 122 patients according to the position of their lymph nodes

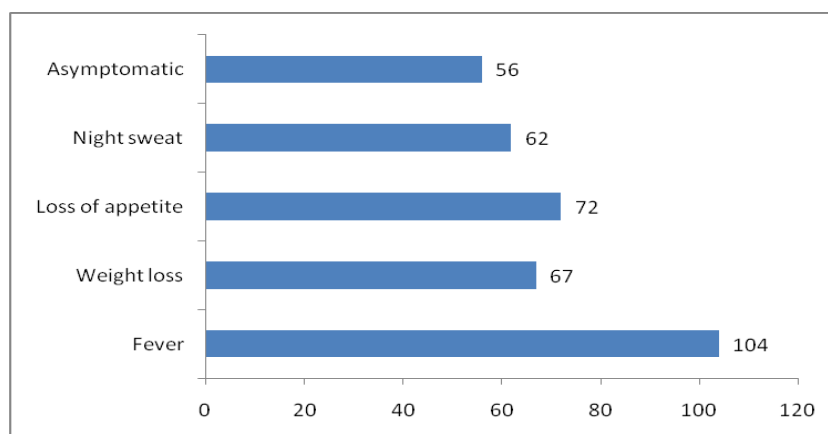


Fig. 2 Bar diagram showing distribution of study cases* according to their systemic features (*Exceed 122 owing to more than one systemic responses/respondent)

Cytomorphological patterns of the TL

The FNA cytomorphology of the patients on ZN stain against AFB tests is shown in a pie diagram below (Fig. 3). A vast majority of the TL exhibited Pattern B, *i.e.* epithelioid granuloma (EG) with caseous necrosis (CN) (n= 71/122; 58%; Fig. 4b), followed by Pattern C, *i.e.*, CN without EG (n= 29/122; 24%; Fig. 4c) and the least common was Pattern A, *i.e.*, EG without CN (n= 22/122; 18%; Fig. 4a).

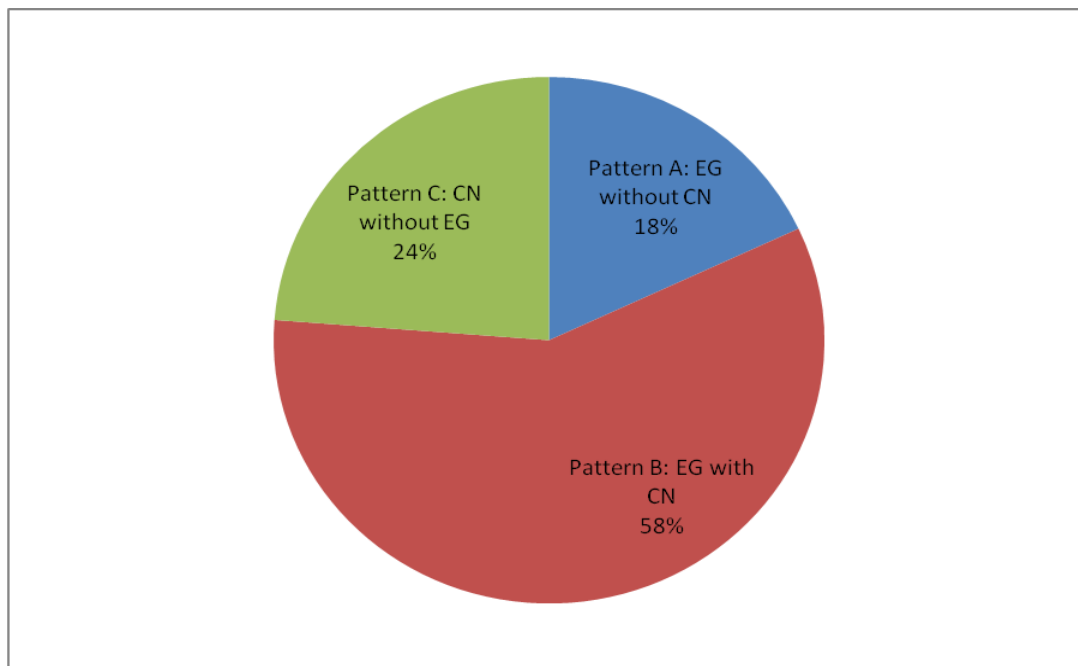


Fig. 3 Pie diagram showing distribution of study cases according to their FNA cytomorphology

