

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

INTRODUCTION - The revised national tuberculosis control program of India has been able to reduce the burden of disease significantly. Still tuberculosis continues to affect a major chunk of the society. To achieve the desired target of national strategic plan 2012-2017 we need to have a focused approach on the disease prevalence and 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 – the disease was microbiologically confirmed in 20% of the suspects. Pulmonary tuberculosis was the predominant type with 28% of the cases followed by 16% for extrapulmonary disease. Overall microscopy by Ziehl Neelsen technique was positive in 18%. Cytology was positive in 67% of the suspects. Biochemical findings were insignificant. Drug resistance was noted in 22% of the isolates of M.tuberculosis. Drug resistance and MDR tuberculosis was more common in pulmonary samples with figures of 27% and 60% than in extrapulmonary 11% and 40%. Females predominated both in the suspect's as 53% and culture confirmed as 76% tuberculosis cases in both pulmonary as 77% and in extrapulmonary as 72%. 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 in men.

CONCLUSION – Our study opens In future further research focusing on as to why the female gender, reproductive and economically active age group is the most

vulnerable has to be carried out. Is it mere an environmental factor?

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

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Right from the time of inception of the National Tuberculosis 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 prevalence of disease 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 has been reduced from 216 / lakh population in 1990 to 176/ lakh population in 2012. Therefore with this great success the RNTCP program 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]. Still almost one third of the world’s population is affected with tuberculosis [2].The global burden of tuberculosis is 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]. Because of its highly contagious nature and the morbidity and mortality associated; the goal of 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 ceases to be a major public health problem in India. The objective of TB {Tuberculosis} control Programme 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]. Another important aspect of the disease which makes it more threatening is its drug resistance nature which is spreading at a great pace in the society. To overcome this issue the government of India with its PMDT {Programmatic

management of drug resistant TB} services has achieved to date screening of 3.25 million multi drug resistant suspects, diagnosed more than 45000 drug resistant tuberculosis cases and initiated treatment for more than 42000 drug resistant tuberculosis cases with the help of 93 drug resistant TB centers [1]. With such an exhaustive data and huge success of TB control program in India we in our simplest efforts have tried to estimate the burden of the disease in non HIV patients at a tertiary care hospital in south India.

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

2. MATERIAL AND METHODS

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

A cross sectional study was carried out From Jan. 2013- Jan. 2015. About two hundred and twenty six samples submitted for suspicion of pulmonary and extra pulmonary form disease were processed for laboratory diagnosis in central laboratories of Princess Esra Hospital Hyderabad .Samples for diagnosis of EPTB { extrapulmonary tuberculosis} were obtained under aseptic techniques by a specialist. In PTB {Pulmonary tuberculosis} BAL { Broncho alveolar lavage} fluid was collected under aseptic precautions by a trained pulmonologist. Sputum samples were collected as per the CDC guidelines under the RNTCP India. Samples were processed by microscopy, culture, biochemical analysis, histopathology and cytology as per the request of the clinician. Microscopy was done using the conventional Ziehl Neelsen [ZN] method for both pulmonary and extra pulmonary samples after concentration by NALC {N acetyl L Cysteine - NAOH} method .Culture was performed using a combination of two different methods solid and liquid as per the WHO guidelines. The conventional solid media used is Lowenstein Jensen media from HI Media Laboratories and the liquid broth media Middle Brook 7H9 from BD MGIT {Mycobacteria growth indicator Tube} 320 systems. Identification as Mycobacterium tuberculosis was achieved using the rapid commercially available test MPT64 Mycobacterial protein 64 KD Antigen test from SD Bioline for M.tb complex {Mycobacterium tuberculosis complex}. 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 Kubica and Kent [6] and as per the manufacturer instructions. HIV testing for HIV 1&2 virus if required was done by rapid test procedure named Tridot Assay with the sensitivity and specificity of 99% commercially available from J.Mitra Co.

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

Was achieved by following the manufacturer instruction 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.

