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Original article

To estimate the point prevalence of Rifampicin resistant tuberculosis in extra pulmonary tuberculosis patients' as detected by CBNAAT in a district hospital and to analyze the data using logistic regression mathematical model

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Abstract

The aim of this study was to estimate the point prevalence of Rifampicin resistant Mycobacteria causing tuberculosis of lymph node as detected by cartridge based nucleic acid amplification test (CBNAAT) and analyzed using logistic regression mathematical modeling. An observational cross-sectional study was carried out in the Department of Otorhinolaryngology from July 2019 to February 2020 in a tertiary healthcare setting. Rifampicin resistant Mycobacteria were identified; data tabulated and analyzed to find the point prevalence of Rifampicin resistant tuberculosis. A total of 37 patients who presented to the OPD were included in the study. Confirmation of the tuberculosis was done either by fine needle aspiration cytology (FNAC) or by direct biopsy. Demographic characteristics of the patients were analyzed. A logical regression mathematical model of Rifampicin resistance in the district was created. Correlation matrix was calculated using Jamovi software. Occurrence of Rifampicin resistance was dependent variable in logistic regression modeling. Levels VA and VB, posterior triangle group lymph nodes were most commonly involved. It was found that there is high prevalence 89.189% ($p<0.01$) of Rifampicin resistant Mycobacteria. Rifampicin resistance have a 2.9672 greater odds per stage (196% higher chance $p=0.0291$) treatment failure as compared to Rifampicin sensitive patients. Females have higher risk of harbouring Rifampicin Resistant Mycobacteria than males.

Key words: Cervical lymph node tuberculosis, Multi-drug resistant tuberculosis, Nucleic acid amplification test, Rifampicin

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Tuberculosis (TB) can affect any organ in the human body with the exception of hair and nails[1]. India is experiencing increasing cases of TB due to diabetes mellitus and human immunodeficiency virus (HIV). Extra pulmonary TB makes up 15–20% of TB cases in India[2]. Extra pulmonary TB occurs more commonly in immune-suppressed patients due to hematogenous spread

of the bacilli[3]. In India tuberculous lymphadenitis is one of the most common presentations of extra pulmonary TB (incidence 34.4% of cases)[4]. In almost 30% (1/3 incidence) of these patients have concurrent pulmonary tuberculosis for which they must be actively screened. The most common infected site is the cervical group of lymph nodes especially the posterior triangle nodes; this is followed by axillary lymph nodes[5]. For some unknown reasons in India the disease is extremely more common in females than males. After confluence of the nodes called the "matting" the nodes break out of the investing layer of deep cervical facia to from a tubercular sinus. The typical TB sinus is described as having "thin, bluish, undermined edges with scanty watery discharge"[6].

It has been observed that there is raising incidence of Rifampicin resistant tuberculosis and muti-drug resistant tuberculosis in India[7]. The connection between drug resistant Mycobacterium causing extra pulmonary lymphadenitis in India is not explored. This research addresses the evolution of drug resistant tuberculosis as a causative agent of extra pulmonary lymph node tuberculosis.

The purpose of this study was to estimate the point prevalence of Rifampicin resistant tuberculosis in extra pulmonary tuberculosis in lymph nodes as detected by cartridge based nucleic amplification test (CBNAAT) in district hospital and to describe the clinical features of cervical TB lymphadenitis who present in ENT OPD. Also, to mathematically model the Rifampicin resistance, Jones and Campbell stage, lymph node size, height, weight, BMI and age using logistic regression (Binomial or Bernoulli distribution and the logit link) and to compute parameters like odds ratio, likelihood ratio, "Z" value, etc. at 95% confidence intervals and draw up inference.

