

1 **Original Research Article**
2 **Tuberculosis burden -In Non HIV patients at a tertiary care hospital**
3 **in Hyderabad Telangana state.**

INTRODUCTION - The revised national tuberculosis control program of India has been able to reduce the disease burden significantly. Despite; TB continues to affect 40% of our population. To achieve the desired goal of NSP {national strategic plan} 2012-2017 we need to have a focused approach on the disease prevalence and the most vulnerable.

AIM – To estimate the burden of tuberculosis both pulmonary and extra pulmonary in non HIV patients at a tertiary care hospital in Hyderabad Telangana state South India.

MATERIAL AND METHODS – Over a period of two years from Jan.2013- Jan.2015 a total of two hundred and twenty six specimens, seventy eight from pulmonary and one hundred forty eight from extra pulmonary tuberculosis suspects were processed by various methods to achieve diagnosis.

RESULTS – Microbiologically the disease was observed in 25% of the studied subjects. Pulmonary tuberculosis accounted for 10 % and extrapulmonary in 15%. Overall smear by ZN stain was positive in 17% and culture in 20%. Cytology could detect disease in 67% of the suspects. Biochemical findings were insignificant. Drug resistance was noted in 4.4% of the cases. Drug resistance and MDR tuberculosis was more common in pulmonary form than in extrapulmonary. Females dominated both in the suspect's and culture confirmed cases as 53% & 76% respectively. The most affected age group for extrapulmonary disease remained as 6-35 years for both the sexes. In case of pulmonary tuberculosis it was noted as 36-50 years for men.

CONCLUSION – TB affects one third of the suspects. In the present study EPTB has exceeded PTB in the proportion of the laboratory confirmed suspects of tuberculosis. Both the forms of disease are more common in females and young age which needs to be prioritized in the control program to achieve the desired target of NSP.

Key words - Tuberculosis, Pulmonary, Extrapulmonary, Microscopy, Mycobacteria growth indicator Tube, Lowenstein Jensen media, Multi drug resistant.

1. INTRODUCTION

Right from the time of inception of the National Tuberculosis Control Program by Government of India in 1962 till today with several modifications in we have seen that the public authorities were able to bring down the disease prevalence substantially from 465 / lakh population in 1990 to 216 lakh per population in 2012 and mortality from 38 / lakh population in 1990 to 22 / lakh population in 2012. Similarly the incidence of the disease too has been reduced from 216 / lakh population in 1990 to 176/ lakh population in 2012. Therefore with this tremendous success the RNTCP has now entered into an ambitious national strategic plan [NSP] for 2012-2017 aimed at providing - Universal access for quality diagnosis and treatment for all tuberculosis patients in the community with the target of “**Reaching the Unreached**” [1]. Despite all these efforts TB still affects almost one third of the world’s population [2]. The global burden of tuberculosis being 9 million new cases and 1.4 million deaths annually as off 2012 report from World Health Organization [3]. The present Indian data on tuberculosis shows that of the 9 million cases globally 2.2 (25%) million are from India, making India the world highest Tb burden country [1]. In other words forty percent of the Indian population is infected with tuberculosis bacterium [1]. Several demographic factors have been held responsible for this high prevalence; overcrowding, female sex and young age being some of them. Overcrowding leads to high transmission rates. Therefore in order to curb its transmission; the goal of the existing strategic vision of the program up to 2015 is to decrease the mortality and morbidity associated with tuberculosis and to cut down the transmission of infection until TB {Tuberculosis} ceases to be a major public health problem in India. The objective of TB control Program is to achieve and maintain cure rate of at least 85% in new sputum positive pulmonary TB patients, and to achieve and maintain detection of at least 70% of such cases [4]. The major obstacle or challenge the disease control authorities right now facing is the emergence of unpredicted number of MDR/XDR {Multi drug resistant and extensively drug resistant} strains of M.tb in the society. Anyways to overcome this threat the government of India has already taken an initiative and with the help of

39 PMDT {Programmatic management of drug resistant TB} services has achieved to
40 date screening of 3.25 million multi drug resistant suspects, diagnosed more than
41 45000 drug resistant tuberculosis cases and initiated treatment for more than 42000
42 drug resistant tuberculosis cases with the help of 93 drug resistant TB centers
43 spread throughout the country [1]. Therefore, with such an exhaustive data in hand
44 and remarkable success of TB control program in India we in our simplest efforts
45 have tried to estimate the burden of the disease in non HIV patients at a tertiary
46 care hospital in south India.

