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COMPARISON OF FINE NEEDLE ASPIRATION CYTOLOGY, ZIEHL-NEELSEN STAINING AND CBNAAT IN SUSPECTED CASES OF TUBERCULAR LYMPHADENOPATHY

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ABSTRACT

Objective: The objective of the study is to assess the usefulness of cytology, ZN staining and CBNAAT in diagnosis of suspected tubercular lymphadenopathy and to compare the results of cytology and ZN staining with CBNAAT findings in the diagnosis of suspected tubercular lymphadenopathy.

Methods: The study was conducted on 100 patients with clinical suspicion of tubercular lymphadenopathy in Department of Pathology in collaboration with Department of Pulmonary Medicine, GMC, Patiala. The study was started after obtaining approval from the Institutional Ethics Committee.

The records of suspected 100 EPTB patients who had under gone FNAC, ZN stain and CB-NAAT test for EPTB diagnosis during the study period of 1 year were analysed.

Results: The results shows male to female ratio of 1:1.4. The youngest patient was 28 days old and oldest was 77 years old. In this study, Granulomatous lymphadenopathy was the most predominant cytological finding (74%) followed by reactive lymphoid hyperplasia (16%) , suppurative (08%), carcinomatous deposits (01%) and lymphoma (01%). Out of the total granulomatous lymphadenopathy i.e 74 cases, ZN stain was positive in 39 cases (52.7%) and CBNAAT was positive in 61 cases (82.4%). Out of the total 100 cases, all 39 ZN-positive cases were diagnosed with tuberculosis on FNAC. Among the 67 CBNAAT-positive cases, 61 were diagnosed with tuberculosis on FNAC, while 4 were diagnosed with suppurative lesions and 2 with reactive lymphoid hyperplasia.

Conclusion: FNAC combined with CBNAAT emerged as a reliable diagnostic approach due to its rapid turnaround time and high sensitivity, facilitating early diagnosis and management of tubercular lymphadenopathy.

Keywords: Fine Needle Aspiration Cytology, Cartridge based nucleic acid amplification test, Ziehl Neelsen Staining, Extrapulmonary tuberculosis.

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1. INTRODUCTION

Tuberculosis is an infectious disease primarily caused by Mycobacterium tuberculosis bacteria. While it commonly affects the lungs, it can also impact other organs. According to the WHO annual report, India alone accounts for an estimated 9.6 million cases annually. Extrapulmonary tuberculosis, which impacts organs beyond the lungs, accounts for 20% of tuberculosis cases in India and continues to represent a significant portion of the global disease burden. [1]

Extrapulmonary tuberculosis refers to tuberculosis affecting parts of the body other than the lungs. Common sites of extrapulmonary tuberculosis include lymph nodes (48.1%), pleura (15.4%), gastrointestinal tract (14.4%), bones/spine (13.5%), and the central nervous system (4.8%). Lymphadenopathy and effusion are typical presentations in many of these cases.[2]

Tubercular lymphadenitis (TBLN) is the most prevalent type of extrapulmonary tuberculosis, comprising approximately 20-40% of all extrapulmonary tuberculosis cases.[3] Lymph node tuberculosis progresses pathologically through five stages.

Hyperplasia, Periadenitis, Cold Abscess, Collar stud abscess and Sinus formation[4]. Extrapulmonary tuberculosis presents challenges due to its paucibacillary nature, meaning there are relatively few bacteria present. This makes early diagnosis difficult, compounded by the limited availability of tests suitable for detecting the disease at its onset.[5]

Cytological smears that show features of tuberculous lymphadenitis are classified into three categories: Granulomas composed of epithelioid cells with necrosis, with or without Langhans giant cells, Granulomas composed of epithelioid cells without necrosis, with or without Langhans giant cells and Presence of necrosis alone, without epithelioid granulomas.[3]

The conventional methods to diagnose EPTB includes fine needle aspiration cytology (FNAC) and demonstration of acid fast bacilli (AFB) using Zeihl- Neelson (ZN) staining.[2] Fine Needle Aspiration Cytology (FNAC) is regarded as a cost-effective and dependable initial investigation for evaluating lymphadenopathy. It has become recognized as an important tool for diagnosis, due to its ability to provide prompt results. FNAC is a straightforward procedure that causes minimal trauma and complications to patients. Combining cytology with confirmation of acid fastness using Ziehl-Neelsen (ZN) staining and Papanicolaou (Pap) stain-induced fluorescence microscopy yields excellent results .[1]

The Cartridge Based Nucleic Acid Amplification Test (CB-NAAT), also known as GeneXpert, is a fully automated, real-time hemi-nested polymerase chain reaction (PCR) system utilizing molecular technology.[2] It operates on the GeneXpert platform, utilizing a disposable plastic cartridge that comes pre-loaded with all necessary reagents.[6]

The WHO has endorsed it as the most sensitive rapid test for diagnosing tuberculosis, particularly in samples with low bacterial counts (paucibacillary samples). CB-NAAT can detect Mycobacterium tuberculosis and rifampicin resistance within two hours. CB-NAAT is known for its high positive predictive value, though its negative predictive value is comparatively lower.[2]

2. METHODS

Patients visiting Outpatient /Inpatient Department of various departments of Rajindra Hospital, Patiala and Department of Pulmonary Medicine, TB and Chest Diseases Hospital, GMC, Patiala.

