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Original Article

Respiratory Medicine Section

Analysis of Fine Needle Non Aspiration Cytology for the Diagnosis of Cervical Lymph Node Tuberculosis: A Cross-sectional Study

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ABSTRACT

Introduction: Conventionally, excision biopsy is performed for the diagnosis of Lymph Node Tuberculosis (LNTB). Comparatively, Fine Needle Non Aspiration Cytology (FNNAC) is a simple, reliable, inexpensive, painless, less invasive, outpatient procedure for the diagnosis of peripheral LNTB and is recommended as the first diagnostic technique for this condition. Fine Needle Non Aspiration Cytology (FNNAC), i.e., fine needle sampling without aspiration, is relatively painless, less traumatic and more patient-friendly compared to FNAC. Although FNNAC is easy to perform, it is not routinely practiced.

Aim: To evaluate the results of FNNAC in cervical lymphadenopathy and determine the various cytomorphological presentations in cervical LNTB.

Materials and Methods: This institutional-based, cross-sectional study was conducted at the Department of Respiratory Medicine, Princess Krishnajammanni TB and Chest Diseases Hospital (PKTB and CDH), Mysore Medical College and Research Institute (MMC and RI) in Mysuru, from January 2019 to September 2022 on presumptive cervical LNTB patients who underwent FNNAC followed by an excision biopsy of the same Lymph Node (LN). The sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy of FNNAC for diagnosing LNTB were estimated.

Results: A total of 415 subjects were studied. The sensitivity, specificity, PPV and NPV rates of FNNAC for Tuberculosis (TB) were 97.92%, 100%, 100% and 99.3%, respectively, with an accuracy of 99.4%. A total of 36 samples (8.7%) were considered Non Diagnostic (ND). Among these 36 ND samples, 31 were of benign origin (4 were TB), while the remaining five ND samples were of malignant origin.

Conclusion: The present study reveals that TB is currently the most common cause of cervical lymphadenopathy, with other common causes being malignant metastasis and reactive lymphadenitis. FNNAC is highly accurate in the diagnosis of LNTB. Therefore, FNNAC is a simple, safe, economical, reliable and accurate method for diagnosing cervical LNTB.

Keywords: Fine needle aspiration, Mycobacterium tuberculosis, Tuberculous cervical lymphadenopathy

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by bacteria of the Mycobacterium Tuberculosis complex (MTBc) [1]. According to the Global TB Report 2022, the annual incidence of TB in 2021 was 10.6 million, with 1.4 million TB deaths reported among Human Immunodeficiency Virus (HIV)–negative individuals and 187,000 deaths among HIV-positive individuals. A total of 87% of the total notified TB cases worldwide are contributed by 30 high-burden countries, with India accounting for 28% [2].

The lungs are the most common site of TB; however, no organ is immune to the disease. In 2021, among the notified TB cases, the proportion of Pulmonary Tuberculosis (PTB) cases was 75.2%, while Extrapulmonary Tuberculosis (EPTB) cases accounted for 24.8% [3]. In India, Lymph Node Tuberculosis (LNTB) is the most common form of EPTB, accounting for 35% of all EPTB cases [4]. Among LNTB cases, cervical lymphadenopathy is the most common, representing more than 70% of cases, followed by the inguinal and axillary groups of lymph nodes [5].

Conventionally, excision biopsy is performed for the diagnosis of LNTB [5]. A conclusive diagnosis of LNTB is established through the demonstration of bacteriological evidence, such as the detection of Acid-Fast Bacilli (AFB) on histopathological sections or stained smears, by Nucleic Acid Amplification Tests (NAAT), or by mycobacterial culture [5]. In comparison, FNAC is a simple, reliable, inexpensive, painless, less invasive outpatient procedure for diagnosing peripheral LNTB [5]. FNAC is recommended as the first diagnostic technique for LNTB [5].

Fine Needle Non Aspiration Cytology (FNNAC), or fine needle sampling without aspiration, was introduced by Brifford M et al., in France in 1982 [6]. The principle of the FNNAC technique is based on the capillary action of the fine needle, making it relatively painless, less traumatic and more patient-friendly. This method is easy to perform but is not routinely practiced [7,8].

