Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 447 - 464
Received 18April2020; accepted 23June2020

Analysis of Diagnostic Trends and Challenges of Tuberculous Lymphadenitis: A 5 years study from Resource Poor Region of South India

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ABSTRACT

Background: Tuberculosis remains one of the major cause of morbidity and mortality in developing countries like India despite intense health campaigning and Government Programmes. Tuberculous lymphadenitis being the most common extra pulmonary manifestation of tuberculosis the incidence still on higher margin especially in resource poor areas which often goes unnoticed and remains as a diagnostic challenge to General Pathologists and sometimes even to cytopathologists.

Aims and Objectives: To document the prevalence of tuberculous lymphadenitis among various patient groups from low socio-demographic profileand also to advocate the best method in early and correct diagnosis of tuberculous lymphadenitis to initiate early appropriate treatment.

Materials &Methodology: In the prospective study conducted in SBV University Hospital, Puducherry a total number of 151 cases clinically suspected of tuberculous LN were included and patients with neoplastic lesion were excluded from this study. The lymph node aspirate collected were analysed for tuberculous cytomorphological changes by using routine cytological stains and observations were correlated with results of various ancillary diagnostic procedures.

Results and Observations: Among 151 cases studied, 120 cases showed tuberculosis indicating high prevalence. 31 aspirates showed classical tuberculous pattern on cytomorphology, correlating well with histopathology (100%), Polymerase chain reaction (PCR) (100%) and culture (92%) respectively. In rest of the 119 cases carrying non tuberculous pattern on cytology, further exploration by ancillary diagnostic procedures revealed up tuberculosis in 79 cases with confirmation by culture, Histopathology and PCR.

CONCLUSION: Our study showed prevalence of tuberculous lymphadenitis is on higher margin especially among low socio-demographic group and cytological procedure in adjunction with ancillary procedures proved to be reliable and helpful to Cytopathologists. PCR procedure could be

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widely employed by the Government especially in endemic regions with resource poor diagnostic facilities

Keywords: Cytology; Polymerase chain reaction; Tuberculous Lymphadenitis; Culture isolates

1. INTRODUCTION

Tuberculosis (TB) being a chronic granulomatous infection still remains a major global health problem affecting millions of population annually worldwide. It is ranks sixth among the top ten causes of mortality worldwide and tops the table in cases of infectious condition. It had been estimated that by the year 2030, TB infection would present in all known manifestations of 10.4 million with average risk of 1.7 million deaths annually. Globally, seven million new incidences of cases of TB were reported in 2018, with an increase in disease prognosis from 6.4 million in 2017[1].

Geographically, In 2018 India accounted for 27% of global total cases. Notification of new cases in India was found to have increased exponentially from 1.2 million cases to 2.0 million cases from 2012 to 2018. With this the increase in TB cases, diagnosis of the prevalence of disease is still at primitive stage due to lack of sufficient financing[2]. India has been ranked first with increase in global report of disease prognosis. Tropical Countries especially India continues to be one among the nation to have found to progress uptrendin TB burden globally, with an reported incidence of 2.81 million laboratory proved TB cases and among that 147000 MDR/RR-TB cases in 2017. Other neighbouring countries of India too are highly susceptible for TB infections [3]. In addition due to various factors and evolvement of new strains, the rise in incidence and prevalence of drug resistance of TB (MDR-TB) had also been reported[4][5]. World Health Organization (WHO) has recognized India as a vulnerable hotspot zone for TB Epidemic and as a leading cause of mortality.Diagnosis and treatment of highly drug resistant TB is alarming [6, 7]. The high level of inconsistency concerning clinical diagnosis and post mortem outcomes is disturbing but consistent with adult autopsy in African patients [8]. Although younger children are highly susceptible to the severe TB [9]. TB strains have unique features making it complicated in the diagnosis of the disease [10].

Regardless ofupsurges in TB warnings, there is a staticand enormouscrackconcerning the total of 7.0 million new cases reported and the expected range of 9.0 million to 11.0 million cases in 2018. This gap is basically in regards to the results in anamalgamation of not reporting the detected cases and most importantly underdiagnosis of disease using sensitive diagnostics tests[11]. Even though with latest treatment based outcome data for new TB cases still diagnostics for testing the TB is still challenging among pathologist[12, 13].

Tuberculosis infection being a T-cell mediated immune infection, it is known to occur in many forms and are broadly categorized into pulmonary and extrapulmonary manifestations of TB[14, 15].Extrapulmonary TB accounts for three-fifth of tuberculous incidence in India with peripheral immune organs especially lymph nodes being the commonest site[16]. if manifestation and it is identified in the form of Tuberculous Lymphadenitis (LNTB)[17].Cervical group of lymph nodes are commonly affected followed by axillary and

inguinal group of nodes[18].An Indian paediatric group study demonstrated prevalence of superficial lymphadenitis as 27.5/1000 children and among the incidence LNTB accounts for 4.61/1000 cases[19].