47 **AIM** To estimate the burden of the disease tuberculosis both pulmonary and extra
48 pulmonary in non HIV patients at a tertiary care hospital of Hyderabad Telangana
49 state.

50 **2. MATERIAL AND METHODS**

51
52 **2.1 CASE DEFINITION** for pulmonary and extra pulmonary tuberculosis was as
53 per the World Health Organization guidelines [WHO] 2002 report [5].

54 A cross sectional study was carried out From Jan. 2013- Jan. 2015. About two
55 hundred and twenty six samples in total submitted for suspicion of pulmonary and
56 extra pulmonary form disease were processed in the central laboratories of Princess
57 Esra Hospital Hyderabad to achieve a proper diagnosis of the disease. Samples for
58 diagnosis of EPTB {extrapulmonary tuberculosis} were obtained under aseptic
59 techniques by a specialist. In PTB {Pulmonary tuberculosis} BAL {Broncho alveolar
60 lavage} fluid was collected under aseptic precautions by a trained pulmonologist.
61 Sputum samples were collected as per the CDC guidelines under the RNTCP India.
62 Samples were processed by microscopy, culture, biochemical analysis,
63 histopathology and cytology as per the request of the clinician. Microscopy was
64 done using the conventional Ziehl Neelsen [ZN] method for both pulmonary and
65 extra pulmonary samples after concentration by NALC {N acetyl L Cysteine -
66 NAOH} method .Culture was performed using a combination of two different
67 methods solid and liquid as per the WHO guidelines. The conventional solid media
68 used is Lowenstein Jensen media from HI Media Laboratories and the liquid broth
69 media Middle Brook 7 H9 from BD MGIT {Mycobacteria growth indicator Tube} 320
70 systems. Identification as Mycobacterium tuberculosis was achieved using the
71 commercially available rapid MPT64 antigen test detecting Mycobacterial protein 64

KD antigen which is specific for M.tb complex {Mycobacterium tuberculosis complex} from SD Bioline. Drug susceptibility test was performed using SIRE kit provided by BD MGIT 320 SYSTEM. All Samples were processed as per the standard guidelines mentioned in diagnostic standards and classification of tuberculosis by American thoracic society [6] and as per the manufacturer instructions. HIV testing for HIV 1& 2 virus was done using commercially available Tridot test from J.Mitra Co. based on the principle of rapid Immuno chromatography procedure with the sensitivity and specificity of 100% each.

2.2 SPECIMEN PROCESSING FOR MYCOBACTERIAL CULTURE AND SMEAR

All the clinical specimens were processed by the standard N-acetyl-L-cysteine sodium hydroxide digestion-decontamination technique for inoculation into MGIT tubes and LJ Medium. The specimens were then utilized for ZN staining. [6, 7]

2.2.1 QUALITY CONTROL

In microbiology was achieved by following the manufacturer instructions from BD MGIT 320 guidelines and test was carried out using the standard strains of M. tb complex H37 RV and ATCC strains.

2.3 COLLECTION OF SPECIMEN FOR CYTOLOGY

FNA {Fine needle aspiration} was performed under aseptic precautions using 18-21 G. needle and slides were fixed in alcohol, followed by Haemotoxylin and Eosin [H&E] staining and microscopy. Other cytological material were processed and stained with H&E followed by microscopy. Body fluids cytology was taken to be suggestive of tuberculosis when it was exudative with protein >3gm% and predominant lymphocytes. FNA cytology of tuberculosis was evidenced by predominant lymphocytosis, necrosis, hypocellularity, and epitheloid granuloma with or without multinucleated giant cells with and without acid fast bacilli.