Both FNNAC and FNAC techniques have their benefits and drawbacks; however, it remains unclear which procedure yields a superior biological sample for accurate cytopathological diagnosis [7]. Multiple studies have revealed contradictory findings between FNNAC and FNAC [7,8]. Hence, the present study was undertaken to evaluate the results of FNNAC in the diagnosis of TB in presumptive cases of cervical LNTB and to determine the various cytomorphological presentations in cervical LNTB.

MATERIALS AND METHODS

The present study was an institutional-based, cross-sectional study conducted at the Department of Respiratory Medicine, Princess Krishnajammanni TB and Chest Diseases Hospital (PKTB and CDH), Mysore Medical College and Research Institute (MMC and RI) in Mysore, from January 2019 to September 2022. Data for the study was collected during this period. Furthermore, the study was planned and executed, including data analysis and interpretation, from October 2022 to October 2023 after obtaining the Institutional

Ethics Committee Clearance Certificate, dated 10th August 2022, EC Reg: ECR/134/Inst/KA/2013/RR-19.

Inclusion and Exclusion criteria: Only selected outpatients or inpatients who presented as presumptive cases of cervical LNTB and underwent FNNAC, followed by excision biopsy of the same cervical lymph node (LN), were included in the study. Subjects with missing FNNAC reports and/or those who could not or did not undergo excision biopsy of the same LN were excluded from the study.

Study Procedure

A total of 415 subjects were included in the study. For each case, detailed socio-demographic information, clinical history and clinical examination findings were recorded. The levels and sublevels of cervical Lymph Nodes (LNs) were defined as per the American Joint Committee on Cancer 2010 [9].

After applying the inclusion and exclusion criteria, data were collected from the medical records of the hospital for each subject, as FNNAC is routinely practiced in the department for the cytopathological examination of LNs. For each subject, a pathologist performed the FNNAC. Informed written consent was obtained from the subject before performing the procedure. The skin in the area of interest was disinfected and the cervical LN was immobilised with the non dominant hand. A 25-gauge needle's hub was held in a pencil grip position and introduced into the cervical LN with the dominant hand. The needle was passed through the cervical LN in the same manner as in FNAC, but no suction was applied with the syringe. The material entering the hub of the needle by capillary action was then expressed onto clean, labelled glass slides after attaching an air-filled syringe to the needle. Multiple smears (2 to 4) were prepared and fixed with 95% alcohol in a Coplin jar for a period of three to five minutes. Among the fixed smears, two were stained with Haematoxylin and Eosin (H&E) stains. Microscopic analysis was conducted after staining. In cases suspicious for malignancy, the remaining smears were subjected to pap staining and studied. Necrotic aspirates, if present, were aspirated and subjected to the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) as per National Tuberculosis Elimination Programme (NTEP) guidelines [10]. Subsequently, a general surgeon performed an excisional biopsy of the same cervical LN under local anesthesia (2% Lignocaine) or sedation, after obtaining written informed consent, to determine a definitive histopathological diagnosis.

The cytopathology reports of FNNAC were categorised into four main categories [11]:

1) Benign diagnosis - recommended for follow-up.
2) Malignant metastatic diagnosis - recommended for searching the primary lesion.
3) Malignant primary lymphoma: Hodgkin Lymphoma (HL) or Non Hodgkin Lymphoma (NHL)- recommended for excision biopsy for confirmation and immune-phenotyping of the lymphoma.
4) Non diagnostic (ND) or Inadequate smears - due to acellular and/or scanty samples.

As described by Das DK et al., based on the cytomorphology of the LNTB, lesions were categorised into three main types as follows:

1) Type I- Epithelioid granuloma without necrosis;
2) Type II- Epithelioid granuloma with necrosis;
3) Type III- Necrosis without epithelioid granuloma [12].

Data were collected on a pre-designed proforma and analysed.

STATISTICAL ANALYSIS

The data was entered into a Microsoft Excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS) software version 22.0 (IBM SPSS Statistics, Somers, NY, USA) version software. Categorical data were represented in the form of frequencies and proportions. After ruling out the ND results, the sensitivity, specificity, PPV and NPV of FNAC for Diagnosing LNTB were calculated.

RESULTS

A total of 415 subjects were included in the present study, with ages ranging from one year to 88 years. Among them, 208 (50.1%) were males and 207 (49.9%) were females. The maximum incidence of cervical lymphadenopathy was found in the 21 to 40 years age group 169/415 (40.7%). The majority of the subjects were underweight 238/415 (57.3%) and illiterate (187/415, 41.5%) [Table/Fig-1].