Mycobacterium Tuberculosis (MTB) being the causative organism of LNTB, its diagnosis remains crucial challenge for clinicians and diagnostic laboratory departments[20]. Revised National Tuberculosis Control Programme (RNTCP) proposed Fine needle aspiration cytology (FNAC) along with demonstration for Acid fast bacilli (AFB)from clinical isolated as an essential screening procedure for any suspected LNTB or superficial lymphadenopathy[21, 22]. The diagnosis can be made on cytomorphology by demonstrating presence of epithelioid cell granuloma with langhans giant cells and caseating type of necrosis[23]. Since past decade of time, FNAC with demonstration of AFB were the validated diagnostic procedure for LNTB owing to its minimal invasive and rapid report inspite of its known limitations[24, 25]. Clinical evaluation of the polymerase chain reaction on pulmonary and extra-pulmonary specimens from these TB patients [26].Many instances cytomorphology had many mimickers with differentials[27]. a multisystem approach is warranted to diagnose LNTB which includes culture studies and recently Genotyping expression of MTB by PCR[28]. Mycobacterium culture being highly sensitive method in terms of sensitivity and specificity, it has its own limitations in terms of duration to show positive growth [29]. PCR came into existence which is based on targeting sequences to detect MTB from clinical isolates at the level of gene complexes and even species [30].Various targeting sequences namely Insertion sequences IS6110,H37RV strain TRC4,hsp60are applied on various specimens like FNAC material, tissue biopsy, peripheral blood mononuclear cells[31]

The various diagnostic techniques which are utilized in detecting LNTB had only added to the already prevailing dilemma concerning to the diagnosis. Single cell RNA seq can precisely resolve cellular states for low cost RNA sequencing of single cells at high throughput [32]. While majority of studies conducted in India focusses on Pulmonary TB and its diagnosis, more light needs to be thrown upon Extrapulmonary manifestations of TB especially LNTB and its diagnostic modalities in terms of sensitivity and specificity [33, 34]. Hence the present study was commissionedin our hospital located in resource poor domain with a novel aim to identify the prevalence of LNTB and to analyse the comparative metric of efficacy of various diagnostic tools including histopathological evaluation of lymph node biopsies adding uniqueness to the study analysis.

2. MATERIALS & METHODOLOGY

Study Design: The prospective cross-sectional study was conducted in the Department of Pathology,SriBalajiVidyapeeth(Deemed to be University) located in South-Eastern Coastal part of India mostly covering low sociodemographic population. The study was conducted for a period of 5 years from 2012 to 2017 after obtaining Institute Ethics committee clearance and informed written consent from the patients.

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Patient Inclusion criteria: (i)Patients referred to Cytology laboratory with superficial lymphadenopathy with strong clinical acumen and suspicion of LNTB including patients of anti-tuberculous treatment. (ii). Significant Lymphadenopathy.

Exclusion criteria: Aspirates which showed features of neoplastic lesions including lymphoma, metastatic deposits on cytomorphology were excluded from the study but directed for further work up for malignancies.

Sample Size: 151 cases with lymphadenitis.
Data & Sample collection: The study was categorized into 2 sections.
First Phase included registration of patient details, sample collection by FNAC, culture study for isolated and Zeil-Neelsen studies(ZN stain).

Sample collection: The lymph nodes from the enrolled patients were palpated and aspiration was performed with 23-23 gauge needle as the standard operating procedure(SOP) of our laboratory. A total of five parallel smears were made of the aspirates and subjected to routine cytochemical stains (i). May Grunwald stain (ii).Papanicalou stain (iii). Two smears for ZN stain for evaluating cytomorphology under light microscope. The staining were performed as per approved guidelines and SOP. A positive case of LNTB on cytomorphology was rendered by presence of epithelioid granuloma, longhand giant cells and caseating necrosis. A positive case on ZN stain is documented by presence of AFB as eosinophilic slender bacilli and confirmed it presence in two consecutive slides Under strict aseptic precautions a portion of the aspirated material (0.1-0.2ml) was subjected for culture study and the sample was inoculated on Lowenstein Jensen slant [LJ] medium and incubated 37^{o}C and the slant was screened for growth.

Polymerase chain reaction (PCR):
A portion of the remaining samples left out in the needle was directed to PCR study-conventional type (Qiagen, Hilden, Germany) for DNA genotyping with an insertion element IS6110 oligonucleotide. Totally 3 primers were used one forward and two reverseset for detection of MTB with species. MTB reference strain (H37RV) grown on LJ agar was used as reference strain to document positive cases. An assay mixture with composition 25µl was formulated with the following (i). Forward primer 0.6µM, reverse primer N-tb&BMB–R each. With a composition of 0.325µl with addition of PCR buffer reagents as provided by the manufacturer kit. The primer composition sequences applied are IS-F 5’ –CCTGCGAGCGTAGGCGTCGG-3’ and IS-R 5’ –CTCGTCCAGCGCCGCTTCGG-3’. The positive controls for every reaction was composed of 2 tubes of DNA of MTB (H37RV strain-Ladder pattern). The PCR procedure was run at the temperature of 95^{o}C with amplification of the 123bp fragment of IS6110. First cycle was run at temperature of 95^{o}C for 10 minutes duration and other 45 cycles at same 95^{o}C & 60^{o}C each for a duration of 30 seconds and 72^{o}C for 40 seconds and at last step brought down to room temperature for cooling down. Various plots were generated by picking up positive and negative controls, appropriate melting points etc.

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Histopathology evaluation of Biopsy samples:
Excision biopsy of lymph nodes were advised in cased where a definite diagnosis could not be arrived on cytology and culture studies and in patients where there was no response to treatment at clinicians discretion. The biopsy specimens obtained were fixed in 10% formalin and processed by automated tissue processor adopting the 24 hours scheduling. Microscopical sections of 3 to 4 microns were cut for conventional histopathological evaluation with haematoxylin and eosin stain. The histopathological diagnosis of tuberculous lymphadenitis was made based on the morphological presence of typical caseating necrosis with epithelioid granuloma,laghans multinucleated giant cells and ZN stain was performed to look for the presence of bacilli.