2.3.1BIOCHEMICAL ANALYSIS

Various body fluids were analyzed biochemically for proteins, sugar, LDH {Lactate Dehydrogenase} and ADA {Adenosine deaminase} levels. Biochemical findings were suggestive of tuberculosis when protein was >3gm%, sugar less than two thirds of the blood levels, ADA with cut off > 20 IU/L and LDH > 130 IU/L.

2.3.2 DATA ANALYSIS

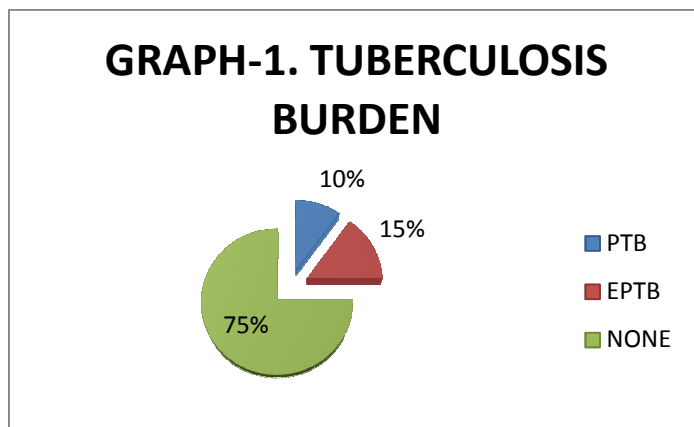
107 The present data was analyzed using the software package from CDC Epi Info
108 version 7.

109 3. RESULTS

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111 3.1 MICROBIOLOGY RESULTS

112 Of the total two hundred and twenty six samples submitted to the microbiology
113 laboratory for diagnosis of tuberculosis 78 or 34.5% were from pulmonary site and
114 148 or 65.5% from extrapulmonary site of the disease. Overall microbiologically 58
115 specimens or 25% of them were positive either by ZN smear, culture, or both. Rest
116 168 or 75% specimens were negative by both. Pulmonary form of disease was
117 noticed in 10% of the cases and extrapulmonary in 15 %.



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119 Altogether culture could detect disease in forty six or (20%) of the samples the
120 remaining 12 samples were positive only by smear. Therefore, 80 % of the samples
121 were negative by culture. None of the patients were reactive for HIV 1&2 virus.

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123 Direct microscopy using ZN stain was positive for 38 (17%) of the samples.

124 Microscopy alone was positive in twelve (5%) of the specimens. Microscopy

125 together with culture could detect tuberculosis in 26 (12%) of the specimens.

126 And the remaining 188 or 83% of the samples were negative by microscopy. The

127 sensitivity and specificity of the ZN stain when calculated with culture positive

128 results as gold standard showed the test to be 58.69% sensitive and 93.96%

129 specific.

130 In the present study we have isolated M. tuberculosis in 20% of the samples using

131 two different culture methods. MGIT alone was positive for thirty two (70%) samples.

LJ alone was positive for three (6%) of the specimens. LJ and MGIT together were positive for eleven (24%) of the samples. Therefore ZN stain missed eight samples which were culture positive. The sensitivity of MGIT in the present study was around 78% when compared to LJ media which was 56%. M. tuberculosis complex was confirmed by using the rapid identification test MPT64 antigen test in all forty six isolates.

Overall, drug resistance was noted in 4.4% of the samples. Mono drug resistance and MDR {Multidrug resistant} each accounted for 2.21% respectively. Drug resistance and MDR were more common in PTB accounting for 2.65% and 1.33% than in EPTB accounting for 1.76% and 0.88%. The details of which are shown in Table 1.

Table 1. Drug susceptibility pattern of the various M. tuberculosis isolates done using SIRE KIT from BD US.