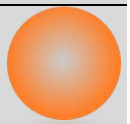
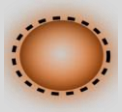
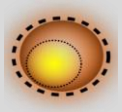
Materials and methods

An observational cross-sectional study was carried out in the Department of Otorhinolaryngology of a major district hospital from July 2019 to February 2020 in a tertiary healthcare setting. A total of 37 (N=37) adult cases were included in this study. Inclusion criteria of cases were adult patients who had clinical features of extra pulmonary tuberculosis cervical lymph node disease. Ultrasound-guided targeted fine needle aspiration cytology (FNAC) or a lymph node excision biopsy for final confirmatory diagnosis of tuberculosis. Aspirated or biopsied material was sent for by cartridge based nucleic amplification test (CBNAAT) in TB clinic of the district hospital. Rifampicin resistant Mycobacteria were identified and data tabulated, analyzed to find the point prevalence of Rifampicin resistant tuberculosis. Consent was obtained from the patients before inclusion in the study.

Clinical history was obtained in all cases which were typical of tuberculosis i.e. loss of weight and appetite, evening raise of temperature, night sweats, cough lasting more than 4 weeks, chest pain, expectoration if it was blood stained, breathlessness, neck swellings and pain in the swelling. A history for pulmonary tuberculosis was included as up to 1/3 of extra pulmonary tuberculosis patients suffer synchronously from a pulmonary tuberculosis focus[2].

Jones and Campbell in 1962 described a classification system for stages of cervical lymph node tuberculosis[8]. This system was used extensively in this study (Table1). The pictures were modeled by the author in Inkscape Vector graphics software package. 3-Dimensional Scalable Vector Graphics (SVG) outputs of the models of lymph nodes can be had from the author.

Table 1: Jones and Campbell classification (1962) of stage of cervical lymph node tuberculosis (drawn by author)[8]

Stage	Physical characteristic of lymph node	Picture
1	Enlarged, firm, mobile, discrete nodes showing non-specific hyperplasia	
2	Large rubbery nodes fixed to surround tissue owing to periadenitis	
3	Central softening due to abscess formation	
4	Collar-stud abscess formation	
5	Sinus tract formation	

Complete blood count (rule out leukemia, anemia), ESR, random blood sugar (to detect type 2 diabetes mellitus) and HIV ELISA Card test (for AIDS, immune suppression) were done. Mantoux test was done in children only in this study; it was performed in those who did not have BCG scar on the upper arm to detect previous exposure to Mycobacterium. Chest X ray was done in all patients.

Sputum for acid fast bacilli (AFB) was done using Ziehl-Neelsen (ZN) stain for only those who had history of cough along with productive sputum.

Ultrasonography (USG) was done in all cases, as it is a non invasive investigation. Anechoic or hypoechoic areas lymph node was the most common radiology picture. These are due to central necrosis and liquefaction to cold abscess. Ultrasound-guided targeted FNAC or a lymph node excision biopsy was used for final confirmation of tuberculosis. Occasionally calcifications inside a lymph node or fully calcified nodes were noted. Fibrosis and fixity to deep cervical facia was also findings in few cases.

Fig 1. Figure shows sinus formation of the right posterior auricular lymph node with massive cold abscess formation in the jugulo-diagastric lymph node behind the angle of the right mandible. CBNAAT of the aspirate yielded Rifampicin resistant mycobacteria.

FNAC was performed using 10ml disposable syringe with 22-gauge needle. Multiple passes were made into the Lymph node while maintaining steady suction. The slides were then alcohol fixed and stained using Papanicolaou stain. In case there were multiple nodes involved which were the majority of cases, FNAC was done from the most fluctuant node. This was done because around 5ml of purulent material to perform CBNAAT which must not contain blood. Care was taken to perform an aseptic aspiration and non dependent manner to avoid secondary infection and sinus formation. In few cases FNAC was inconclusive and these were commenced on a 10 days course of antibiotics. Standard guideline drug therapy of Penicillin or Amoxicillin twice or thrice a day was administered for 10 days (Clindamycin or Co-trimoxazole were other approved choices)[9]. Both these antibiotics are not effective against tuberculosis and would not interfere with the outcome. Persistent antibiotic unresponsive neck lymphadenopathy was treated by excision biopsy (for small node) or an incision biopsy (for a large, matted node). All the diagnosed cases of TB in this research were notified, given a TB card and DOTS for TB was started in accordance with the Revised National Tuberculosis Control Program (RNTCP) for India[10].