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INCLUSION CRITERIA –

Patients of both sexes and all age groups who were suspected to have extrapulmonary tuberculosis.

EXCLUSION CRITERIA –

Patients already took treatment of Tuberculosis, with active and suspected malignancy and already diagnosed cases of reactive lymphadenitis.

PROCEDURE –

Participants of study were those coming from various departments of Rajindra hospital, Patiala with clinical suspicion of tubercular lymphadenopathy. Patients were explained about the procedure and complications of procedure before hand. Written informed consent was taken from all the patients. FNAC involves using a 18-20 gauge needle connected to a 10 ml syringe, guided by palpation to access the lymph node and collect adequate material. Smears were then prepared using Haematoxylin and Eosin (H&E), May-Grunwald Giemsa (MGG), Papanicolaou (Pap) and Ziehl-Neelsen (ZN) staining methods. For MGG staining, the smear was air-dried, while for H&E and Papanicolaou (Pap) staining, the smear was fixed in ethanol. These smears were evaluated for adequacy, arrangement of cells, individual cell characters and for the presence of three granulomatous lesion patterns – 1) Granuloma without necrosis, 2) Granuloma with necrosis. 3) Presence of necrotic debris alone.

These patterns aid in diagnosing tubercular lymphadenitis cytologically, guiding clinical management effectively.

ZN smears were examined for the bright pink,beaded curved bacilli against a bluish background and were reported as positive or negative for acid-fast bacilli. The remaining aspirate was rinsed into 0.7 ml sterile phosphate-buffered saline, incubated, and processed for CBNAAT testing, which detects Mycobacterium tuberculosis nucleic acids. The CBNAAT result was displayed as TB detected or not detected as well as showed the status of rifampicin resistance in the CBNAAT software.

3. RESULTS AND DISCUSSION

The present study involved 100 patients suspected with tubercular lymphadenopathy. FNAC findings were compared with Ziehl-Neelsen (ZN) staining and CBNAAT findings. The patients ranged in age from 28 days to 77 years, with the majority (28 cases) ranging from 21 to 30 years. The male-to-female ratio was 1:1.4. The FNAC aspirated material was grossly categorized as - 74 cases were blood-stained, 20 cases were purulent, and 6 cases were cheesy.

Cytological findings observed included predominantly granulomatous pathology (74%), reactive patterns (16%), suppurative patterns (08%), carcinomatous deposits (01%) and lymphoma (01%). (Table 1). Out of 74 cases with granulomatous lymphadenopathy, they were further classified into three categories : Granuloma without necrosis (43.2%), Granuloma with necrosis (41.8%), and only necrosis (14.8%). (Table 2). Among the 74 cases of granulomatous pathology, 39 (52.7%) were positive for AFB and 35(47.3%) were negative for AFB. Among the 16 cases of reactive lymphoid hyperplasia, all of them were negative for AFB. Among the 8 cases of suppurative pathology and 02 malignant cases, all were negative for AFB. Overall, p value by fisher exact test came out to be < 0.001 (<0.05) showing statistically highly significant results. (Table 3). Granulomatous lesion showed 82.4 % CBNAAT positive result, reactive lymph node 12.5% and suppurative lesion showed 50.0 %. Overall, p value by fisher

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exact test came out to be < 0.001 (<0.05) showing statistically highly significant results.(Table 4).

Table 5 shows comparison of cytological diagnosis with ZN staining and CBNAAT showing that among the 74 granulomatous cases, 39(52.7%) tested positive with ZN stain while 61(82.4%) tested positive with CBNAAT. Among the reactive lymphoid hyperplasia cases (16), no case was positive on ZN and 02 (12.5%) of the cases were positive on CBNAAT. Among the 08 suppurative cases, 04 (50%) tested positive with CBNAAT. The p value came out <0.05 (statistically significant) in cases of Granulomatous pathology.

Table 6 shows correlation of subtypes of granulomatous pathology with ZN and CBNAAT. In the study of granulomatous lesions, the results showed that among cases with necrosis (14.8% of cases), 64.5% tested positive for AFB (acid-fast bacilli) and 93.5% tested positive for CBNAAT. For cases without necrosis (43.2% of cases), 31.2% were positive for AFB and 68.8% were positive for CBNAAT. Additionally, smears showing only necrosis (14.8% cases), 81.8% were positive for ZN and 90% were positive for CBNAAT. The p value came out <0.05 (statistically significant) in pattern A, whereas > 0.05 (unsatisfactory) in pattern B and C.