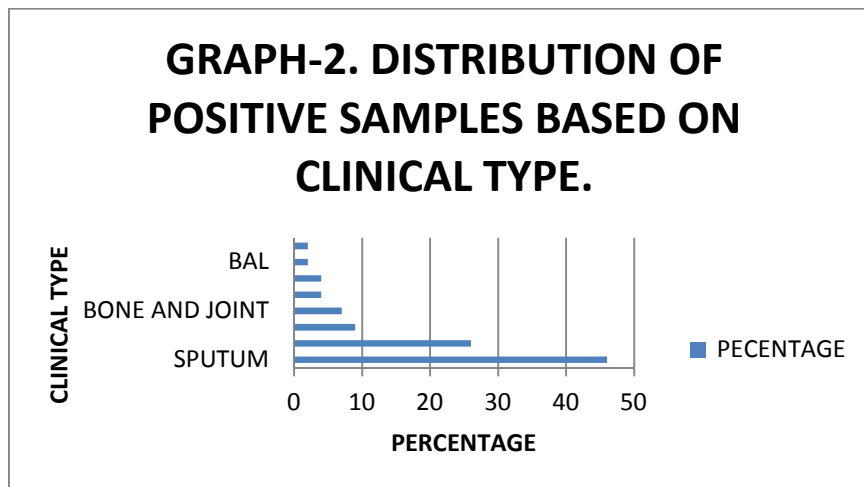
Susceptibility to first line anti tuberculosis drugs	Pulmonary isolates	Extra pulmonary isolates	Total
Pan susceptible	16 (44%)	20 (56%)	36 (78%)
Resistant	6 (60%)	4 (40%)	10 (22%)
Mono resistance			
Rifampicin	1 (16.6%)	1 (25%)	2 (20%)
Streptomycin	1 (16.6%)	0	1 (10%)
Isoniazid	1 (16.6%)	1 (25%)	2 (20%)
Multi Drug Resistant			
INH + STREP	0	1 (25%)	1 (10%)
INH +RIF	2 (33.33%)	1 (25%)	3 (30%)
INH+RIF+EMB	1 (16.6%)	0	1 (10%)

Distribution of samples based on site of involvement showed that of the total seventy eight pulmonary samples received for processing two lung biopsies ,twenty one were BAL fluid and rest sputum. Of the various extrapulmonary samples received for processing seventy three were pleural fluids, thirty nine as lymphnodes biopsies or fine needle aspirates, seventeen as gastrointestinal, eleven from bone and joints, five as pus and three as genitourinary respectively. Out of the total 46 culture positive samples 48% were from pulmonary form of disease and the remaining 52 % from extrapulmonary disease. In 22 PTB culture positive specimens majority of the samples were from sputum followed by BAL. In 24 EPTB positive

156 samples twelve were from lymphnodes, four pleural fluids, three samples from bone
157 and joint, two each from gastrointestinal tract and pus and one from genitourinary
158 tract. Over all maximum number of isolates were from sputum sample and the least
159 from BAL as shown in Graph -2 below.

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164 The most common site for EPTB was lymph node accounting for 50% followed by
165 pleura accounting for 16.6%, bone and joint accounting for 12.5% then pus and
166 gastrointestinal each accounting for 8.3% and in the last genitor urinary accounting
167 for 4% respectively. Moreover, in the lymph node tuberculosis 91.1% of the cases
168 were from cervical lymphnode followed by axillary .

169

170 **3.2 BIOCHEMICAL RESULTS**

171 Of the ninety body fluids submitted for biochemical analysis we have found that
172 nearly fifty percent of them had proteins raised with values of >2gm% none where
173 having proteins levels of more than 3gm% . Sugar was less than two third of the
174 blood sugar levels in cent percent. Adenosine deaminase and Lactate
175 dehydrogenase was raised in all .Hence any significant correlation could not be
176 established with these findings as culture was positive only in forty six of the
177 specimens.

178 **3.3 HISTOPATHOLOGY AND CYTOLOGY RESULTS**

179 Of the total 148 specimen submitted for cytological diagnosis of extra pulmonary
 180 tuberculosis in pathology laboratory during the study period, seventy three were
 181 pleural fluids, thirty nine lymph node material obtained by FNA, seventeen were
 182 peritoneal fluids, five were pus samples, eleven were from bone and joint and three
 183 genitourinary. Cytology was suggestive of tuberculosis in 99 or 67% with the
 184 sensitivity and specificity of 76% and 71%. Out of this 35 percent of the specimens
 185 were positive by both cytology and culture. Smear and cytology was positive for
 186 forty one [41%]. Another twenty three percent were only cytology positive.
 187 Remaining 49 percent were negative by both.