Statistical analysis

Categorical variables were compared using the chi-square test or the Mantel-Haenzsel chi-square test for trend. Sampling strategy used was population based simple random sampling. This whole study used Jamovi software package which is a graphical front end for R programming language to do biostatistical analysis[11,12]. Statistically significant association was taken to be $p < 0.05$ at 95% confidence interval for this whole research. Correlation matrix was calculated. Occurrence of Rifampicin resistance is dependent variable used for logistic regression modeling. Logistic regression modeling which uses binomial (Bernoulli) distribution (only 2 outcomes yes or no) was used to analyze and create a mathematical model the Rifampicin resistance data. The mathematical model was charted and odds ratios and 95% ($p=0.05$) confidence intervals were computed. Logistic regression assumes that the observations are independent of each other and one observation does not interfere with another. Schema of coding the exposure variable was as follows: Rifampicin resistance was taken as 1 (hit) and sensitive as 0 (miss), and the odds ratio, Model fit measures, Omnibus likelihood ratio test, Chi square test were analyzed at 95% ($p<0.05$) confidence interval. Computer generated model coefficients for Rifampicin resistance was calculated and odds ratio inferred.

Results

Demographic, clinical, social information of the study participants are presented in table 2.

Table 2: Demographic characteristics of study population

Characteristic		Median	p value
Age (Adults >14 years of age) in years		26	<0.001
Sex	Male	13	<0.001
	Female	24	
Education level	High school or less	32	0.09
	Higher education	7	
Employment	Employed	27	0.03
	Unemployed	10	
Other diseases	Type 2 diabetes mellitus	6	0.421
	HIV positive	12	0.032
	Malnutrition	12	0.127
Below poverty line		34	0.00216
Place	Palanpur city urban	16	0.017
	Surrounding villages	21	0.033
Size of lymph node (cm) Assumed 1 cm and less nodes not palpable clinically, USG neck was done in all cases		2.7	0.002
Height (cm)		163	0.389
Weight (kg)		55	0.717
Body mass index (BMI) (kg/m^2)		21.5	0.087
Rifampicin resistance		33/37= 89.189%	<0.01

Table 3: Pattern of involvement of neck lymph nodes by tuberculosis

Type of lymph nodes	Cases	Mean size of lymph node (cm)	p value
Levels VA and VB: posterior triangle group	20 (54%)	3.2	<0.01
Levels IA and IB: submental and submandibular groups	3 (8%)	1.5	0.562
Levels IIA and IIB: upper jugular group	16 (43.24%)	2.5	0.050
Level III: middle jugular group	13 (35.13%)	1.9	0.278
Level IV: lower jugular group	8 (21.62%)	2.1	0.01
Level VI: anterior (central) compartment group	0 (0%)	-	-

Location and type of nodes

The most common causes of acute cervical lymphadenopathy are viral upper respiratory infections, acute pyogenic bacterial infections by Streptococcus pyogenes (group A Beta hemolytic streptococcus) and Staphylococcus aureus. These nodes are mostly tender. Subacute or chronic cervical lymphadenopathy is caused by tuberculosis, non-tuberculous mycobacteria (NTM), actinomycosis and nocardiosis. Nocardia infected lymphnodes are unusual, that they are tender.

Lymph nodes affected by Mycobacteria were classified in accordance with American Academy of Otolaryngology[13]. Levels VA and VB, posterior triangle group were most commonly involved. VA and VB are separated by an imaginary horizontal plane from inferior border of the arch of the cricoid cartilage. Paradoxical lymph node enlargement and new lymph node involvement, sinus formation in middle of tuberculosis drug therapy was observed in 37.84% cases. This was managed by local therapy Magnesium sulphate pack application and reassurances that these will disappear once course of drugs is completed. The observed pattern of involvement of cervical lymph nodes is depicted in the table 3.