In the study, it was found that sensitivity of CBNAAT (82.43%) was higher as compared to FNA AFB (52.7%). The overall diagnostic accuracy of CBNAAT (81%) was higher as compared to FNA AFB (65%). (Table 7).

TABLE 1 – DISTRIBUTION OF STUDY POPULATION ACCORDING TO CYTOLOGICAL FINDINGS (N=100)

CYTOLOGICAL FINDINGS	NO. OF CASES	PERCENTAGE
Granulomatous	74	74%
Reactive	16	16%
Suppurative	08	08%
Carcinomatous deposits	01	01%
Lymphoma	01	01%
Total	100	100%

TABLE 2 - DISTRIBUTION OF STUDY POPULATION ACCORDING TO PATTERNS OF GRANULOMATOUS PATHOLOGY (N=74)

PATTERNS	NO. OF CASES	PERCENTAGE
Granuloma without necrosis (Pattern A)	32	43.2%
Granuloma with necrosis (Pattern B)	31	41.8%
Only necrosis (Pattern C)	11	14.8%
Total	74	100%

TABLE 3 –CORRELATION OF CYTOLOGICAL FINDINGS WITH ZN STAINING (N=100)

FNAC findings	TOTAL	ZN +	%	ZN -	%
Granulomatous	74	39	52.7%	35	47.3%
Reactive	16	00	00	16	100%
Suppurative	08	00	00	08	100%
Carcinomatous deposits	01	00	00	01	100%

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Lymphoma	01	00	00	01	100%
TOTAL	100	39		61	
Fisher Exact Value	24.968				
P value	<0.001				
Significance	HS				

TABLE 4 – CORRELATION OF CYTOLOGICAL FINDINGS WITH CBNAAT RESULTS (N=100)

CYTOLOGICAL FINDINGS	CBNAAT +	%	CBNAAT	%
Granulomatous (74)	61	82.4%	13	17.5%
Reactive(16)	02	12.5%	14	87.5%
Suppurative (08)	04	50.0%	04	50.0%
Carcinomatous deposits(01)	00	-	01	100%
Lymphoma (01)	00	-	01	100%
Total	67	-	33	-
Fisher Exact value	33.395			
P value	<0.001			
Significance	HS			

TABLE 5 – COMPARISON OF CYTOLOGICAL DIAGNOSIS WITH ZN STAINING AND CBNAAT (N=100)

CYTOLOGICAL DIAGNOSIS	Total	Positive on ZN		Positive on CBNAAT		P value	Significance
		No.	%	No.	%		
Granulomatous	74	39	52.7	61	82.4	0.028	S
Reactive	16	00	0.0	02	12.5	-	-
Suppurative	08	00	0.0	04	50.0	-	-
Carcinomatous deposits	01	00	0.0	00	0.0	-	-
Lymphoma	01	00	0.0	00	0.0	-	-
TOTAL	100	**39**	39.0	**67**	67.0	-	-

TABLE 6 – CORRELATION OF SUBTYPES OF GRANULOMATOUS PATHOLOGY WITH ZN AND CBNAAT RESULTS (N=74)

PATTERNS	No. of cases	ZN+		CBNAAT +		P value	Significance
		No.	%	No.	%		
Granuloma without necrosis (Pattern A)	32 (43.2%)	10	31.2	22	68.8	0.034	S

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Granuloma with necrosis (Pattern B)	31(41.8%)	20	64.5	29	93.5	0.199	NS
Only necrosis (Pattern C)	11(14.8%)	09	81.8	10	90.0	0.819	NS
Total	74	39	52.7	61	82.4		

TABLE 7 – SENSITIVITY AND SPECIFICITY OF CBNAAT

Variable	FNA -AFB	FNA-CBNAAT
Sensitivity	52.70	82.43
Specificity	100.0	76.92
PPV	100.0	91.04
NPV	42.62	60.61
Accuracy	65.00	81.00