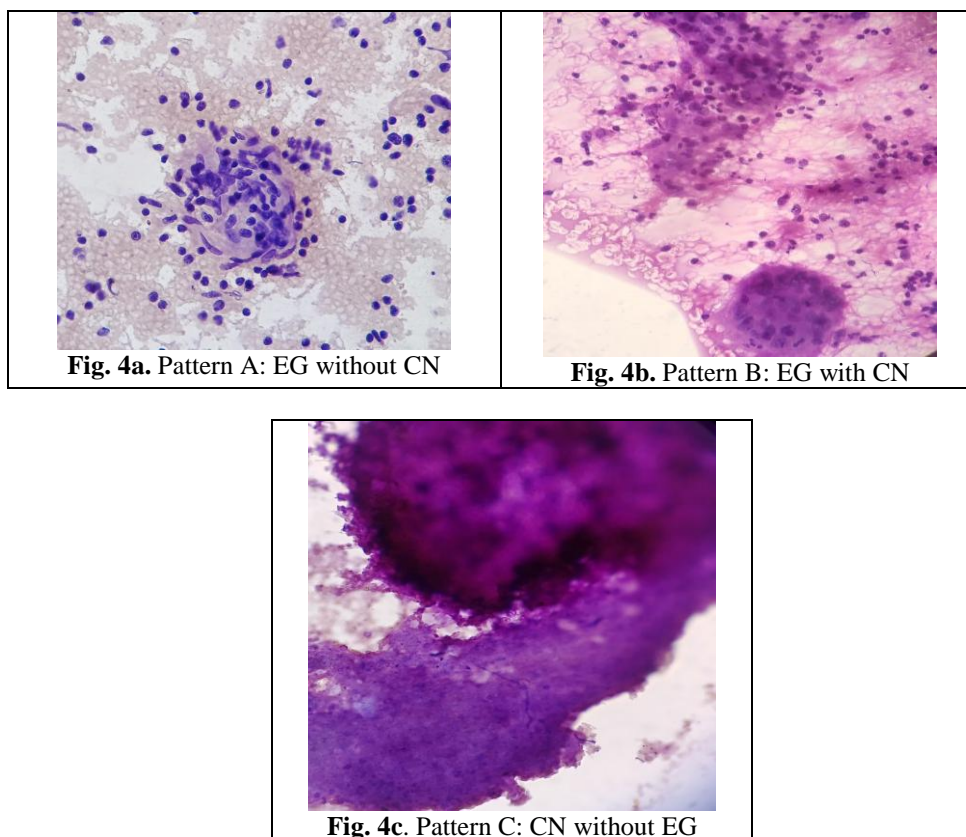


Fig. 4 Cytomorphological patterns of the TL in the studied subjects

FNAC diagnostic tests

Data on cytomorphology patterns and their responses against AFB tests are presented in Table 5. Overall, FNAC was found AFB positive for TL in 61/122 or 50.0% cases; of which Pattern A (EG without CN) was 17/22 or 77.27%, Pattern B (EG with CN) was 24/71 or 33.80%; and Pattern C (CN without EG) was 20/29 or 68.97%. For Pattern A, sensitivity, specificity, positive predictive value, negative predictive value and

diagnostic accuracy of FNAC were 92.31%, 73.33%, 66.67%, 94.29% and **80.28%**, respectively. The corresponding values for Patterns B 89.47%, 33.33%, 89.47%, 33.33% and **81.82%**, and C were and 86.96%, 66.67%, 90.91%, 57.14% and **82.76%**, respectively. Diagnostic accuracies above 80% in all three cytomorphology patterns indicated a quite satisfactory performance of FNAC in the diagnosis of TL in the experimental patients under study. AFB positivity was statistically insignificant for Pattern A ($\chi^2=1.144$; $P>0.05$), but highly significant for Pattern B ($\chi^2= 28.407$; $P<0.001$) and Pattern C ($\chi^2=7.472$; $P<0.01$).

Table 5 Cytomorphology patterns against AFB positivity tests used for the diagnosis of TL and calculating diagnostic accuracies of FNAC

| Cytomorphology patterns | | | AFB | AFB | Total | χ^2 values |
|-------------------------|----------|--|----------|----------|-------|-----------------|
| Pattern A | | | Positive | Negative | | |
| EG without CN | Positive | | 17 | 2 | 19 | 1.144 |
| EG without CN | Negative | | 2 | 1 | 3 | >0.05ns |
| (n=22) | Total | | 19 | 3 | 22 | |
| Pattern B | | | Positive | Negative | Total | |
| EG with CN | Positive | | 24 | 2 | 26 | 28.407 |
| EG with CN | Negative | | 12 | 33 | 45 | <0.001 |
| (n=71) | Total | | 36 | 35 | 71 | |
| Pattern C | | | AFB | AFB | Total | χ^2 values |
| | | | Positive | Negative | | |
| CN without EG | Positive | | 20 | 3 | 23 | 7.472 |
| CN without EG | Negative | | 2 | 4 | 6 | <0.01 |
| (n=29) | Total | | 22 | 7 | 29 | |

EG= Epithelioid granuloma; CN= caseous necrosis; AFB= Acid fast bacilli test; TL= Tuberculous lymphadenitis; Pearson's chi-square tests were performed against cytomorphology as gold standard; ns= not significant.