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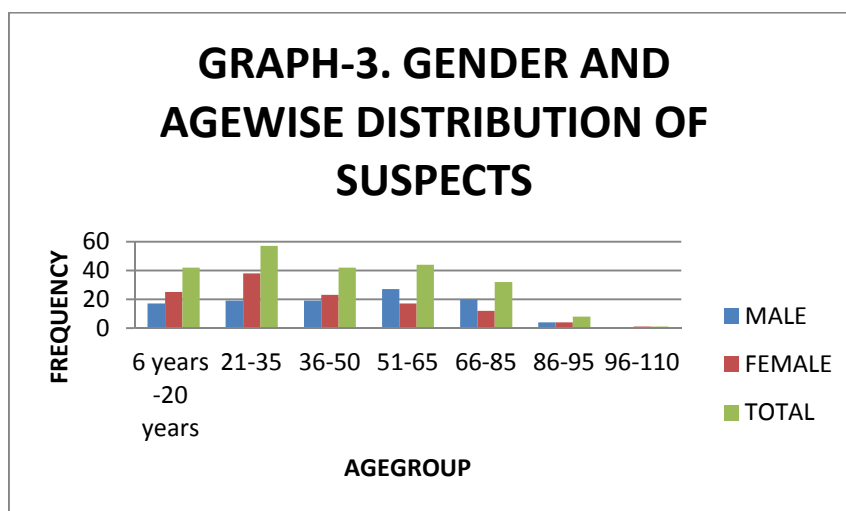
189 4. DEMOGRAPHIC FACTORS STUDIED.

190 4.1 AGE -The overall mean age for the suspects in the study was 43.66 ± 22.18 , for
 191 males it was noted as 48.17 ± 22.26 and for females 39.45 ± 21.08 respectively.

192 Significant variation in the mean age of the suspects with regards to clinical type of
 193 tuberculosis was observed. Especially in pulmonary form of disease, the mean age
 194 for females was 36.07 ± 11.56 and for males 51.60 ± 14.02 with the two tailed P
 195 value of 0.002 when determined using Chi squared T test by Yates. In extra
 196 pulmonary form of disease no such variation was observed; the mean age being
 197 41.13 ± 16.91 for females and 46.26 ± 15.68 for males.

198 Further majority of the samples around one hundred or 44% of them submitted for
 199 processing were in the age group 6-35 years of age as shown in graph 3 below.

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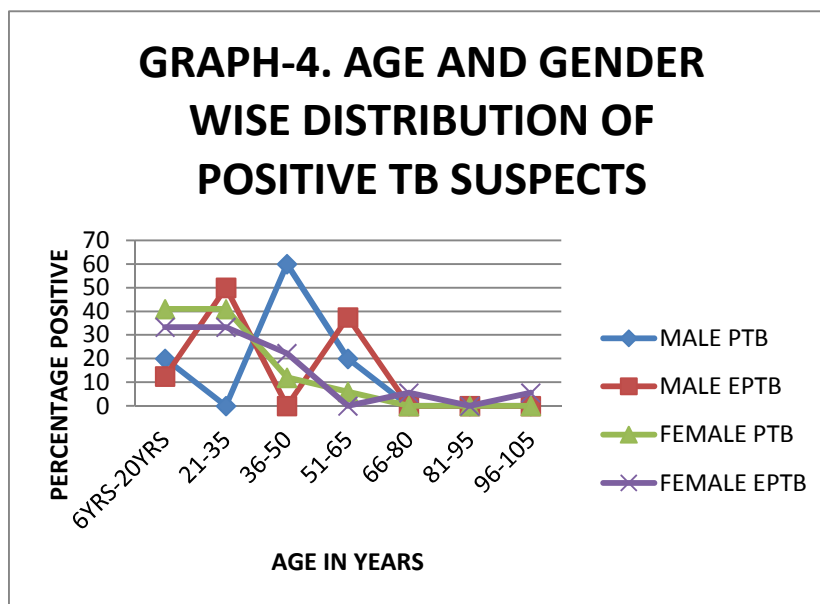
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203 Hence it is evident that clinical suspects are more in the reproductive and
204 economically active age group. Except for PTB; similar pattern of age distribution
205 was observed even in culture positive as well. Almost 70 % of the positive cases for
206 EPTB were in the age group of 6-35 years in both the sexes. But in PTB majority of
207 the positive cases 60% in males were seen in the age group 36-50 years. No such
208 variation was noticed in case of females as shown in graph 4.