Characteristics	Number (n=415)	Percentage (%)
Gender		
Male	208	50.1
Female	207	49.9
Age (in years)		
1-20	77	18.55
21-40	169	40.72
41-60	104	25.06
61-80	62	14.93
81-100	3	0.72
Marital status		
Single	140	33.73
Married	202	48.67
Divorced	6	1.44
Widowed	54	13.01
Separated	13	3.13
Body Mass Index (kg/m²)		
Under weight	238	57.34
Normal	136	32.77
Preobese	29	6.98
Obesity class 1	3	0.72
Obesity class 2	7	1.68
Obesity class 3	2	0.48
Qualification		
Illiterate	187	45.06
<10th standard	84	20.24
10-12th standard	42	10.12
Undergraduate	51	12.28
Postgraduate	51	12.28

[Table/Fig-1]: Socio-demographic characteristics of the subjects participated in the study.

Right-sided cervical lymph nodes were more commonly affected (233/415, 56.1%). Most subjects presented with lymph node sizes of less than 3 centimeters 274/415 (66%). The most common lymph node consistency encountered in the study was firm 201/415 (48.4%) and the involved level/sublevel of cervical lymph nodes was level IV 134/415 (32.3%). Apart from the cervical group, other lymph node groups were not involved in 328 (79%) subjects. The majority of the subjects presented with discrete lymph nodes 270/415 (65.1%) and many had a significant history of contact 208/415 (50.1%). In addition to neck swelling, associated symptoms were observed in 377/415 (90.8%) of subjects, whereas 38/415 (9.2%) had no associated symptomatology. Furthermore, 197/415 (47.5%) of the subjects had no co-morbid illnesses. A history of past TB was reported, among whom 97 (23.4%) had PTB and 90 (21.6%) had EPTB [Table/Fig-2].

The Fine Needle Non Aspiration Cytology (FNNAC) was diagnostic in 379/415 (91.3%) of subjects, among which TB was diagnosed in 242/415 (58.3%), Non TB diagnosis were made in 137/415 (33%) and results were not diagnostic (ND) in 36/415 (8.7%) [Table/Fig-3].

Characteristics	Number (N=415)	Percentage (%)
Side of cervical LN		
Right	233	56.1
Left	115	27.7
Bilateral	67	16.1
Size of LN		
<3 cm (centimeter)	274	66
3-6 cm	115	27.7
>6 cm	26	6.3
Consistency of the cervical LN		
Firm	201	48.4
Soft	113	27.2
Stone hard	60	14.5
Variable	16	3.9
Rubbery	14	3.4
Cystic	11	2.7
Level of cervical LN		
IA	19	4.6
IB	16	3.9
IIA	21	5.1
IIB	17	4.1
III	121	29.2
IV	134	32.3
VA	19	4.6
VB	19	4.6
VI	48	11.6
VII	1	0.2
Other groups of LN if involved		
Axillary	54	13
Inguinal	10	2.4
Abdominal	8	1.9
Thoracic	15	3.6
Cervical only	328	79
Character of cervical LN		
Matting	145	34.9
Discrete	270	65.1
History of contact with PTB		
Yes	208	50.1
No	207	49.9
Associated symptoms		
Fever	162	39
Loss of weight	81	19.5
Haemoptysis	35	8.4
Loss of appetite	28	6.7
Dry cough	27	6.5
Productive cough	19	4.6
Throat pain	16	3.9
Others (malaise, fatigue, night sweating)	9	2.2
Nil	38	9.2
Co-morbidity		
Chronic Obstructive Pulmonary Obstruction (COPD)	70	16.9
Diabetes Mellitus (DM)	46	11.1
Anaemia	34	8.2
HIV	31	7.5
Systemic Hypertension (HTN)	12	2.9
Chronic Renal Disease (CRD)	10	2.4
Malignancy	9	2.2
Interstitial Lung Disease (ILD)	4	1
Chronic Liver Disease (CLD)	2	0.5
Nil	197	47.5
Past history of TB		
Yes	187	45.1
No	228	54.9

[Table/Fig-2]: Clinical characteristics of the subjects participated in the study.