3. RESULTS

The study analysed various diagnostic modalities LNTBon 151 suspected patients enrolled in the study. The age incidence ranged between 2 years to 60 years with high incidence from 3rd decade to 5th decade as shown in Graph-1. With regard to gender, male preponderance was observed upto fourth decade with average of Male: Female ration of 2:1 and the ratio started to flatten in equal proportion after 4th decade as shown in Graph-1

With context to site distribution, cervical group of nodes topped the table(60%) followed by axillary (27%) and Inguinal group (13%) as depicted in Graph -2.The duration of lymphadenopathy ranged between 20 days to 24 months indicating the chronicity of the infection.

The cytomorphological patterns on the smears were categorized into two broad spectrums(i).classic granulomatous tubercular pattern constituting 31 cases

(ii).non-tubercular pattern-which included non-necrotizing (20 cases), suppurative (35 cases), reactive (55 cases) and non-diagnostic(09 cases).Patients with non-tubercular pattern who were on antibiotics started empirically showed poor response to treatment thereby warranting subsequent biopsy and further explorations. The cytomorphological patterns correlated and varied in equal proportion with subsequent Histopathology report of lymph node biopsy and PCR reports from isolated which were elaborated in Table-1. Substantial variations were observed in case of reactive lymphadenitis where 55 cases showing reactive picture on cytology turned out to be LNTB on subsequent biopsy (N=15) and PCR revealed LNTB in 29 cases (n=55). Similar variations were observed in case of acute suppurative and non-

necrotizing granulomatous lymphadenitis thereby directing cytopathologists to be judicious while dealing with non-tuberculous pattern on cytology smears.

Classic LNTB on cytomorphology correlated well with subsequent reports whereas non-classical pattern varied significantly reiterating the need for additional diagnostic studies to rule out possibility of LNTB. Interestingly, 8 out of 9 cases which were reported as non-diagnostic on cytology proved to be LNTB on PCR and confirmed on biopsy thereby emphasizing the limitations of cytology.

The data from culture, ZN stain were compared individually and also comparative metric keeping PCR results as reference standard as tabulated in Table-2, from which it is evident that ZN stain has low pick up rate whereas culture study though significant also proved to vary in few cases thereby emphasizing the demerits of standalone procedures.

Statistical analysis

The data obtained by various diagnostic tests were analyzed in terms of sensitivity, specificity, positive predictive value and the diagnostic odds ratio for prevalence.

Keeping histopathological evaluation of lymph node excision biopsy as gold standard methods (=80/150) and PCR as standard reference methods, the parameters were calculated for TB lymphadenitis

N=120; True positive (TP) = 108; True negative (TN) =30; False positive (FP) =3; False negative (FN)=25

Prevalence of the disease= $T^{disease}$ /Total X 100(133/151X100= 89%)

Prevalence of Disease = 89%

Positive predictive value(PPV) of the disease: A/ (A+B) X100

PPV= 97%

Negative predictive value of disease:(NPV): D/ (D+C) X100

NPV= 56%

Sensitivity and specificity

The parameters sensitivity and specificity being characteristics of tests and calculated by,

Sensitivity: A/(A+C)X100 ; Specificity: D/(D+B)X100.

All the diagnostic test had significant p value with sensitivity and specificity as depicted in Table-4. When combined diagnostic tests are performed it proved to be significant and reject 'null hypothesis'(with 95% confidence interval) than standalone procedure in diagnosis for LNTB as evident from **Table-5** which shows the statistical analysis in quanta. The observations showed the 'power value of the study rejected Type-2 error which is more among studies in tropical countries

4. DISCUSSION

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The incidence of extra pulmonary manifestation of tuberculosis is highly on increasing margin as proposed by WHO with high morbidity and mortality with emergence of Multi drug resistance [MDR] especially among rural populations[35].FNAC procedure is useful rapid diagnostic method for detection of etiology of lymphadenitis[36]. It is considered to be simple, rapid and economical method. Inflammation and swelling of lymph node is one of the common clinical presentation and an indicator of various pathological conditions and it is often treated as a systemic disease [37].In India till date, the diagnosis of LNTB is based primarily on clinical evaluation and lab diagnosis with culture study with many pros and cons[38]. The presentstudy was also aimed to analyse the efficacy of various diagnostic methods in diagnosing classic as well as non-classicalcases with underlying evidence of tuberculous lymphadenitis in high prevalence areas[28].

Researchers have proposed that effective diagnosis of LNTB could be achieved with a multi-diagnostic approach rather than standalone procedures. Lymphadenitis of granulomatous inflammation is of chronic nature known to yield good amount material on aspirates due to high proliferation of lymphoid population of cells as immune response [39].

Cytomorphology detection of LNTB is based on demonstration of epithelioid granuloma with caseating necrosis (Fig-1), but it has its own limitation with a sensitivity and specificity of 65 % and 89% respectively as evident from the present study concords with prior studies done [12]. The reasons attributed to the varied efficacy are multiple factors like foci of aspiration, nature of aspirate, technical expertise, mimickers of several other granulomatous lesions on microscopy thus owning its limitations in clinical situations. Lymphadenitis due to non-MTB are usually resistant to anti-tubercular drugs and very often categorized as MDR-TB. Nevertheless this technique paves way for collecting materials for further additional examinations [40].

ZeihlNeelsen (ZN) staining is a technique to demonstrate Acid-Fast bacilli (MTB) which has high specificity (96%)despite very low pick up rate (sensitivity-65%) as observed in our study concurring with previous studies done (Fig-2) [41].The overall positivity of AFB in aspirates ranges from 36 % to 60 % as seen in the present study as well.The reason for the low sensitivity and low negative predictive value is the bacterial load, foci of aspiration and treatment history[42]. The concentration of bacterial load is directly proportional to sensitivity of ZN stain and it has been explored that that a minimum of 10000 bacilli/UL is required for demonstration for ZN stain which is highly uncommon in majority of cases as observed in the study[43].