2.3.1 BIOCHEMICAL 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.

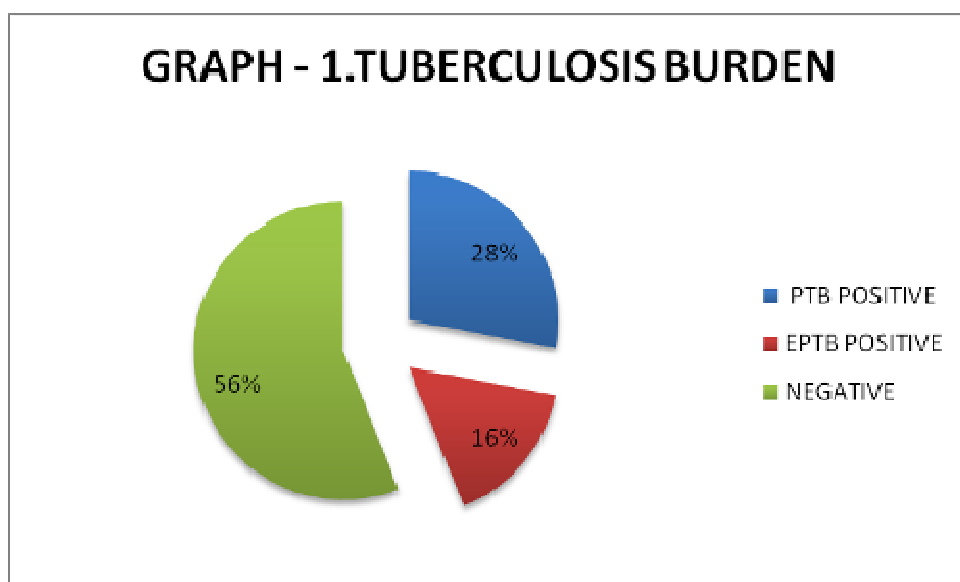
2.3.2 DATA ANALYSIS

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

3. RESULTS

3.1 MICROBIOLOGY RESULTS

109 Of the total two hundred and twenty six samples submitted to the microbiology
 110 laboratory for diagnosis of tuberculosis. Seventy eight [34.5%] were from pulmonary
 111 site and one hundred and forty eight [65.5%] from extrapulmonary site of the
 112 disease. Overall tuberculosis was confirmed by culture in forty six or (20%) of the
 113 samples. Pulmonary form of disease was the commonest with twenty two or [28%]
 114 positive cases and twenty four or [16%] of cases from extrapulmonary form of the
 115 disease. The remaining one hundred eighty samples were negative for both as
 116 shown in graph 1. None of the patients were reactive for HIV 1&2.

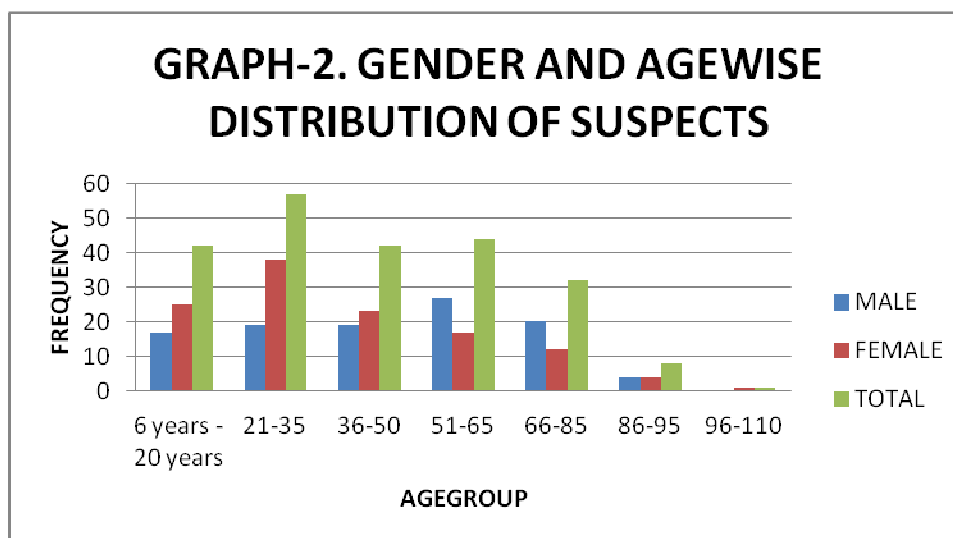


117
 118

119 Direct microscopy using ZN stain was positive for forty one (18%) of the samples.
 120 Microscopy alone was positive in twenty three (10%) of the specimens. Microscopy
 121 together with culture could detect tuberculosis in eighteen (8%) of the specimens.
 122 Distribution of samples based on site of involvement showed that of seventy eight
 123 pulmonary samples received for processing twenty one was BAL fluid, two lung
 124 biopsies and rest sputum. The various extrapulmonary samples received for
 125 processing were seventy three as pleural fluids , thirty nine as lymphnodes
 126 biopsies or fine needle aspirates, seventeen as gastrointestinal, eleven from bone
 127 and joints, five as pus and three as genitourinary respectively.
 128 Significant variation in sex and age distribution was noticed with reference to the
 129 clinical type of tuberculosis in suspects. Gender wise majority of the samples, one
 130 hundred twenty were from females followed by one hundred and six from males