Correlations between various study variables were done using Spearman's rank correlation metric as data was non-parametric. There was strong positive correlation between Jones and Campbell stage and size of lymph node Spearman's rank correlation was 0.434 ($p<0.01$) highly significant. This was logically expected as bigger nodes were likely to be at a higher stage of disease. This also validates the Jones and Campbell (1962) classification of stage of cervical lymph node tuberculosis as a valid clinical tool in staging lymph node spread of disease. Body mass index was strongly (spuriously) correlated with weight and negatively correlated with height, which is understandable as it is a dependent variable of the two (Spearman's rank=0.761 $p<0.001$). Body mass index was negatively correlated with Rifampicin resistant tuberculosis but was not statistically significant (Spearman's rank =-0.110, $p=0.742$).

Mathematical modeling using logistic regression analysis

Logistic regression analysis done found that Rifampicin resistance is significantly associated with body mass index (Chi square value 5.852, df=1, $p=0.016$), height (Chi square value 6.001, df=1, $p=0.014$), and weight (Chi square value 5.769, df=1, $p=0.016$).

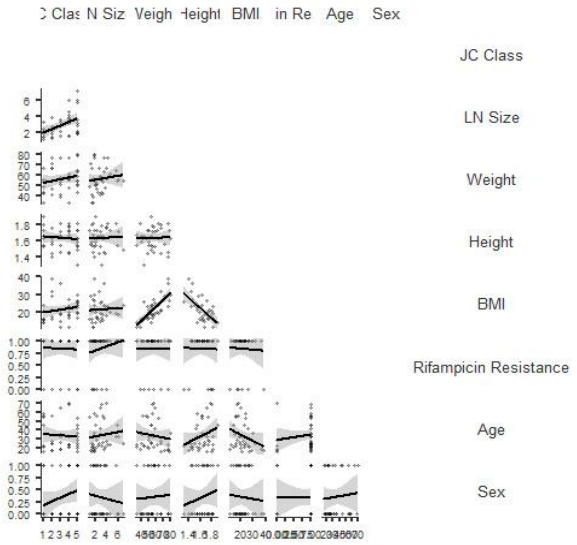


Fig 2. Figure shows the correlation between Jones and Campbell Class, lymph node size, weight, height, BMI, age sex and Rifampicin resistance using Spearman's rank correlation

Mathematical model generated coefficients for Rifampicin resistance, odds ratio for height was 4.09X 10^{-17} (p=0.036) here Log_e odds was -16.3883. There is a negative connection between height and Rifampicin resistance. Likewise BMI odds ratio was 0.284 (p=0.037) meaning 71% higher risk of occurrence of Rifampicin resistance with decreasing BMI. While for weight odds ratio was 1.650 (p=0.040) which means higher 65% risk of Rifampicin resistance in higher weight patients.

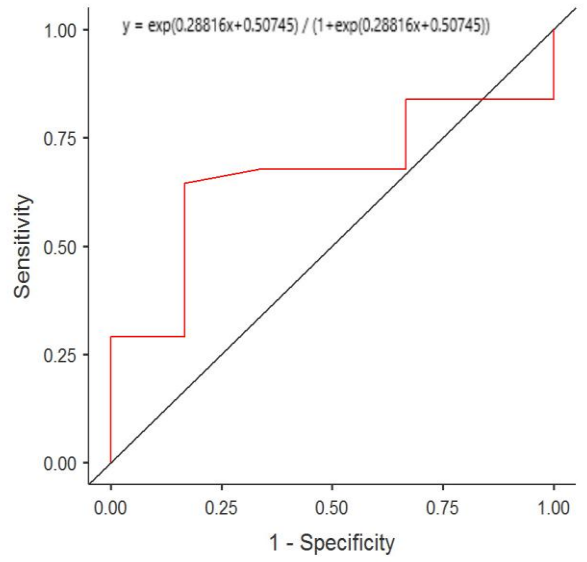


Fig 3. Mathematical Logistic regression modeling of the obtained data

There was no significant contribution to development of Rifampicin resistance by Jones and Campbell stage, size of lymph node, age and sex of the patient. Individuals with CBNAAT detected Rifampicin resistance have a 2.9672 greater odds per stage (196% higher chance p=0.0291) treatment failure as compared to individuals with sensitive patients.