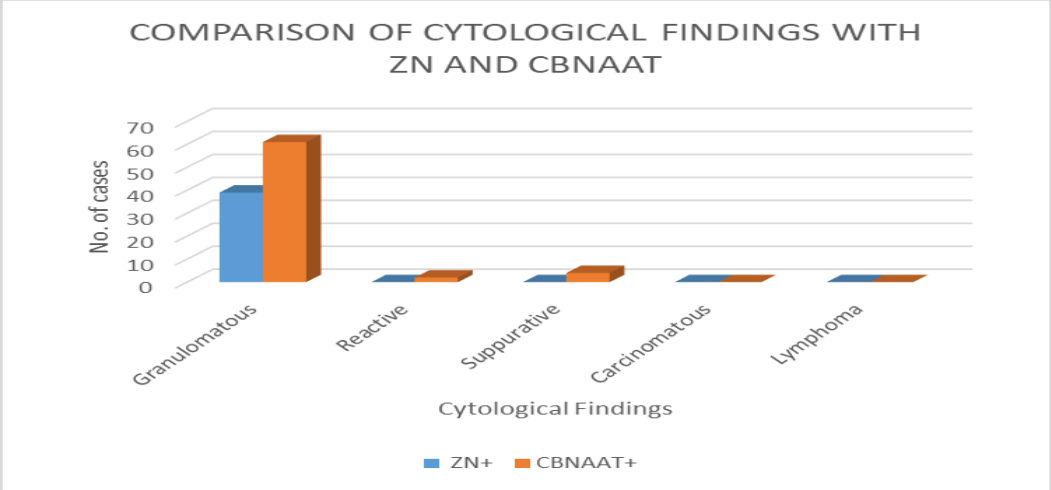

FIGURE 1 – CORRELATION OF CYTOLOGICAL FINDINGS WITH ZN AND CBNAAT

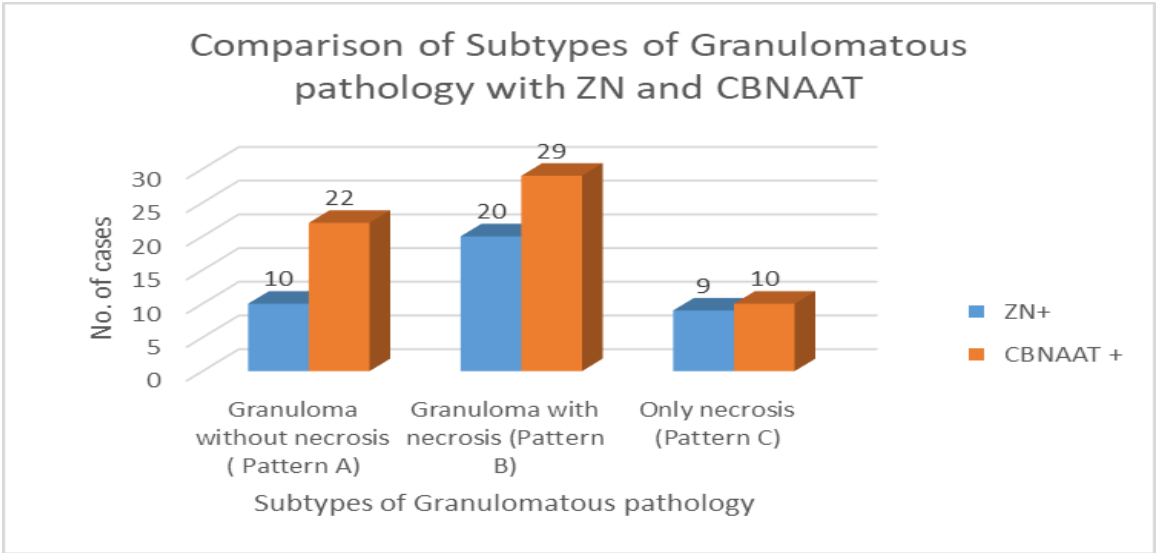

FIGURE 2 – CORRELATION OF SUBTYPE OF GRANULOMATOUS LESION WITH ZN AND CBNAAT RESULTS

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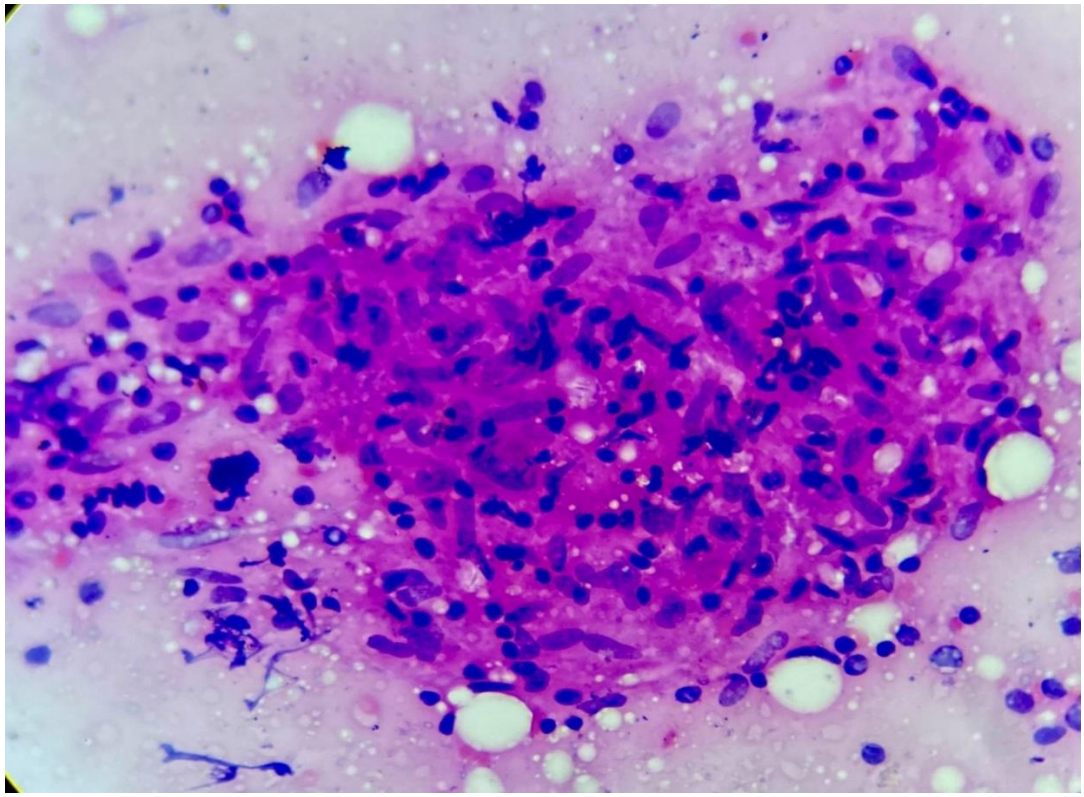