131 Females predominated in the clinical suspects with figures of 51% in pulmonary
132 form and 54% in extrapulmonary form of disease. Overall the female to male ratio
133 was 1.1. Clinical wise in PTB the ratio was 1.05 and in EPTB as 2.0. Therefore it is
134 evident that the number of female suspects is exactly the double of males as far as
135 EPTB is concerned.

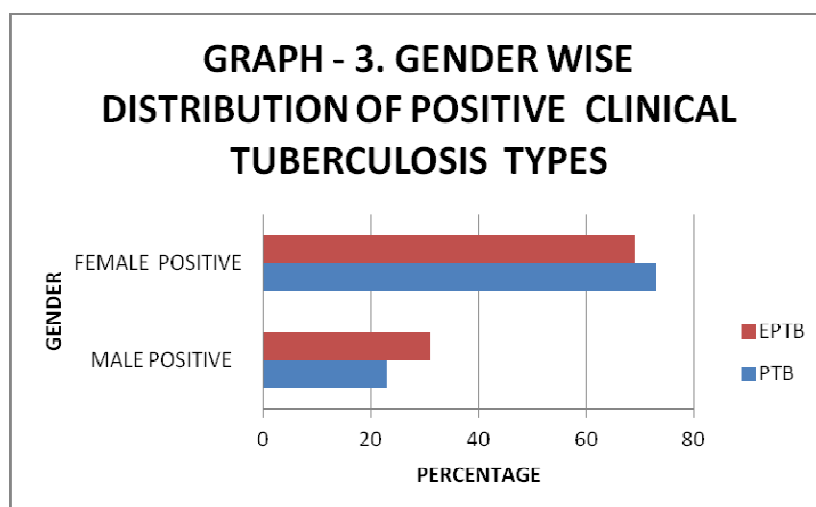
136 Further with respect to age it is observed that nearly half (44%) of the samples i.e.
137 around hundred submitted for processing were in the age group 6-35 years of age
138 as shown in graph 2 below.



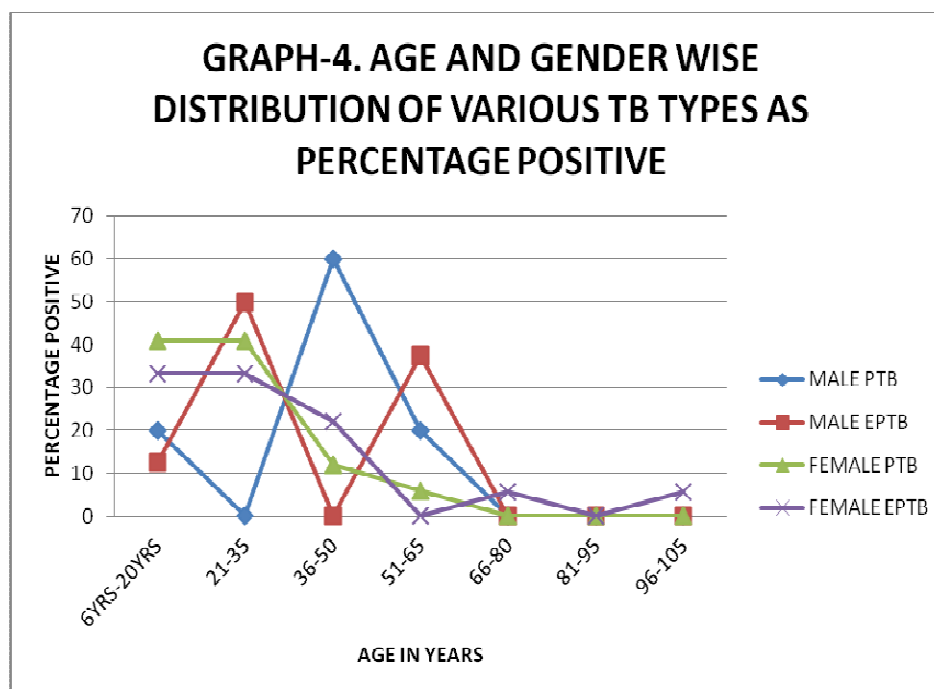
139 It makes clear that the clinical suspects are more in the reproductive and
140 economically active age group.
141