LN FNNAC report	Number	Percentage (%)
TB	242	58.3
Non TB	137	33.0
Non Diagnostic (ND)	36	8.7

[Table/Fig-3]: Diagnosis of cervical Lymph Node (LN) FNAAC.

Among the 242 diagnosed TB cases of LNTB, the most common histological pattern was Type-II, found in 164/242 (67.76%), followed by Type-III at 60/242 (24.79%), with Type-I being the least common at 18/242 (7.43%) [Table/Fig-4].

If LNTB on FNAAC	Number	Percentage (%)
Type-I	18	7.43
Type-II	164	67.76
Type-III	60	24.79
Total	242	

[Table/Fig-4]: Distribution of cytomorphology of cervical LNTB.

Cytopathological specimens were subjected to CBNAAT in 54% (224/415) of the studied subjects. Among these, Mycobacterium Tuberculosis (MTB) was detected in 57/224 (25.44%) of subjects and Rifampicin Resistance (RR) was detected in 1/57 (1.75%) of subjects.

The cytopathological report was suggestive of benign disease in 285/415 (68.7%), malignant metastasis in 66/415 (15.9%), non diagnostic (ND) in 36/415 (8.7%) and both Hodgkin's Lymphoma (HL) and Non Hodgkin's Lymphoma (NHL) in each 14/415 (3.4%) [Table/Fig-5,6].

Cytopathology report	Number	Percentage (%)
Benign (including TB)	285	68.7
Malignant metastasis	66	15.9
NHL	14	3.4
HL	14	3.4
Non diagnostic	36	8.7

[Table/Fig-5]: Cytopathology report of cervical Lymph Node (LN) FNAAC.
TB: Tuberculosis; NHL: Non Hodgkin's lymphoma; HL: Hodgkin's lymphoma

If benign on FNAAC	Number	Percentage (%)
TB	242	58.3
Reactive lymphadenitis	26	6.3
Suppurative lymphadenitis	10	2.4
Kikuchi lymphadenitis	4	1.0
Others (cystic inflammation)	3	0.7
Not applicable	130	31.3

[Table/Fig-6]: Cytopathological reports of benign conditions diagnosed by FNAAC.

The cytopathological results of the lymph nodes from FNAC were compared with the histopathological results obtained from excision biopsy of the same cervical lymph nodes [Table/Fig-7].

The sensitivity, specificity, PPV and NPV of FNAC for the diagnosis of cervical LNTB, after excluding ND results, were 97.92%, 100%,

Cytopathological diagnosis by FNAAC	Histopathological diagnosis by excision biopsy								
	LNTB	Suppurative LN	Reactive LN	Kikuchi LN	Others	Malignant metastasis	NHL	HL	Total
LNTB	242	-	-	-	-	-	-	-	242
Suppurative LN	-	10	-	-	-	-	-	-	10
Reactive LN	-	-	26	-	-	-	-	-	26
Kikuchi LN	-	-	-	4	-	-	-	-	4
Others	-	-	-	-	3	-	-	-	3
Malignant metastasis	-	-	-	-	-	66	-	-	66
NHL	-	-	-	-	-	-	14	-	14
HL	-	-	-	-	-	-		14	14
ND	4	3	19	5	0	4	0	1	36
Total	246	13	45	9	3	70	14	15	415

[Table/Fig-7]: Cytopathological reports by FNAAC to HPE reports by excision biopsy.

100% and 99.30%, respectively, with an overall accuracy of 99.47% (95% Confidence Interval).

DISCUSSION

In the present study, out of 415 subjects, the socio-demographic data reveal that the majority were males 208 (50.1%). Cervical lymphadenopathy was most common among the 21 to 40 years age group, accounting for 169/415 (40.7%). The most common cause of cervical lymphadenopathy was TB, followed by malignant metastasis and reactive lymphadenitis. The accuracy of FNNAC for the diagnosis of cervical LNTB was 99.47%.

The comparison of the FNAC and FNNAC techniques by Srikanth S et al., in head and neck swellings revealed that the cellular yield was adequate for a definitive diagnosis, with better retention of tissue architecture in smears from LN lesions using the FNNAC technique [13]. The cytopathological diagnosis of LNTB depends on the demonstration of AFB or classical epithelioid granuloma with caseation necrosis—i.e., "soft granuloma"—or epithelioid granuloma without necrosis. Other cellular components, such as Langhans giant cells, polymorphonuclear cells and lymphocytes, may or may not be present [10]. Subjects in which cytopathological smears contained only necrotic material without any evidence of epithelioid granuloma have also been considered as having tuberculosis, as the authors have done in present study [14].