Culture studies is which being more sensitive and specific is considered as gold standard method to demonstrate MTB along with biochemical test.In the present study culture study has a sensitivity of 88.4% and specificity of 90.5% (Fig -3). Prior studies proposes that sensitivity and specificity of culture varies between 40% to 90% in clinically suspected cases of LNTB and our observation falls into the reported range [44]. Although being highly efficient, it requires 6 to 8 weeks before a positive visual growth is noted.Another major

shortcoming in culture study is that it cannot differentiate and categorize speciation of the genus of the mycobacterium thus unhelpful to the Clinicians to initiate treatment since the course of treatment varies according to species [45].In 5 cases culture study was negative where PCR and biopsy was positive for LNTB due to the reason that the patient was already on ATT. All these shortcomings are inherent and BacTsystem could be advocated in resource poor areas to avoid longer duration[46].

In past decade, various studies had been carried out to emphasize PCR from FNAC aspirates or subsequent excision biopsy of lymph node could improve the sensitivity of diagnosis of LNTB to 99% when compared to conventional techniques alone[47]. PCR being highly sensitive method for detection of MTB in Extra pulmonary LNTB, it has a sensitivity and specificity of 95 % and 96% as evident from our study. (Fig -4)The reason for high efficacy is that it could detect MTB even in very low bacterial load concentration of 100/µL whereas other techniques require at least 1 millionbacilli/µl to show positive results. The efficacy of PCR was found to be at par with Histopathological evaluation of LNTB(Fig-5).Few cases(5 cases) in the present study were found be negative in PCR with positive on culture studies. The reason was unlike culture, PCR does not distinguish between live and dead MTB, which is also a major limitation as it could not be applied for screening for viable MTB in samples like dairy products, exposure to antibiotics which is known to inhibit MTB. Another major limitation of PCR is challenge to get expertize and financial support as it is relatively expensive procedure[48].

5. CONCLUSION

In the Modern era of Medicine, the incidence of tuberculous lymphadenitis is on higher margin than assumed and expected among the study population and diagnosis still remains a challenging task to clinicians and Pathologists. Combination of diagnostic procedures could serve as a novel method in detection of LNTB especially in patients with poor response to treatment in resource poor areas. Application of molecular procedures for routine diagnosis in country like India depends on varied factors. We conclude with a novelty that even conventional PCR with IS6110 is a robust rapid, economical, sensitive method, it be could be advocated as routine diagnostic tool in primary care centres which warrants minimal infrastructure to complete assays in areas with minimal resources.

Graph-1

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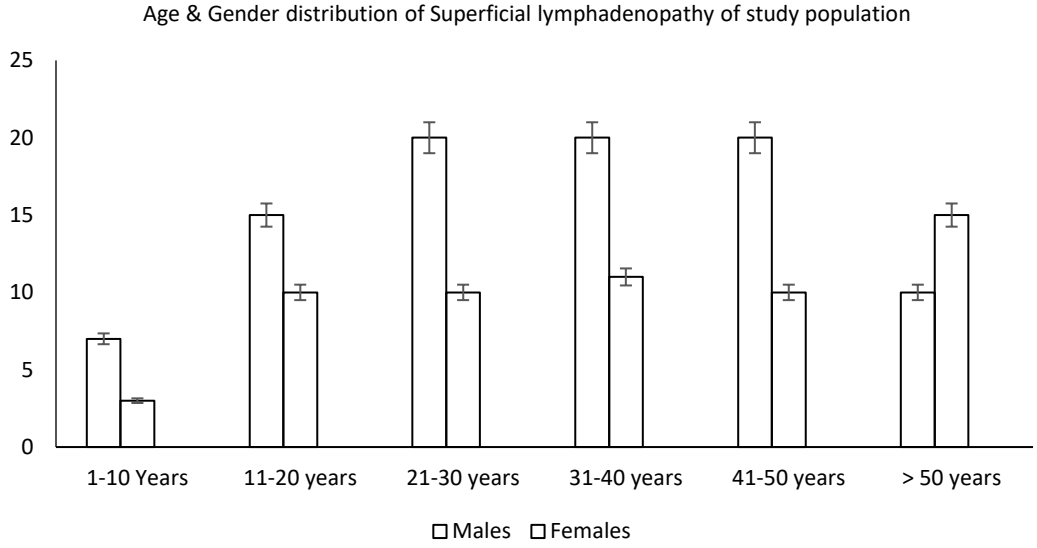
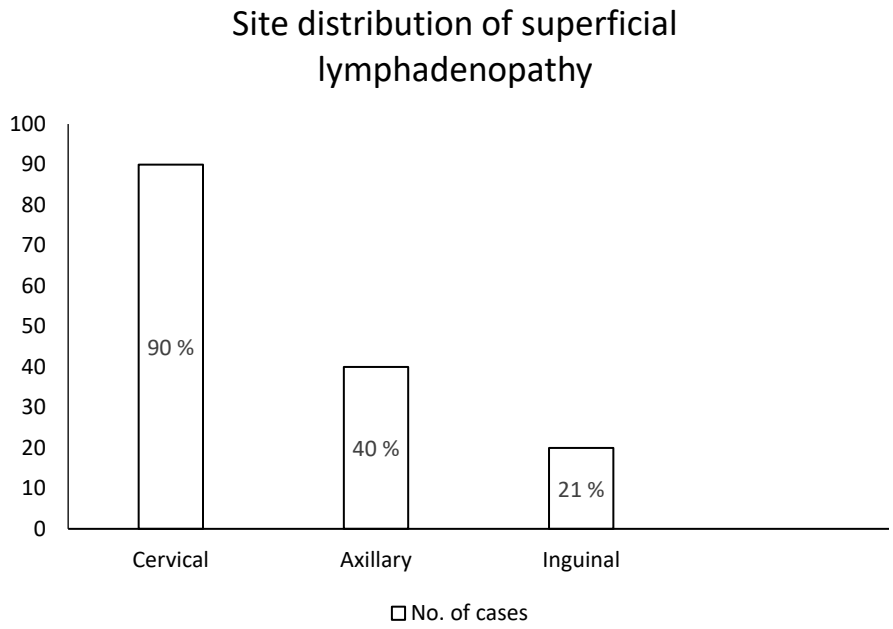


Graph-1:Age& Gender distribution of superficial lymphadenopathy of study population.