142 The overall mean age for the suspects in the study for males was 48.17 ± 22.26
143 and for females 39.45 ± 21.08 . Further, we have noticed that there was a significant
144 variation in the mean age of the suspects with respect to the clinical type of
145 tuberculosis and sex. Especially in pulmonary form of the disease as noted the
146 mean age for females was 36.07 ± 11.56 and for males 51.60 ± 14.02 with the two
147 tailed P value of 0.002 when determined using Chi squared T test by Yates. In extra
148 pulmonary form of disease no such variation was observed the mean age being
149 41.13 ± 16.91 for females and 46.26 ± 15.68 for males.

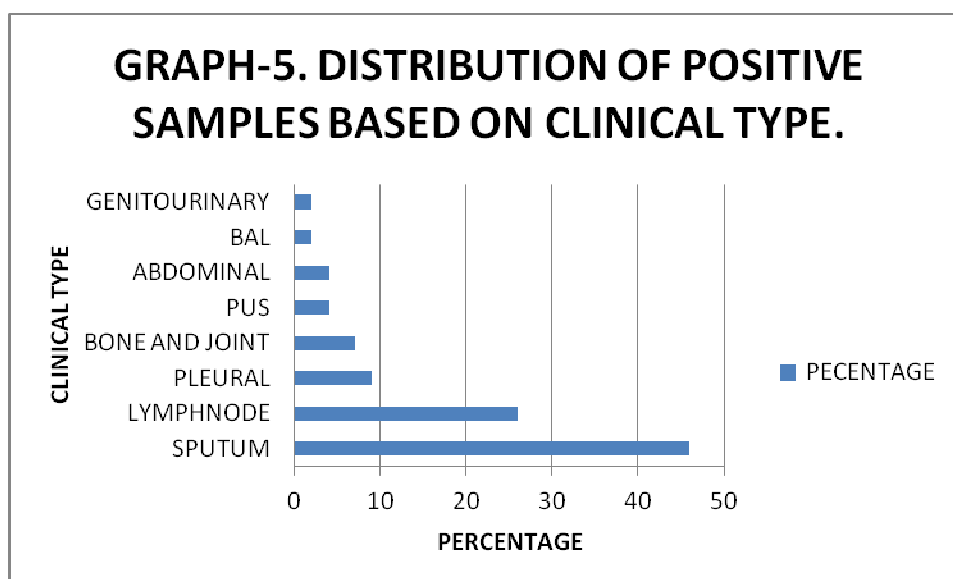
150 In laboratory confirmed tuberculosis cases again females dominated for both
151 pulmonary and extra pulmonary form of disease accounting to 35 /46 total culture
152 positive cases with a distribution of as follows 17/22 from the PTB and 18/24 from
153 the EPTB cases as shown in graph 3. .



154 Therefore whether pulmonary or extrapulmonary form of disease females are at the
 155 greater risk of acquiring the disease with a statistically significant two tailed P value
 156 of 0.008 calculated using Chi squared T test of Yates
 157
 158 Age wise distribution of culture confirmed tuberculosis cases revealed a difference
 159 in the predisposing age group for the two forms of tuberculosis with regards to
 160 gender. It was noted that in extra pulmonary tuberculosis for both the sexes 70 % of
 161 the positive cases were in the age group 6-35 years of age. But with reference to
 162 pulmonary form of disease there was a significant difference in the predisposing age
 163 as majority of the positive cases in males were seen in the age group 36-50 years.
 164 Whereas for females it was the same age group 6- 35 years as the most sensitive
 165 one as shown in graph 4.