Discussion

The most common site of extra pulmonary tuberculosis in India is lymph nodes (as well in many other parts of the developing world. GeneXpert MTB is a new molecular diagnostic test for TB, in addition to being highly sensitive for diagnosis of tuberculosis and Rifampicin drug resistance. This is particularly true in high-burden areas like North Gujarat. Drug susceptibility testing (DST) by cultures growing Mycobacterium often takes 8–10 weeks for a result which is simply too long for practical use. Line probe assay (LPA) which detects Isoniazid (INH) and Rifampicin (Ri) resistance is not available widespread in India hence most practical and quick method of diagnosing of tuberculosis and drug resistance is by GeneXpert MTB CBNAAT. Results are expeditiously obtained in about 2 hours which saves time of the health worker and also the patient from defaulting. The test is completely automated so the chances of contamination and laboratory error are minimal. Sensitivity was 95% which means the test accurately identifies 95 tuberculosis patients out of 100 patients and specificity was 98% which means the test results are negative for 98 normal patients out of 100 normal patients. These values re-establish the protagonist role of GeneXpert MTB CBNAAT in diagnosis of drug resistant tuberculosis.

Females have higher risk of harboring Rifampicin resistant Mycobacteria than males (p<0.48). Firstly, this may have to do with the fact that in Indian population the incidence of malnutrition is more in female children than males. Secondly, due to nature of lympho-hematogenous reaction in TB lymph node involvement is more common in females.

In this research Rifampicin resistance was found to be 89.189% (p<0.01) this is disproportionately high prevalence of resistant mycobacteria. This phenomenon of resistance was connected with body mass index, lower the BMI higher the chance of harboring resistant Mycobacteria.

Limitations of the study

Limitations of this study were that it was done in a district hospital setting and limited role of private practitioners' were ignored. Sample size was adequate at 37 though not very large; this was due to inclusion of only lymph node tuberculosis cases.

Interferon-gamma release assays (IGRA) has high diagnostic accuracy in TB lymphadenitis in areas of higher prevalence. This investigation was not studied and any benefit of this new test is yet to be seen. Another emerging investigation is positron emission tomography (PET) scan which according to some papers is useful for studying response to anti-tuberculosis therapy and differentiation of patterns of pulmonary tuberculosis. The high cost (around the time of publication 25,000 rupees) and radiation exposure precludes routine use of this tool in tuberculosis imaging.

Conclusion

There is high prevalence 89.189% of Rifampicin resistant tuberculosis in our district. This study is a wakeup call to the stake holders and policy makers to the increasing cases of multidrug resistant (MDR) tuberculosis in India. Females have higher risk of harboring Rifampicin resistant Mycobacteria than males ($p<0.48$). Rifampicin resistance is negatively correlated with height and body mass index. Malnourished and stunted patients have statistically significant higher risk of harboring Rifampicin resistant tuberculosis Mycobacteria ($p=0.037$) as per our mathematical model. Rifampicin resistance have a 2.9672 greater odds per stage (196% higher chance $p=0.0291$) treatment failure as compared to individuals with sensitive patients. Erythrocyte sedimentation rate (ESR) is elevated in most cases but is not specific for tuberculosis. The Jones and Campbell (1962) classification of stage of cervical lymph node tuberculosis is validated as a valuable clinical tool in the staging of lymph node spread of Mycobacteria. Paradoxical lymph node enlargement and new lymph node involvement, sinus formation in middle of tuberculosis drug therapy was observed in 37.84% cases. These can be conservatively managed.

Ethical consideration: Clearance was given by the Ethics Committee of Banas Medical College and Research Institute, Palanpur Civil Hospital.

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