In the current study, among the 242 diagnosed cases of LNTB, the most common histological pattern was Type II, i.e., epithelioid granuloma with necrosis, found in 67.76% (164/242) of the cases. LNTB was confirmed in all 164 cases by Histopathological Examination (HPE) of the excised lymph nodes. The sensitivity, specificity, PPV, NPV and accuracy of FNNAC for the diagnosis of TB, after omitting ND values, were 97.92%, 100%, 100%, 99.3% and 99.47%, respectively, which is comparable to several other studies conducted worldwide [Table/Fig-8] [15-18].

Author	Country	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Muyanja D et al., [15]	Uganda	93.1	100	78.9	100
el Hag IA et al., [16]	Saudi Arabia	97	100	93	100
Adhikari P et al., [17]	Nepal	80	100	82	100
Sellami M et al., [18]	Tunisia	83.3	83.3	78.9	100
Present study	India	97.92	100	99.3	100

[Table/Fig-8]: Results of fine-needle aspiration for the diagnosis of Tuberculosis (TB) [15-18].

These findings differ from a study conducted at a public hospital in the United States, a retrospective 5-year study that examined 238 lymph node FNACs in patients with mycobacterial infection. It was found to have a low sensitivity of 53% in the diagnosis of tuberculosis [19].

In the present study, the ND rate was 8.7% (36/415); however, in the literature, the ND rate for FNAC is highly variable, ranging from 0.9% to 48% [15,20]. In a study by Rammeh S et al., on factors influencing the failure rate of cervical lymph node FNAC, among 272 samples, it was revealed that 20.6% were ND. This was found to depend on the size of the lymph node (≤1 cm), the location of the lymph node (submandibular and jugulodigastric) and the experience of the health worker performing the FNAC [21]. Four out of the 36 ND cases by FNNAC were diagnosed as tuberculosis by HPE; extensive necrosis and fibrosis may explain this in the present study.

The experience of the health worker is a crucial factor that determines the quality of FNNAC. A study by Singh N et al., analysed 5,226 FNAC samples from six common sites. Inadequate rates in FNAC were compared, concluding that the ND rate was lowest when FNAC was performed by a cytopathologist (12%) and highest when performed by a non cytopathologist (32%) [22]. In the present study, all the FNNACs were conducted by the same pathologist, which explains the low ND rate.

The use of Ultrasonography (USG)-guided FNAC is known to reduce the ND rate. However, USG-guided FNAC is a more expensive technique compared to conventional palpation-guided FNAC. USG-guided FNAC should be preferred for lymphadenopathy cases where the size of the lymph node is small or when the lymph node is situated in difficult locations [23]. In the current study, all subjects underwent the palpation-guided FNNAC technique.

Repeating FNNAC is very useful and should especially be considered in ND cases. A prospective study by Shykhon M et al., studied 57 subjects, each of whom was sampled twice, with the repeat (second) FNAC performed just prior to surgery in the operation theatre. The ND rate was 48% in the first FNAC, which dropped to 32% after the second FNAC [20].

Limitation(s)

The limitations of the study include the inability to confirm tuberculosis by performing mycobacterial cultures due to a lack of funds and infrastructure; however, all necrotic aspirates were subjected to CBNAAT. Secondly, repeat FNNAC was not considered in the ND cases in the current study.

CONCLUSION(S)

To conclude, the present study revealed that the most common cause of cervical lymphadenopathy was tuberculosis (TB), with the most prevalent cytomorphological pattern of presentation being Type II, i.e., epithelioid granuloma with necrosis. FNNAC was highly accurate in the diagnosis of LNTB, with sensitivity,

specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy of 97.92%, 100%, 100%, 99.30% and 99.40%, respectively. This accuracy can significantly impact patient management, thereby preventing unnecessary excision biopsies for the diagnosis of cervical lymphadenopathy. Therefore, FNNAC is simple, safe, economical, reliable and accurate in the diagnosis of cervical LNTB.

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