166 M. tuberculosis complex was confirmed by using the rapid identification test
 167 provided from SD Bioline from Germany in all forty six isolates. In the present study
 168 the sensitivity of the two different culture methods used showed that MGIT alone
 169 was positive for thirty two (70%) samples. LJ alone was positive for three (6%) of
 170 the specimens. LJ and MGIT together were positive for eleven (24%) of the
 171 samples.
 172
 173 The distribution of culture positive isolates showed that 22/46 or 48.9% of the
 174 isolates were from pulmonary form of disease and the remaining 52.1 % from
 175 extrapulmonary disease. Maximum of isolates 46% were from sputum sample and
 176 the least from BAL.
 177 In the present study EPTB isolates accounted for 24/46 or 51.1% of the total. Their
 178 distribution was as follows twelve were lymphnodes, four pleural fluids, three
 179 samples from bone and joint, two each from gastrointestinal tract and pus and one
 180 from genitourinary tract as shown in Graph -5 below.



181
182 The most common site for EPTB was lymph node accounting for 50% followed by
183 pleura accounting for 16.6%, bone and joint accounting for 12.5% then pus and
184 gastrointestinal each accounting for 8.3% and in the last genitor urinary accounting
185 for 4% of the total 24 isolates of EPTB respectively. Moreover, in the lymph node
186 tuberculosis 91.1% of the cases were from cervical lymphnode followed by axillary.

187 The drug susceptibility pattern of the 46 isolates of *M. tuberculosis* showed that
188 overall ten strains 22% were resistant to the first line anti- tuberculous drugs used in
189 management of the disease. Mono resistance was seen in five isolates i.e. 11%.
190 Multidrug resistant [MDR] strains of *Mycobacterium* were noticed in another 11% of
191 the total isolates. MDR was more common in PTB than EPTB. The details of which
192 are shown in Table 1.

193

194 Table 1. Drug suseptibilty pattern of the various *M. tuberculosis* isolates done using
195 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%)

3.2 BIOCHEMICAL RESULTS

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

3.3 HISTOPATHOLOGY AND CYTOLOGY RESULTS

Of the total 148 specimen submitted for cytological diagnosis of extra pulmonary tuberculosis in pathology laboratory during the study period, seventy three were pleural fluids, thirty nine lymph node material obtained by FNA, seventeen were peritoneal fluids, five were pus samples, eleven were from bone and joint and three genitourinary. Cytology was suggestive of tuberculosis in 99 [67 %]. Forty six percent of the specimens were positive by both cytology and culture. Smear and cytology was positive for forty one [41%]. Another twelve percent were only cytology positive. Remaining 49 percent were negative by both.

4. DISCUSSION

Tuberculosis after HIV is the next important killer worldwide [3]. In spite of the global efforts to contain its spread it remains a major threat to public health. Pulmonary form of the disease continues to be the most common followed by extra pulmonary and receives great attention due to its highly contagious nature [8]. PTB can be diagnosed without any difficulty because of the ease in the accessibility and availability of the sample. On the contrary EPTB remains the most neglected form of disease for reasons like vague symptomatology, less accessible nature of the sample that has to be collected by an expert using invasive procedure moreover which cannot be repeated and the paucibacillary nature of the disease which increases the chances of false negative results [9, 10].In the present study we have tried to assess the burden of tuberculosis in a tertiary care hospital in Hyderabad. Our findings show that overall twenty percent of the samples received for laboratory

diagnosis by culture and microscopy were positive for tuberculosis. The prevalence of pulmonary form of disease is higher 28% than the extrapulmonary form 16% consistent with the reports by Sree ram reddy CT et al, Selma AG et al and SK Sharma et al [11-13]. Further, it is noticed that females outnumbered males both in the clinical suspects for various forms of tuberculosis and laboratory confirmed case of disease with a percentage of 53% and 76 % respectively [11, 14-15].

Females were more vulnerable to acquiring disease at a younger age group of 6-35 years than males both in pulmonary and extrapulmonary form of disease for several unexplained reasons [11,14-16]. On the other hand the most sensitive age for males in PTB was 36-50 years [11].