Graph 2



Graph-2: Site Distribution of superficial lymphadenopathy of enrolled patients.

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TABLE -1: Comparison of FNAC, histopathology and PCR in patients with superficial lymphadenopathy

Disease group on FNAC Impression	**LNTB on Histo-pathology(n=80)**	**TB positive on PCR (n=120)**
Reactive lymphadenitis (N=55)	15 (N=15)	29
Acute suppurative lymphadenitis (N=35)	25 (N=25)	32
Classical tuberculous lymphadenitis (N=31)	10 (N=10)	31
Non-necrotizing Granulomatouslymphadenitis (N=20)	20 (N=20)	20
Others/NDA (N=09)	09	08

Table-2: Comparative metric of conventional procedures with PCR

Diagnostic method (Conventional Procedures)	**Total number of clinical sample from isolates**	**PCR Positive cases**	**PCR negative cases**
TB related cases(n=120)	(N=151)	(N=151)	(N=151)
ZN stain Positive	55	54	05
ZN stain negative	65	66	31
LJ positive for MTB	97	90	10
LJ Negative for MTB	23	25	05
ZN positive	30	30	--
LJ positive	29	30	--
ZN negative	25	25	05
LJ positive	25	22	05
ZN negative	65	25	05
LJ negative	23	05	05

Table-3: Statistical parameters analysis

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A TP= 108	**B** FP=03
C FN=25	**D** TN=30
T disease= 133	**T Non disease=33**

Table-4: Statistical Analysis of data (keeping Histopathology as gold standard)

Diagnostic procedures	**Sensitivity (%)**	**Specificity(%)**	**P value**
PCR	89	96	0.002
LJ Culture study for MTB	80	86	0.004
ZN staining	57	86	0.006

Table- 5: Summary of statistical analysis and comparative metric values of various diagnostic tools in detection of LNTB (N=120 LNTB)

Diagnostic procedure				**Conventional method to be compared with**		**Conventional method to be compared with**	
				Culture	**Culture**	**ZN stain**	**ZN stain**
				++	--	++	--
PCR IS 6110 H37RV	**Result**		**Total**	**97**	**23**	**55**	**65**
¤	**(+)**		**120**	**115**	**5**	**53**	**65**
	(-)		**05**	**5**	**-**	**02**	**05**
					95% of confidence level (with 5% error)		**95% of confidence level (with 5% error)**
Sensitivity				**88.4%**	**90.5 to 96%**	**55.9%**	**70.5 to 79%**
Specificity				**90.5%**	**91.5 to 96%**	**84.9%**	**91.5 to 96%**
Positive predictive				**90.9%**	**82.5 to 66%**	**86%**	**82.5 to 92.5%**

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value							
Negative predictive value				**97.9%**	**80.5 to 86%**	**65%**	**60.5 to 70.2%**
Disease prevalence				**78%**	**60.5 to 76%**	**52%**	**70.5 to 76%**
AFB smear	**(+)**		**55**	**54**	**-**		
	(-)		**65**	**01**	**05**		
					95% of confidence level (with 5% error)		
Sensitivity				**65%**	**54.5 to 75%**		
Specificity				**96%**	**80.4 to 92%**		
Positive predictive value				**90.5%**	**89.2 to 95%**		
Negative predictive value				**52.5%**	**45 to 55.5%**		
Disease prevalence				**70.2%**	**70.1 to 81%**		

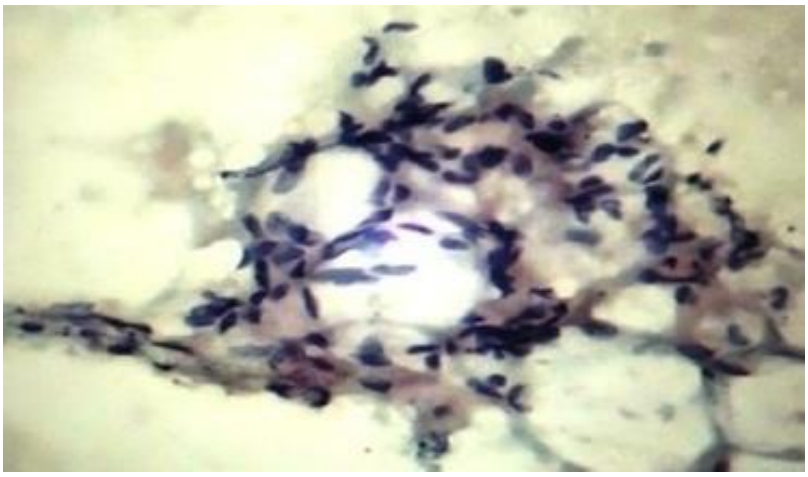

Figure-1: Cytomorphology of lymph node showingepithelioid cell granuloma, Pap-40X

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Figure-2: Zeihl-Neelsen stain showing AFB, ZN-Oil Immersion,100X