An easy and the most commonly used diagnostic tool for TL is FNAC, followed by ZN staining and AFB test, and culture for the causal agent *Mycobacterium tuberculosis*^{25,26}. TL is one of the most common causes of lymph node enlargement in developing countries like Bangladesh, where FNAC has played an important role in the diagnosis of the disease^{6,8}. Here we report the sociodemographic features of the TL patients along with their characteristics, cytomorphological features of the TL and diagnostic accuracy of the FNAC.

The number of female TL patients in the present study was significantly higher than that of their male counterparts. This is consistent with previous reports from Sylhet Medical College, Bangladesh⁵, BSMMU, Dhaka, Bangladesh⁶ and Lahore, Pakistan¹⁸. Likewise, females had a higher prevalence of pulmonary TB than males in Uganda²⁷, suggesting the cases of female preponderance in general. But the present finding is contrasting to a couple of reports on the prevalence of TB in males in Bangladesh^{7,9}. The majority of the present TL patients belonged to rural areas and low socio-economic backgrounds, which lend support to a previous study from the country⁵.

The size of the TL in most respondents was <3 cm in diameter, and single nodes with solid texture dominated over multiple nodes and abscess or discharging sinus. These findings are similar to those of Sylhet Medical College patients mentioned above⁵. Cervical TL had the highest frequency as compared to those at other positions, which is in good agreement with several previous reports from home^{6,8} and abroad^{15,18,21}. Majority of the TL patients of the present study suffered from such systemic features as fever, loss of appetite, noticeable weight loss and night sweat, which were similar to those observed in patients from Bangladesh⁶ and Uganda²⁷.

In recent years, FNAC followed by cytopathological patterns of the lymph node tuberculosis have been utilized to diagnose extrapulmonary TB in patients in Bangladesh⁸, India¹²⁻¹⁵ and Nepal²¹. Three major cytomorphological patterns of the TL include: Pattern A with central caseous necrosis (CN) surrounded by the aggregation of epithelioid granuloma (EG), Pattern B with EG but without CN and Pattern C with CN but without EG^{21,28}. Majority of the TL patients in our study belonged to Pattern A (58.20%), followed by Pattern B (23.77%) and Pattern C (18.03%), showing the maximum bacillary load in Pattern A. AFB positivity of the above three patterns were 33.80%, 77.27% and 68.97%, respectively. Variable frequencies of the cytomorphological patterns have been reported by several investigators. For example, in Karnataka, India, Patterns A, B and C had 56.0%, 19.33% and 22.67% cases, respectively¹³, whereas in Haryana, India¹⁴ and Jammu and Kashmir, India¹⁵, the Patterns had 66.0%, 26.6% and 7.4% and 49.2%, 20.1% and 30.6% cases, respectively. The present FNAC showed AFB positivity for TL in overall 61/122 or 50.0% cases, which varied from 83.9%⁵, 23.3%⁸, 73.5%¹³, 21.6%¹⁴, 64%¹⁵ and 44.0%¹⁸ cases reported earlier. The apparent variations in

the frequencies of the cytomorphological patterns and AFB positivity might have resulted from the genetic and/or racial differences between the human subjects under study.

Sensitivity, specificity, diagnostic accuracy, and negative and positive predictive values are vital indicators for the evaluation of FNAC. For Pattern A, these values were 92.31%, 73.33%, 66.67%, 94.29% and 80.28%, respectively. The corresponding values for Patterns B and C were 89.47%, 33.33%, 89.47%, 33.33% and 81.82%, and 86.96%, 66.67%, 90.91%, 57.14% and 82.76%, respectively, which are quite consistent with previous reports from Dhaka, Bangladesh⁸, Tamil Nadu, India¹² and Lahore, Pakistan¹⁷. Diagnostic accuracies above 80% in all three cytomorphology patterns therefore indicated a quite satisfactory performance of FNAC in the diagnosis of TL in the experimental patients under study.

IV. CONCLUSIONS

TB is a global health problem nowadays and TL is the most common presentation of extrapulmonary TB in the world. FNAC is not only a simple and important diagnostic tool in the evaluation of lymph node lesions, but it is also an inexpensive, easy to perform and the first choice investigation for patients in developing countries like Bangladesh. It has gained a wide acceptance to both physicians and patients because of its greater diagnostic efficacy^{8,10,12}. The present diagnostic accuracy of FNAC provided an excellent example for the TL patients, who with cytomorphology on ZN stain followed by AFB test, can avoid unnecessary surgery. In addition, FNAC is a useful and reliable diagnostic tool that also appears to be a safe and minimally invasive procedure, which provides preoperative information for appropriate management. However, more experimental and clinical studies in different settings are needed in the future to support the widespread clinical use of FNAC for the diagnosis and effective treatment of TL patients in the country.

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Disclosure of conflict of interest: Authors declare no conflict of interest.

Statement of ethical approval: Ethical clearance was sought from the DAMC, Faridpur, and subsequently, written permission was obtained from the hospital authority.

Statement of informed consent: Informed consent was obtained from all the respondents who participated in the study. All their information was kept highly confidential.

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