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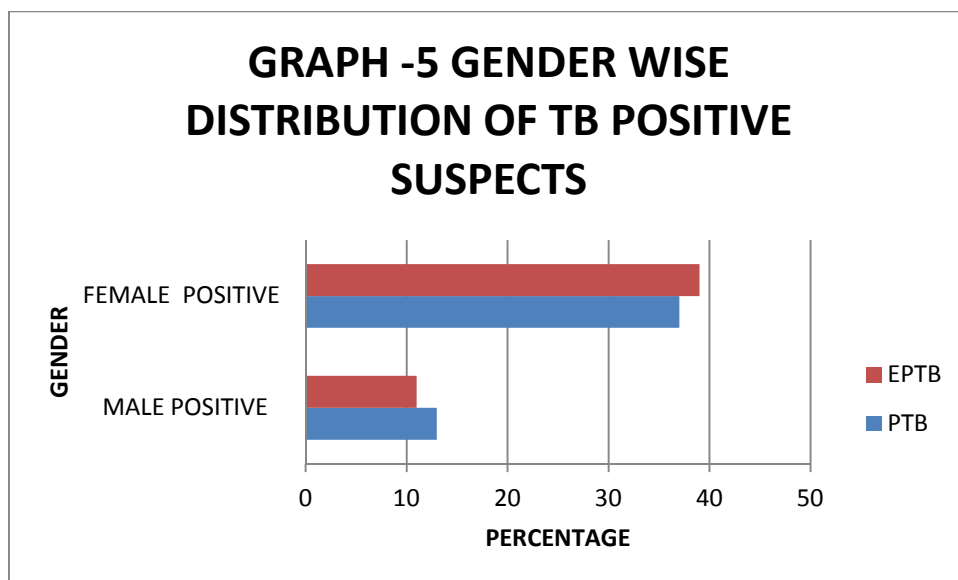
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213 **4.2 GENDER** - Overall the female to male ratio was 1.1. In PTB being 1.05 and
214 EPTB being 1.17. Significant variation in disease prevalence was noticed with
215 reference to gender. Majority of the samples received for processing were from
216 females accounting to one hundred twenty or 53%, with pulmonary being 17.6% and
217 extrapulmonary being 35.4 % respectively. Moreover, in laboratory confirmed
218 tuberculosis cases too females accounted for majority with the figure as 76% of the
219 total culture positive cases. The distribution being 36.9% for PTB and 39.1% for
220 EPTB as shown in graph 5.

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223 Therefore our findings suggest that whether pulmonary or extrapulmonary form of
224 disease females are at the greater risk of acquiring the disease with a statistically
225 significant two tailed P value of 0.008 calculated using Chi squared T test of Yates