Figure-3: LJ culture slant showing grey yellow growth for MTB

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Figure-4: Conventional PCR with IS6110M sequence and H37RV strain with Ladder pattern

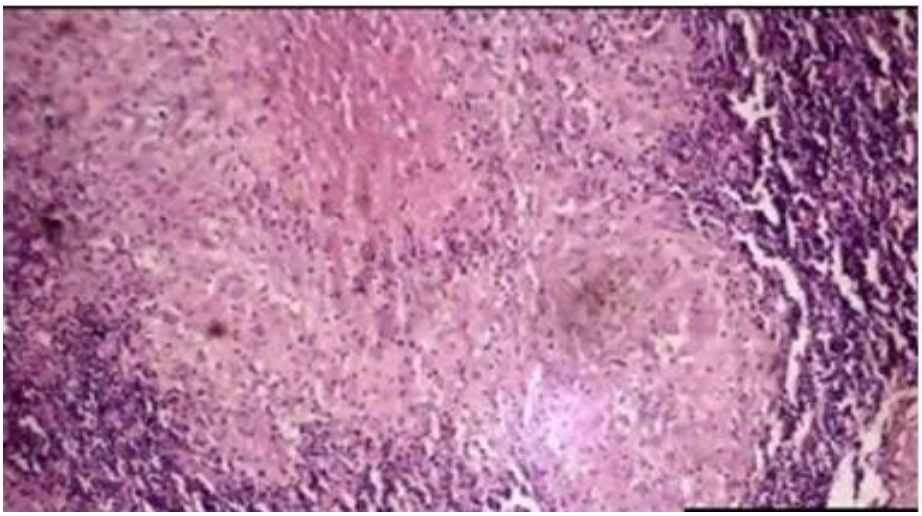

Figure-5: Histopathology of LNTB showing caseating necrosis, H&E-40X-High Power

Conflicts of Interest:NIL

References

1. WHO (2019) World Health Organisation. Global Tuberculosis Report.
2. Graham, S. M., Sismanidis, C., Menzies, H. J., Marais, B. J., Detjen, A. K. & Black, R. E. (2014) Importance of tuberculosis control to address child survival, Lancet. 383, 1605-1607.
3. Nunn, A. J., Phillips, P. P. J., Meredith, S. K., Chiang, C.-Y., Conradie, F., Dalai, D., van Deun, A., Dat, P.-T., Lan, N., Master, I., Mebrahtu, T., Meressa, D., Moodliar, R., Ngubane,

N., Sanders, K., Squire, S. B., Torrea, G., Tsogt, B. & Rusen, I. D. (2019) A Trial of a Shorter Regimen for Rifampin-Resistant Tuberculosis, New England Journal of Medicine. 380, 1201-1213.
4. Das, P., Ganguly, S., Mandal, B. & Khan, A. (2018) Prevalence of rifampicin-resistant pediatric tuberculosis by cartridge-based nucleic acid amplification test at the intermediate reference laboratory under revised national tuberculosis control program India: A multidimensional approach, Biomedical and Biotechnology Research Journal (BBRJ). 2, 300-305.
5. Atherton, R. R., Cresswell, F. V., Ellis, J., Kitaka, S. B. & Boulware, D. R. (2019) Xpert MTB/RIF Ultra for Tuberculosis Testing in Children: A Mini-Review and Commentary, Front Pediatr. 7, 34-34.
6. Conradie, F., Diacon, A. H., Ngubane, N., Howell, P., Everitt, D., Crook, A. M., Mendel, C. M., Egizi, E., Moreira, J., Timm, J., McHugh, T. D., Wills, G. H., Bateson, A., Hunt, R., Van Niekerk, C., Li, M., Olugbosi, M., Spigelman, M. & Nix, T. B. T. T. (2020) Treatment of Highly Drug-Resistant Pulmonary Tuberculosis, N Engl J Med. 382, 893-902.
7. WHO (2015) World Health Organisation. The End TB Strategy, WHO.
8. Bates, M., Shibemba, A., Mudenda, V., Chimoga, C., Tembo, J., Kabwe, M., Chilufya, M., Hoelscher, M., Maeurer, M., Sinyangwe, S., Mwaba, P., Kapata, N. & Zumla, A. (2016) Burden of respiratory tract infections at post mortem in Zambian children, BMC Medicine. 14, 99.
9. Dodd, P. J., Yuen, C. M., Sismanidis, C., Seddon, J. A. & Jenkins, H. E. (2017) The global burden of tuberculosis mortality in children: a mathematical modelling study, Lancet Glob Health. 5, e898-e906.
10. Devrim, I., Aktürk, H., Bayram, N., Apa, H., Tulumoğlu, S., Devrim, F., Erdem, T., Gulfidan, G., Ayhan, Y., Tamsel, I., Can, D. & Alper, H. (2014) Differences between pediatric extra-pulmonary and pulmonary tuberculosis: a warning sign for the future, Mediterr J Hematol Infect Dis. 6, e2014058-e2014058.
11. Pattnaik, S. (2018) Analysis of tuberculosis case report in Hyderabad district of Telangana state, Journal of Family Medicine and Primary Care. 7, 561-564.
12. Kaur, R., Kachroo, K., Sharma, J. K., Vatturi, S. M. & Dang, A. (2016) Diagnostic Accuracy of Xpert Test in Tuberculosis Detection: A Systematic Review and Meta-analysis, J Glob Infect Dis. 8, 32-40.
13. Stival, A., Chiappini, E., Montagnani, C., Orlandini, E., Buzzoni, C., Galli, L. & de Martino, M. (2014) Sexual Dimorphism in Tuberculosis Incidence: Children Cases Compared to Adult Cases in Tuscany from 1997 to 2011, PLOS ONE. 9, e105277.
14. Lee, J. Y. (2015) Diagnosis and treatment of extrapulmonary tuberculosis, Tuberc Respir Dis (Seoul). 78, 47-55.
15. Joosten, S. A., Ottenhoff, T. H. M., Lewinsohn, D. M., Hoft, D. F., Moody, D. B., Seshadri, C., Collaboration for Tuberculosis Vaccine Discovery - Donor-Unrestricted T-cells Working Group, B. & Melinda Gates, F. (2019) Harnessing donor unrestricted T-cells for new vaccines against tuberculosis, Vaccine. 37, 3022-3030.
16. Kulchavenya, E. (2014) Extrapulmonary tuberculosis: are statistical reports accurate?, Ther Adv Infect Dis. 2, 61-70.