The existing data on EPTB shows that the disease is more common in females Yang Z et al and Gunal S. Et al [11, 15, 17-21, 22-23] and in young age group between 6-35 years of age Weir et al, Hopewell et al Musellim et al Ilgazli et al [24-28] . LNTB { Lymphnode tuberculosis } 50% was the most common type followed by pleura then bone and joint, abdominal , pus and genitourinary in the last. Further, in LNTB cervical LNTB 92% is the most commonest as reported by Sree ram reddy CT et al, Weir et al, Hope well et al and Eric Walter et al [11,24,25,28]

Diagnosis of tuberculosis by direct microscopy is considered as the most feasible and rapid technique in resource poor settings by several authors Heubner et al and Aber VR et al [29-30]. The results of which are always dependent on skilled hands and quality of the specimen submitted to the laboratory. The sensitivity of which is variable some have reported it to be between 22-80% Nolte FS et al [31] and others 60% Siddiqui et al and Apers et al [32-33] . Others have reported it to be less sensitive with values of 22 -43% while using single smear Toman et al 2005 [34] . In the present study it is 18 %. Anyhow, the sensitivity of the direct smear is always less when compared to culture as culture positive are in our study are 20%.

Attaching significance to the direct microscopy results again is influenced by several factors as it is noted that 55% of the smear positive samples are negative by culture and 30% of culture positive samples are negative by smear U Y et al 1988 and Bass et al [35-36]. Hence a combination of different methods with strong clinical suspicion helps in decision making.

258 Bacteriological yield of Mycobacteria was more in pulmonary samples than in
 259 extrapulmonary which is consistent with the report of [31, 37]. Our study showed
 260 that recovery of mycobacterium using MGIT 320 system from BD US has helped us
 261 in detecting majority of the samples almost 70% with the shortest turnaround time
 262 for smear positive samples as 5- 9 days. And in smear negative samples 15-30
 263 days which is consistent with the findings of Bungar et al and Rodrigues et al [38-
 264 39]. MGIT together with LJ media can detect 24% of the samples and LJ alone 6%.
 265 Hence it is evident that use of liquid media and automated Tb culture system like
 266 MGIT 320 is most suitable and economical for diagnosis of tuberculosis in resource
 267 poor settings with high tuberculosis burden. The drug susceptibility results are also
 268 available in a minimum period of twelve days time after recovery of Mycobacteria in
 269 culture which helps the clinician in selecting appropriate drugs for management of
 270 the disease. Therefore these newer techniques expedite the time needed in
 271 diagnosis and initiation of anti tuberculous treatment which is of significance.

272 In the present study an overall drug resistance of 22% was detected. More number
 273 of isolates [27%] were from pulmonary form of disease than from EPTB [17%]. Sethi
 274 S et al has reported it as 20.9% for pulmonary and 11.8% in extrapulmonary in his
 275 study [40]. Of the total 10 drug resistant strains 11% of the strains were showing
 276 mono resistance to one of the first line anti tuberculosis drugs and another 11 %
 277 were MDR Tb bacteria. Of which majority 60% of the isolates were from PTB and
 278 rest from EPTB samples 40%. Parmasivan et al has reported MDR prevalence to
 279 vary from 25-100% [41].

280 In the present study biochemical findings for diagnosis of extrapulmonary
 281 tuberculosis could not correlate with microscopy and culture results. The sensitivity
 282 of histopathology and cytology results is variable. As different authors have
 283 reported variable results in our study we have found histopathology and cytology to
 284 be positive in 67% of the specimens similar results are reported by [42]. Out of
 285 which forty six percent of the samples have given consistent results with
 286 microbiological findings but others authors have reported it to be as 53.2% by Oztop
 287 A et al, Noertjojo K et al 70.5% [43-44]. Therefore diagnosis of extrapulmonary form
 288 of disease must always be done using a combination of different methods with
 289 strong radiological and clinical findings as per WHO guidelines [45]. Diagnosis of

290 EPTB based on the results of a single test alone may either lead to over diagnosis
291 or under diagnosis of the disease both of which are of significance in tuberculosis.

292

293 **5. CONCLUSION**

294

295 We conclude saying that pulmonary form of tuberculosis is the commonest followed
296 by extrapulmonary form of disease. For reasons stated but not established the
297 disease remains more common in female gender and the economically active and
298 reproductive age group. Is it a mere environmental factor which is acting as a
299 predisposing agent or is there any underlying genetic association or are there any
300 microbial factors related to it? Further studies with focus on this aspects is needed
301 which may help us in controlling the disease burden and the associated mortality
302 and morbidity.