FIGURE 3: PHOTOMICROGRAPH SHOWING EPITHELIOID CELL GRANULOMAS IN TUBERCULAR LYMPHADENOPATHY – PATTERN A (H&E,X400)

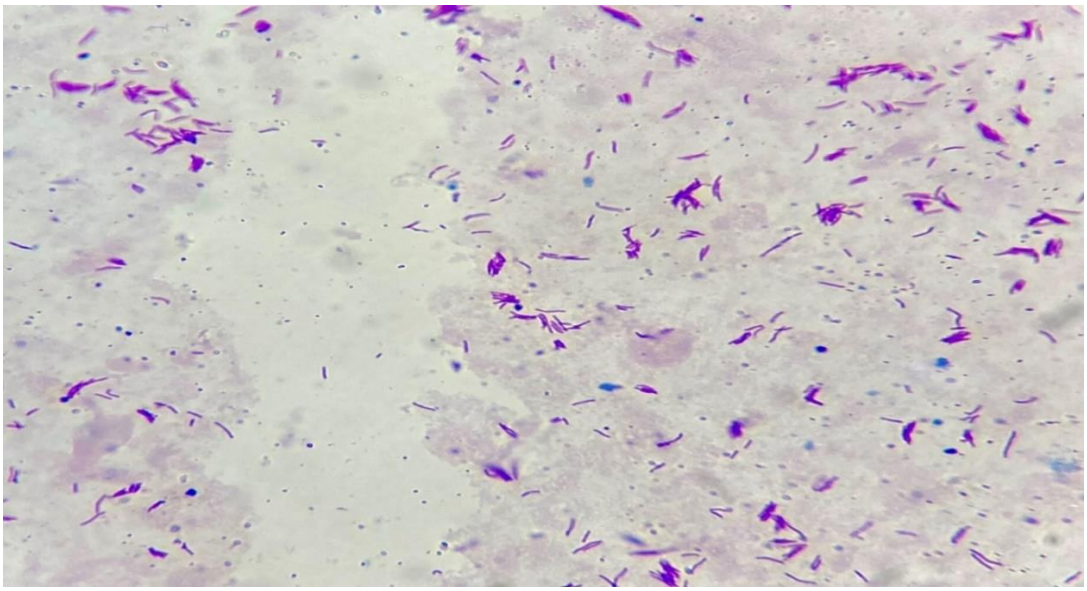

FIGURE 4: PHOTOMICROGRAPH SHOWING MANY ACID FAST BACILLI (ZIEHL-NEELSON STAIN X OIL IMMERSION).