226 **4. DISCUSSION**

227 Tuberculosis after HIV is the next important killer worldwide [3]. In spite of the global
228 efforts to contain its spread it remains a major threat to public health. In the present
229 study the overall disease prevalence is noted as 25 % which is consistent with the
230 reports of Surendar K & Sharma et al [8]. PTB accounted for 10% and EPTB for 15
231 %. Earlier reports were suggestive of PTB being more common than EPTB in India
232 [9-10]; but our study shows a change in the trend with EPTB exceeding PTB in
233 proportion. The reasons for which could be improved diagnostic facilities and
234 presence of latent infection in the society which later in life manifest as
235 extrapulmonary form of disease. EPTB though being detected in increasing number
236 of suspects it remains the most neglected form of disease for reasons like vague
237 symptomatology, less accessible nature of the sample that has to be collected by an
238 expert using invasive procedure and which cannot be repeated. In the last but not
239 the least is the paucibacillary nature of the disease which increases the chances of
240 false negative results [11-13]. On the contrary PTB has received much attention for
241 reasons like its highly contagious nature, overt symptoms of the disease and ease in
242 diagnosis [14]. The sample can be easily accessed and repeated if required.

243 Detection of mycobacteria in smear and culture is taken definitive diagnosis of
244 tuberculosis. Direct microscopy is considered as the most feasible, economical and
245 rapid technique in resource poor settings as mentioned by Collins CH et al 2014
246 [15]. The sensitivity of ZN stain is questionable and is influenced by several factors.
247 Nolte FS et al have reported it to be in the range of 22-80% [16]. Siddiqui et al and
248 Apers et al reported it to be as 60%, [17-18]. Toman et al 2005 [19] found it to be
249 less sensitive with values of 22 -43% while using a single smear. In the present
250 study it was found to be 56. 52 % sensitive and 93.96 % specific which correlates
251 well with the findings of 51% and 100% by Ziaee M et al 2008 , 48% and 99.64% by
252 KP Kaur et al 2011 and 61% by Ulukanligil M et al 2000 [20-22]. Anyhow, the
253 sensitivity of the direct smear is always less when compared to culture as noticed in
254 our study as 17% and 20% respectively. Further attaching significance to the direct
255 microscopy results alone is a debated topic because U Y et al 2008 and Kumar A et
256 al 2014 has found that 55% of the smear positive samples are negative by culture
257 and 30% of culture positive samples are negative by smear [23-24]. Therefore it is
258 always better to go for a combination of two methods.

259 Isolation of mycobacteria in culture is taken as the gold standard technique for
260 establishing diagnosis of tuberculosis. Culture was positive in 20% of the suspects
261 The conventional LJ media in use since decades, in the present study showed a
262 sensitivity of 56% which is consistent with the reports of KP Kaur et al of 58%, 59%
263 by LU de et al and 60% and Idigoras et al [20,25-26]. The use of liquid culture media
264 for isolation of Mycobacterium has not only enhanced the yield substantially but
265 even has reduced the recovery time from 6-8 weeks on conventional media to 2-3
266 weeks in liquid media. In the present study MGIT was found to be 78% sensitive
267 while other have reported a sensitivity of 93% KP Kaur et al and Badak F et al. SS
268 Negi et al 2005 found it to be less sensitive as 55.8% [20, 27-28]. Our study showed
269 that recovery of mycobacterium using MGIT 320 system from BD US has helped us
270 in detecting majority of the positive samples almost 70% with the shortest
271 turnaround time for smear positive samples as 9- 12 days. And in smear negative
272 samples as 15-30 days which is consistent with the findings of Bungar et al and
273 Rodrigues et al [29-30]. The added advantage being the drug susceptibility results is
274 available in less than twelve days time after recovery of mycobacteria in culture.

275 Therefore these newer techniques expedite the time needed in diagnosis and
276 initiation of anti tuberculosis treatment which is of prime significance. Moreover, it
277 allows the clinician to select the most appropriate drug without any apprehension of
278 drug resistance related issues. Therefore we can substantiate that use of liquid
279 media and automated Tb culture system together like, MGIT 320 is most suitable
280 and economical for diagnosis of tuberculosis in resource poor settings with high
281 tuberculosis burden.

282 In the present study an overall drug resistance of 4.4% was noted. More number of
283 cases as seen 2.6% were from pulmonary form of disease than from EPTB as 1.76
284 %. Mono drug resistance and MDR each accounted for 2.21%. Further it was
285 observed that both monodrug resistance and MDR were high in newly diagnosed
286 PTB cases accounting for 1.33% each than in EPTB accounting for 0.88% each.
287 This is in accordance with the report by Surrender K et al 2013 [8].