303

304

305 **REFERENCES**

306

- 307 1. 1. Tb India 2014 revised national Tb control programme .Annual status
308 report. Central TB Division Directorate General of Health Services, Ministry of
309 Health and Family Welfare, Nirman Bhavan, New Delhi–110108 www.tbcindia.nic.in.
- 310 2 2. Sudhr P, Tendam G, Kochi A.Tuberculosis : a global overview of the
311 situation today.Bull World Health organ 1992,70:149-59.
- 312 3. 3. World health organization Tb fact sheet 2014 update.
313 <http://www.who.int/mediacentre/factsheets/fs104/en/>.
- 314 4. 4. Strategic vision for TB control for the country up to 2015 Goals and
315 Objectives for TB control in India up to 2015.
316 <http://www.tbcindia.nic.in/pdfs/Strategic%20Vision%20for%20the%20country%2005-2015%20%20Final.pdf>.
317
- 318 5. Global tuberculosis control. Surveillance, planning financing .IN WHO Report
319 Geneva .World Health Organization; 2002.

- 320 6. Kent PT, Kubica GP public health Mycobacteriology – a guide for the level III
321 laboratory. Department of health and Human services centres for disease control
322 and prevention. Atlanta 1985. 207
- 323 7. Needham DM, Godfrey Fausett P, Foster SD. Barriers to tuberculosis control in
324 urban Zambia. The economic impact and burden on patients prior to diagnosis .int. j.
325 Tuberc. Lung dis. 1998;2 (10) 811-17.
- 326 8. World Health Organization report. World Health Organization global tuberculosis
327 report.http://www.who.int/tb/publications/global_repot/archive/en/.
- 328 9.Gonzalez OY, Adams G, Teeter LD etal .Extrapulmonary manifestations in a
329 large metropolitan area with low incidence of tuberculosis.Int.J. Tuberc. Lung.
330 Dis.2003; 7:1178-85.
- 331 10.Chakravorthy S, Sen MK, Tyagi JS.Diagnosi of extrapulmonary tuberculosis by
332 smear,culture and PCR using universal sample processing
333 technology.J.ClinMicrobiol2005;43:4357-62.
- 334 11. Sree ram reddy ct, panduru kv, verma sc. Comparison of pulmonary and
335 extrapulmonary tuberculosis in Nepal a hospital based retrospective study.BMC
336 Infectious Dis.2008;8:1471-2334.
- 337 12. Selma AG,Fulsen B, Mehmet F.Evaluation of pulmonary and extrapulmonary
338 tuberculosis in immunocompetetn adults .A retrospective case series analysis.Med
339 Princ. Pract.2015;24:75-79.
- 340 13. Sharma S.K. Mohan A. Extrapulmonary tuberculosis Review Article Indian J
341 Med Res 120 (4), October 2004, pp 316-353.
- 342
- 343 14.Othman GQ, Ibrahim MM Ijham, Raja YA. Comparison of the clinical and
344 sociodemographical factors in pulmonary and extrapulmonary tuberculosis patients
345 in Yemen.J of clin. Diag.Res. 2011; April vol -5(2): 191-195.
- 346 15. Peto HM, Prat RH, Harrington TA. Epidemiology of extrapulmonary tuberculosis
347 in the United States, 1993- 2006.clin.infect. Dis. 2009; 49:1350-7.
- 348 16. Ullah S, Shah SH, Rehman AU. Extrapulmonary tuberculosis in aldy reading
349 hospital Peshawar.nwfp,Pakistan : survey of biopsy results .J.Ayub Med. Coll.
350 Abottabad 2008;20(2) 43-45.