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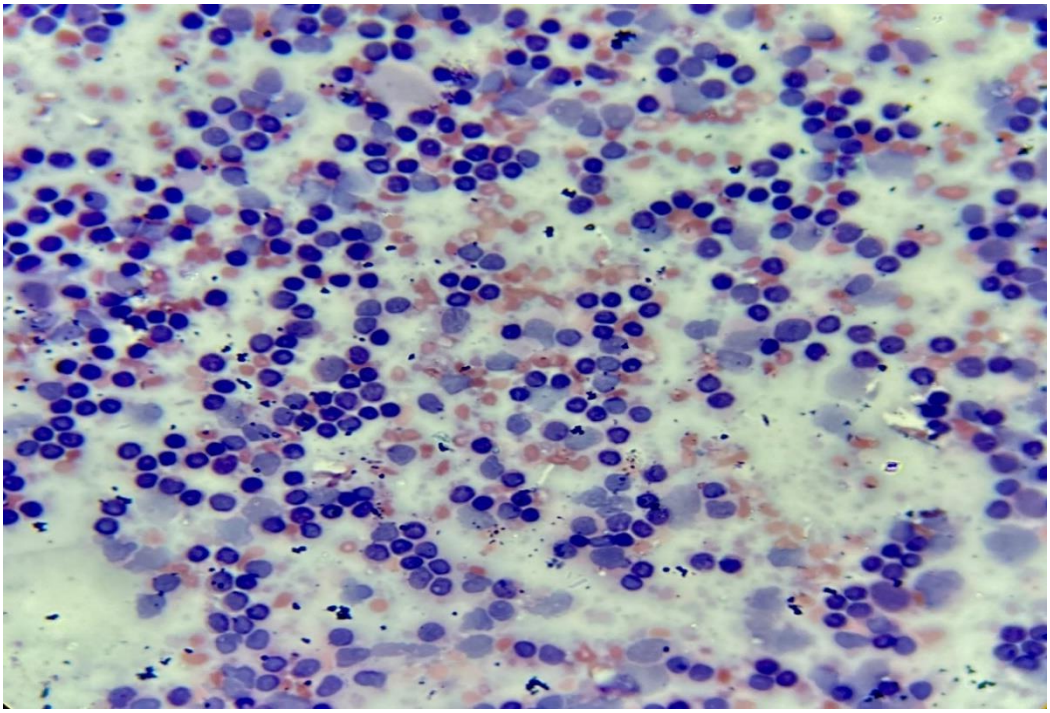

FIGURE 5: PHOTOMICROGRAPH SHOWING POLYMORPHIC POPULATION OF LYMPHOID SERIES OF CELLS IN REACTIVE LYMPHOID HYPERPLASIA. (PAP, X400).

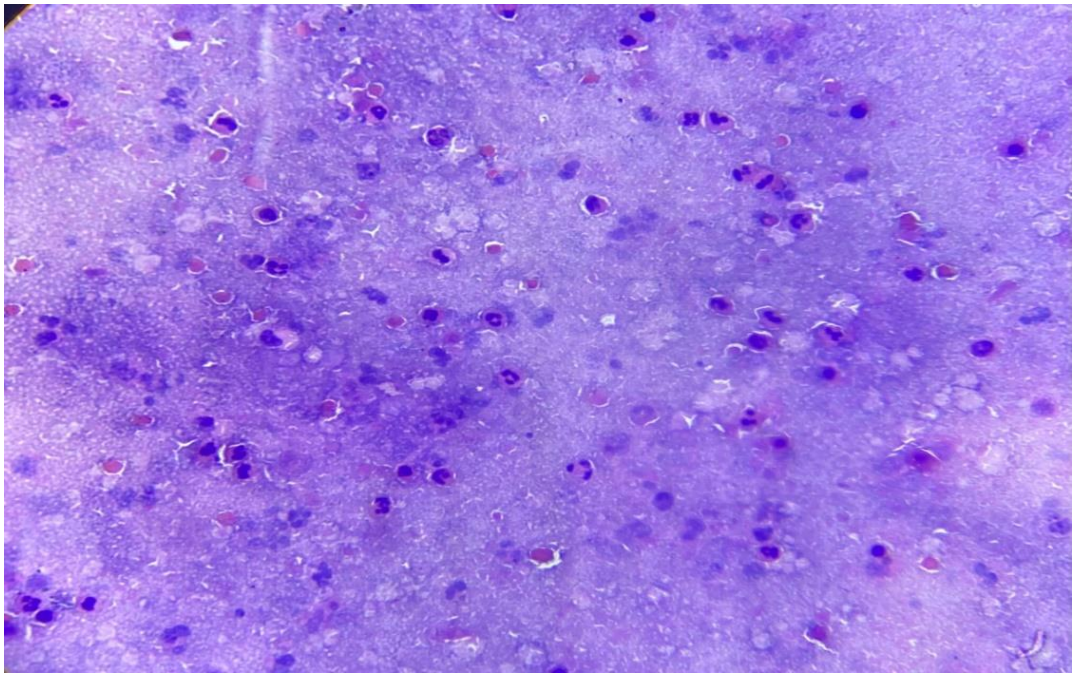

FIGURE 6: PHOTOMICROGRAPH SHOWING NEUTROPHILS IN THE BACKGROUND OF NECROSIS IN ACUTE SUPPURATIVE PATHOLOGY (H&E, X400).