17. Revised National Tuberculosis Control Programme [RNTCP] (2016) Technical and operational control for tuberculosis and management, Status Report of tuberculosis in India.
18. Kala Yadhav .M. L, V. M. (2018) Role of GeneXpert in Rapid Molecular Detection of Extrapulmonary Tuberculosis in Tertiary Care Hospital, 5. 6.
19. Narang, P., Narang, R., Narang, R., Mendiratta, D. K., Sharma, S. M. & Tyagi, N. K. (2005) Prevalence of tuberculous lymphadenitis in children in Wardha district, Maharashtra State, India, The International Journal of Tuberculosis and Lung Disease. 9, 188-194.
20. Azadi, D., Motallebirad, T., Ghaffari, K. & Shojaei, H. (2018) Mycobacteriosis and Tuberculosis: Laboratory Diagnosis, Open Microbiol J. 12, 41-58.
21. Nidhi P1, S. T., Shalini M, Kumud G. (2011) FNAC in tuberculous lymphadenitis: experience from a tertiary level referral centre., Indian J Tuberc. 58, 102-7.
22. Hasaneen, N. A., Zaki, M. E., Shalaby, H. M. & El-Morsi, A. S. (2003) Polymerase Chain Reaction of Pleural Biopsy Is a Rapid and Sensitive Method for the Diagnosis of Tuberculous Pleural Effusion, CHEST. 124, 2105-2111.
23. Kumar, S. N., Prasad, T. S., Narayan, P. A. & Muruganandhan, J. (2013) Granuloma with langhans giant cells: An overview, J Oral Maxillofac Pathol. 17, 420-423.
24. Raizada, N., Khaparde, S. D., Rao, R., Kalra, A., Sarin, S., Salhotra, V. S., Swaminathan, S., Khanna, A., Chopra, K. K., Hanif, M., Singh, V., Umadevi, K. R., Nair, S. A., Huddart, S., Tripathi, R., Surya Prakash, C. H., Saha, B. K., Denkinger, C. M. & Boehme, C. (2018) Upfront Xpert MTB/RIF testing on various specimen types for presumptive infant TB cases for early and appropriate treatment initiation, PLOS ONE. 13, e0202085.
25. Chand, P., Dogra, R., Chauhan, N., Gupta, R. & Khare, P. (2014) Cytopathological Pattern of Tubercular Lymphadenopathy on FNAC: Analysis of 550 Consecutive Cases, J Clin Diagn Res. 8, FC16-FC19.
26. Cheng, V. C. C., Yam, W. C., Hung, I. F. N., Woo, P. C. Y., Lau, S. K. P., Tang, B. S. F. & Yuen, K. Y. (2004) Clinical evaluation of the polymerase chain reaction for the rapid diagnosis of tuberculosis, J Clin Pathol. 57, 281-285.
27. Nurwidya, F., Handayani, D., Burhan, E. & Yunus, F. (2018) Molecular Diagnosis of Tuberculosis, Chonnam Med J. 54, 1-9.
28. Deveci, H. S., Kule, M., Kule, Z. A. & Habesoglu, T. E. (2016) Diagnostic challenges in cervical tuberculous lymphadenitis: A review, North Clin Istanb. 3, 150-155.
29. Muddaiah, R. K., James, P. M. & Lingegowda, R. K. (2013) Comparative study of Smear Microscopy, Rapid Slide Culture, and Lowenstein - Jensen culture in cases of pulmonary tuberculosis in a tertiary care hospital, J Res Med Sci. 18, 767-771.
30. Park, H., Jang, H., Kim, C., Chung, B., Chang, C. L., Park, S. K. & Song, S. (2000) Detection and identification of mycobacteria by amplification of the internal transcribed spacer regions with genus- and species-specific PCR primers, J Clin Microbiol. 38, 4080-4085.
31. Sallin, M. A., Kauffman, K. D., Riou, C., Du Bruyn, E., Foreman, T. W., Sakai, S., Hoft, S. G., Myers, T. G., Gardina, P. J., Sher, A., Moore, R., Wilder-Kofie, T., Moore, I. N., Sette, A., Lindestam Arlehamn, C. S., Wilkinson, R. J. & Barber, D. L. (2018) Host resistance to pulmonary Mycobacterium tuberculosis infection requires CD153 expression, Nature Microbiology. 3, 1198-1205.