288 The influence of various demographic factors studied like age and sex on the
289 disease and its type showed that female's outnumbered males both in the clinical
290 suspects and laboratory confirmed case of disease with a percentage of 53% and
291 76 % respectively which is consistent with the findings of [9, 31-32]. The female to
292 male ratio being 1.05 in PTB and 1.17 in EPTB which is consistent with the report of
293 Othman GQ et al of 1.2 and 1.6 respectively [31]. As mentioned in previous studies
294 females were the most vulnerable to the disease at a young age of 6-35 years than
295 males both in pulmonary and extrapulmonary form of disease for reasons
296 unexplained same has been observed in our study too [9, 31-35]. On the other hand
297 the most sensitive age for males in PTB in our study was 36-50 years which is
298 consistent with the report by [9, 10, 30, 35].

299 The existing data on EPTB shows that the disease is more common in females
300 Yang Z et al and Gunal S. et al [23, 25, 36-37] and in young age group between 6-
301 35 years of age as reported by Neortjojo et al, Hopewell et al, Musellim et al and
302 Ilgazli et al [35, 38-41]. LNTB { Lymphnode tuberculosis } 50% was the most
303 common type followed by pleura then bone and joint, abdominal, pus and
304 genitourinary in the last. Further, in LNTB cervical LNTB 92% is the most
305 commonest as reported by Sree ram reddy CT et al, Neortjojo et al, Hope well et al
306 and Eric Walter et al [9, 35, 38, 41, 42]

307 In the present study biochemical findings for diagnosis of extrapulmonary
308 tuberculosis could not correlate with microscopy and culture results. The sensitivity
309 of histopathology and cytology results is variable. In the present study it was found
310 to be positive in 67% of the samples with a sensitivity and specificity of 76% and
311 70% similar results were reported by Y. Zenebe et al [43]. Brijesh T et al reported it
312 to be as 62% sensitive and Yogesh M et al as 76.6% [44, 45]. But only thirty five
313 percent of which has given consistent results with culture. Others have reported it
314 to be 80.5% by Noertjojo K et al and 53.2% by Oztop A et al, [35, 46].

315 Therefore diagnosis of extrapulmonary form of disease must always be done using
316 a combination of different methods with strong radiological and clinical findings as
317 per WHO guidelines [47]. Diagnosis of EPTB based on the results of a single test
318 alone may either lead to over diagnosis or under diagnosis of the disease both of
319 which are of significant.

320

321 **5. CONCLUSION**

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323 The overall disease prevalence noted is 25% with extra pulmonary form being more
324 common than pulmonary form of disease. For reasons stated but not established
325 the disease remains more common in female gender and the economically active
326 and reproductive age group. Is it a mere an environmental factor which is acting as
327 a predisposing agent or is there any underlying genetic association or are there any
328 microbial factors related to it? Further studies with focus on these aspects are
329 needed to apply a target approach towards disease control.

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ABBREVIATIONS

RNTCP – Revised National Tuberculosis control program

NSP- National Strategic Plan

TB- Tuberculosis

469 **PTB**- Pulmonary tuberculosis
470 **EPTB** – Extrapulmonary tuberculosis
471 **BAL** - Broncho alveolar lavage
472 **MGIT**- Mycobacteria growth indicator tube
473 **LJ Media** – Lowenstein Jensen media
474 **ZN**- Ziehl Neelsen
475 **FNA** – Fine needle aspiration
476 **LNTB** – Lymph node tuberculosis
477 **SIRE KIT**– Streptomycin, Isoniazid, Rifampicin and Ethambutol.
478 **MDR** – Multi drug resistant
479 **XDR** – Extensively drug resistant
480 **WHO**- World Health Organization
481 **CDC** – Centers for Disease Control Atlanta.
482 **APPENDIX**
483 **TITLE**
484 **ABSTRACT**
485 **MANUSCRIPT**
486 **INTRODUCTION**
487 **AIM**
488 **MATERIALANDMETHODS**
489 **RESULTS**
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