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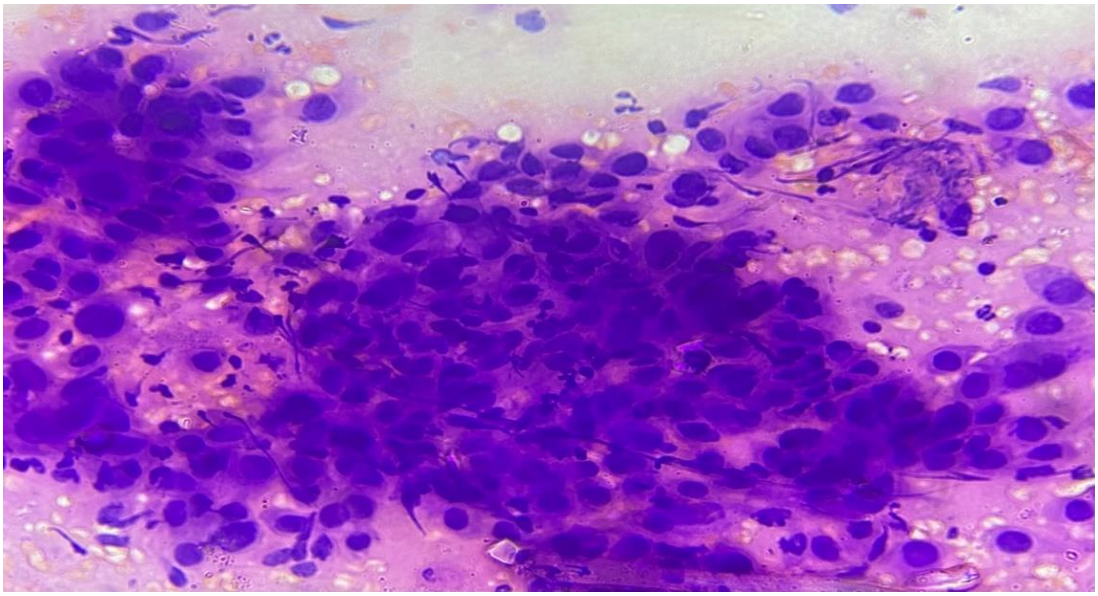

FIGURE 7: PHOTOMICROGRAPH SHOWING CARCINOMATOUS DEPOSITS (SCC) (H&E, X400).

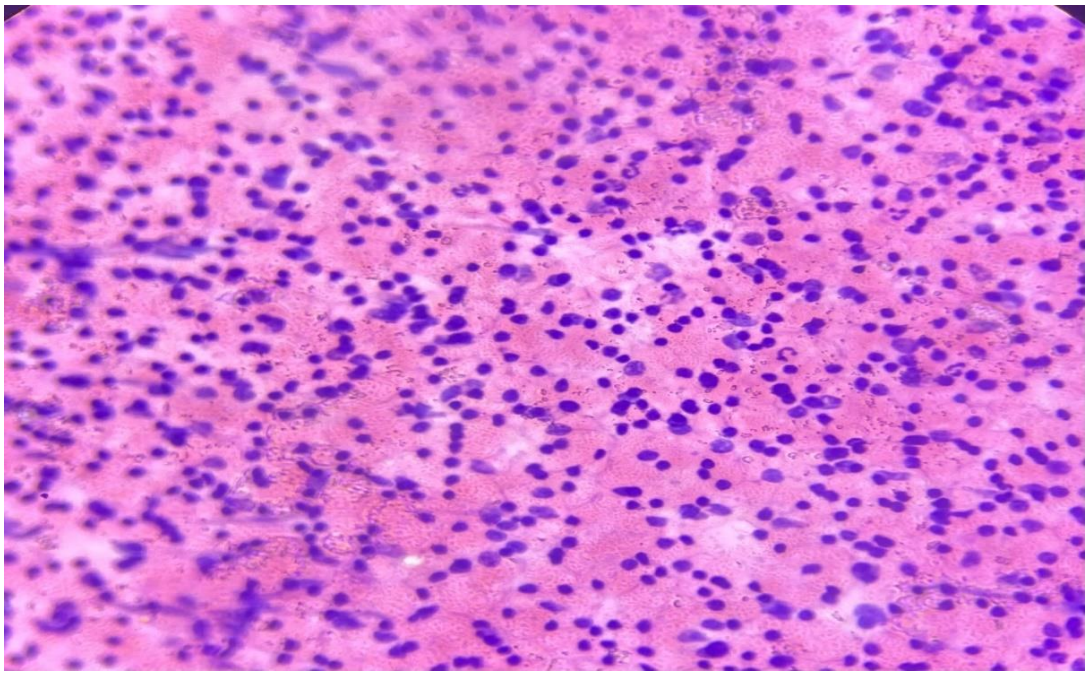

FIGURE 8: PHOTOMICROGRAPH SHOWING DISCRETE ROUND MONOMORPHIC POPULATION OF CELLS IN NON HODGKIN LYMPHOMA (H&E, X400).