32. Gierahn, T. M., Wadsworth, M. H., Hughes, T. K., Bryson, B. D., Butler, A., Satija, R., Fortune, S., Love, J. C. & Shalek, A. K. (2017) Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput, Nature Methods. 14, 395-398.
33. Sharma, S. K., Ryan, H., Khaparde, S., Sachdeva, K. S., Singh, A. D., Mohan, A., Sarin, R., Paramasivan, C. N., Kumar, P., Nischal, N., Khatiwada, S., Garner, P. & Tharyan, P. (2017) Index-TB guidelines: Guidelines on extrapulmonary tuberculosis for India, Indian J Med Res. 145, 448-463.
34. Tatar, D., Senol, G., Alptekin, S., Gunes, E., Aydin, M. & Gunes, O. (2016) Assessment of Extrapulmonary Tuberculosis in Two Provinces of Turkey, Iran J Public Health. 45, 305-313.
35. Raviglione, M. & Sulis, G. (2016) Tuberculosis 2015: Burden, Challenges and Strategy for Control and Elimination, Infect Dis Rep. 8, 6570-6570.
36. Gupta, V. & Bhake, A. (2017) Molecular Diagnosis of Tubercular Lymphadenopathy from Fine-Needle Aspirates in Pediatric Patients, Acta Cytologica. 61, 173-178.
37. Martin, C. J., Cadena, A. M., Leung, V. W., Lin, P. L., Maiello, P., Hicks, N., Chase, M. R., Flynn, J. L. & Fortune, S. M. (2017) Digitally Barcoding Mycobacterium tuberculosis Reveals In Vivo Infection Dynamics in the Macaque Model of Tuberculosis, mBio. 8, e00312-17.
38. Qaqish, A., Huang, D., Chen, C. Y., Zhang, Z., Wang, R., Li, S., Yang, E., Lu, Y., Larsen, M. H., Jacobs, W. R., Jr., Qian, L., Frencher, J., Shen, L. & Chen, Z. W. (2017) Adoptive Transfer of Phosphoantigen-Specific γδ T Cell Subset Attenuates Mycobacterium tuberculosis Infection in Nonhuman Primates, J Immunol. 198, 4753-4763.
39. Chhajed, P. N., Vaidya, P. J., Mandovra, N. P., Chavhan, V. B., Lele, T. T., Nair, R., Leuppi, J. D. & Saha, A. (2019) EBUS-TBNA in the rapid microbiological diagnosis of drug-resistant mediastinal tuberculous lymphadenopathy, ERJ Open Res. 5, 00008-2019.
40. Jason, H. L. (2020) Modern pathology, nature 33, 317-458.
41. Krishna, M. & Gole, S. G. (2017) Comparison of Conventional Ziehl-Neelsen Method of Acid Fast Bacilli with Modified Bleach Method in Tuberculous Lymphadenitis, J Cytol. 34, 188-192.
42. Masilamani, S., Arul, P. & Akshatha, C. (2015) Correlation of cytomorphological patterns and acid-fast Bacilli positivity in tuberculous lymphadenitis in a rural population of southern India, J Nat Sci Biol Med. 6, S134-S138.
43. Krishna, M. & Gole, S. (2017) Comparison of conventional Ziehl–Neelsen method of acid fast bacilli with modified bleach method in tuberculous lymphadenitis, J Cytol. 34, 188-192.
44. Agrawal, M., Bajaj, A., Bhatia, V. & Dutt, S. (2016) Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis, J Clin Diagn Res. 10, DC09-DC12.
45. Wolf, F. A., Angerer, P. & Theis, F. J. (2018) SCANPY: large-scale single-cell gene expression data analysis, Genome Biology. 19, 15.
46. Goel, M. M., Ranjan, V., Dhole, T. N., Srivastava, A. N., Mehrotra, A., Kushwaha, M. R. S. & Jain, A. (2001) Polymerase Chain Reaction vs. Conventional Diagnosis in Fine Needle Aspirates of Tuberculous Lymph Nodes, Acta Cytologica. 45, 333-340.

47. Zewdie, O., Abebe, T., Mihret, A., Hirpa, E. & Ameni, G. (2017) Concentration of fine needle aspirates similar to molecular method improves sensitivity of the diagnosis of tuberculous lymphadenitis in Addis Ababa, Ethiopia, BMC Infectious Diseases. 17, 77.
48. Eom, J. S., Mok, J. H., Lee, M. K., Lee, K., Kim, M. J., Jang, S. M., Na, H. J., Song, S. E., Lee, G., Jo, E.-J., Kim, M.-H., Kim, K. U. & Park, H.-K. (2015) Efficacy of TB-PCR using EBUS-TBNA samples in patients with intrathoracic granulomatous lymphadenopathy, BMC Pulmonary Medicine. 15, 166.
49. Differential expression of Helios, Neuropilin-1 and FoxP3 in head and neck squamous cell carcinoma (HNSCC) patients A.A.Mohamed Adil, Anil Kumar Bommanabonia, AnandrajVaithy, Sateesh Kumar 3biotech 9 (178)
50. Protagonist of Immuno-Profiling, Immuno-Scoring, and Immunotherapy Towards Colitis-Associated Cancer: Systematic Review, Mohamed Adil a.a, AK Pandurangan, M Waseem, N Ahmed Diagnostic and Treatment Methods for Ulcerative Colitis and Colitis 2020
51. Emerging Role of Mitophagy in Inflammatory Diseases: Cellular and Molecular Episodes, Mohamed Adil AA, S Ameenudeen, A Kumar, S Hemalatha, N Ahmed, N Ali 2020 Curr Pharm Des. 2020;26(4):485-491. doi: 10.2174/1381612826666200107144810
52. Increased Expression of TGF-β and IFN-γ in Peripheral Blood Mononuclear Cells (PBMCs) Cultured in Conditioned Medium (CM) of K562 Cell Culture AAM Adil, L Vallinayagam, K Chitra, S Jamal, AK Pandurangan, N Ahmed Journal of Environmental Pathology, Toxicology and Oncology 38 (2)
53. Cancer immunotherapy: Targeting immunosuppressive tumor microenvironment NA A.A Mohamed Adil Oncobiology and Targets 2014