Present study showed male to female ratio was 1:1.4 with female preponderance. The findings were consistent with study conducted by Chadrakar J et al in 2020[1] and Siddegowada MS et al in 2020[2].

Tubercular lymphadenopathy can manifest across all age groups. In our study, patients ranged from as young as 28 days to as old as 77 years. The highest proportion of patients fell within the 21-30 age group (28%). This trend mirrors findings observed in studies by Ahmad et al. (2005)[7] and Hemalatha A et al (2014)[9] and Patil S B et al[14].

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Cervical region was the most commonly affected site of lymphadenopathy (69%). Studies conducted by Siddegowada MS et al (2020)[2], Ahmad S S et al (2005)[7], Qadri SK et al (2012)[8]also identified the cervical region as the predominant site of involvement.

On the basis of appearance of aspirate, blood mixed aspirate was noted more commonly (74%) followed by purulent/ pus material (20%) and caseous or cheesy material (6%) in our study. The similar results were seen in a study by Hemalata A et al (2014)[9] and Masilamani S et al (2015)[11]. But in contrast to study by Shetty D et al (2022)[16], purulent aspirate was the most common and caseous the least common aspirate noted.

The various cytological findings seen in our study composed of predominantly granulomatous LN (74%), reactive LN (16%), suppurative LN (08%), carcinomatous deposits (01%) and lymphoma (01%). This aligns with the findings of Chadrakar J et al (2020)[1]

Among 100 cases, 74 showed cytological findings indicative of a tubercular etiology. Among these, epithelioid granulomas without necrosis (Pattern A) were observed in 32 cases (43.2%), while epithelioid granuloma with necrosis (Pattern B) were observed in 31 cases (41.8%) and necrosis without granuloma (pattern C) accounted for 11 cases (14.8%). Most common cytomorphological pattern in our study was epithelioid granulomas without necrosis (43.2%) cases. This result was consistent with the findings of study by Siddegowada MS et al (2020)[2] and Chand P et al (2014)[10].

Out of the total granulomatous lymphadenopathy i.e 74 cases, ZN stain was positive in 39 cases (52.7%) and CBNAAT was positive in 61 cases (82.4%).

Out of the total 100 cases, all 39 ZN-positive cases were diagnosed with tuberculosis on FNAC. Among the 67 CBNAAT-positive cases, 61 were diagnosed with tuberculosis on FNAC, while 4 were diagnosed with suppurative lesions and 2 with reactive lymphoid hyperplasia. The findings were in concordance with study by Bajaj D et al (2022)[15].

Upon combining the results, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) between FNA-AFB and FNA-CBNAAT were determined to be 82.43%, 76.92%, 91.04%, and 60.61% respectively. This comparison highlights that CBNAAT offers higher sensitivity (82.43%) compared to FNA AFB smear (52.7%) in diagnosing tubercular lymphadenopathy.

CBNAAT of FNAC samples from lymph nodes demonstrated superior sensitivity and accuracy compared to FNA AFB smear microscopy. This was in agreement with study by Goyal V K et al (2019)[12], where CBNAAT showed a sensitivity of 77.27% whereas FNA AFB smear microscopy had a sensitivity of 45.45%. In terms of accuracy, CBNAAT achieved 74.51% compared to 62.75% for FNA AFB smear microscopy.

The table below illustrates the comparison of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with findings from other studies."

TABLE 36 – COMPARISON OF SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE (PPV) AND NEGATIVE PREDICTIVE VALUE (NPV) -

STUDY	Sensitivity	Specificity	PPV	NPV
Goyal V K et al (2019)[12]	77.27	57.1	91.89	28.57
Bajaj D et al (2022)[15]	84.61	39.72	93.54	20.0
Siddegowda M S et al (2020)[2]	85.7	73.8	63.8	90.5
Lavanya G et al (2019)[13]	28.75	88.7	74.1	52.5
MD Manju et al (2020)[4]	79.49	100	100	72.41
Present	82.43	76.92	91.04	60.61

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4. CONCLUSION

- FNAC and ZN staining was the primary diagnostic method. CBNAAT being the latest and less time-consuming technique but had an advantage of detecting FNAC missed cases, especially suppurative abscess and can detect TB even if bacilli were less in number and also it gave information about rifampicin resistance. But CBNAAT had a disadvantage of being costly and maintenance was high.
- FNAC combined with CBNAAT emerged as a reliable diagnostic approach due to its rapid turnaround time and high sensitivity, facilitating early diagnosis and management of tubercular lymphadenopathy.
- The present study concludes that, there is a statistical correlation between the results of cytology, ZN staining and CBNAAT findings in the diagnosis of suspected cases of tubercular lymphadenopathy.

CONFLICTS OF INTERESTS -
None.
AUTHORS FINDINGS –
Nil.

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