- 351 17. Fanning A, tuberculosis 6 Extrapulmonary disease CMAJ/ 1999;160 (11):1597-
352 603.
- 353 18.Report from Med. Res. Council [ICMR] tuberculosis and chest disease unit.
354 National survey of tuberculosis notifications in England and wales in 1983;
355 characteristics of disease. Tubercle 1988; 68 (1):19-32.
- 356 19.American thoracic society. Diagnsotic standards and classification of tuberculosis
357 in adults and children .Am, j. respir crit care Med. 2000;161 (4 Pt 1):1376-95.
- 358 20.Snider DE, JR Roper WL. The new tuberculosis Engl. J. Med. 1992;326
359 (10):703-705.
- 360 21.Ptichenik AE, Fertel D, Mycobacterial disease; epidemiology diagnosis treatment
361 and prevention. Clin chest med. 1988;9 (3):425-4.
- 362 22. Yang Z, Kong Y,Wilson Fetal. Identification of risk factors for extrapulmonary
363 tuberculosis .Clin.Infect.Dis 2004;38:199-205
- 364 23. Gunal S, Yang Z, AgarwalM. Demographic and microbial characteristics of
365 extrapulmonary tuberculosis cases diagnosed in Malatya .Turkey, 2001-
366 2007.BMC.Public Health;11:154.
- 367 24. Weir MR,Thorton G,F.Extrapulmonary tuberculosis .Experience of a community
368 hospital and review of the literature.Am.J.Med 1985;79:467-78.
- 369 25.HopewellPC, Chaison RE.Tuberculosis and human immunodeficiency virus
370 infection .In Reichman LB,Hershfield ESeds.Tuberculosis .A comprehensive
371 international approach.2nd ed.New Yrok.Marcel Dekker Inc.200:525-552.
- 372 26.Musellim B,Erturan S, Sonnez Dunman E.Comparison of extra pulmonary and
373 pulmonary tuberculosis cases.factors influencing the site of reactivation
374 .Int.J.Tuberc.Lung Dis.2005;9:1220-23.
- 375 27.Ilgazli A,Boyaci H, Basyigit I .Extrapulmonary tuberculosis clinical and
376 epidemiological spectrum of 636 cases .Arch. Med.Res.2004;35:435-41.
- 377 28. Eric Walter PY, Pascal KA, Balkisson AD.Clinical forms and determinants of
378 different locations of Extrapulmonary tuberculosis in an African country.Indian J. of
379 Tuberc.2013;60:107-13.
- 380 29. Heubner RE, Good RC, Tokers JI.Current practices in Microbiology.Results of
381 survey of state public health laboratories .J.Clin.Microbiol 1993; 31(4)771-75.

- 382 30. Aber VR, Allen BW, Mitchison DA. Quality control in tuberculosis bacteriology. 1.
383 Laboratory studies on isolated positive cultures and the efficiency of direct smear
384 examination. *Tubercle*. 1980;61(3):123-33.
- 385 31. Nolte FS, Beverly M. *Mycobacterium*. Murray PR, Baron EJ, Pfaller MA, (Eds).
386 *Manual of clinical Microbiology* 6th ed. ASM Press Washington 1995;400-438.
- 387 32. Siddiqi K, Lambert ML, Walley J. Clinical diagnosis of smear negative pulmonary
388 tuberculosis in low income countries :the current evidence . *Lancet Infect*.
389 *Dis*. 2003;3(5) 288.
- 390 33. Apers L, Mutsvangwa J, Magwenzi J. A comparison of direct microscopy , the
391 concentration method and mycobacteria growth indicator tube for the examination
392 of sputum for acid fast bacilli . *Int. J. Tuberc. Lung Dis*. 2003;7(4):376-81.
- 393 34. Toman K, Freiden T. *Tomans Tuberculosis: case detection treatment and*
394 *monitoring : question and answers . Occupational and environmental*
395 *Med*. 2005;62(1):70.
- 396 35. Uy R, Yu C, Juco M. Chlorox concentration technique for the demonstration of
397 acid fast bacilli in the sputum . *J. Microbiol*. 2008; 17(1):13-18.
- 398 36. Bass J Jr, Farer L, Hopewell P. Diagnostic standards and classification of
399 tuberculosis . *Am. J. Resp. Critical Care Med*. 1990;142: (3) 725-35.
- 400 37. Pehme L, Hollo V, Rahu M et al . Tuberculosis during fundamental societal
401 changes in Estonia with special reference to extrapulmonary manifestations. *Chest*
402 2005; 127:1289-95.
- 403 38. Bunker R, Varsha A, Singh Avneet . Evaluation of BACTEC MGIT with
404 Lowenstein Jensen media for detection of mycobacteria in clinically suspected
405 patients of EPTB in a tertiary care hospital at Mullana Ambala . *Med. Microbiol.*
406 *Diagnosis*. 2013; 2 (123).
- 407 39. Rodrigues C, Shenai S, Sadani M. Evaluation of the BACTEC MGIT 960 TB
408 system for recovery and identification of *Mycobacterium tuberculosis* complex in
409 a high throughput tertiary care centre . *Ind. J. Microbiol*. 2009 Jul-Sep;27(3):217-21.
- 410 40. Sunil Sethi, Manisha Biswal, Shiv Shekhar Chatterjee. Susceptibility pattern
411 among pulmonary and extrapulmonary isolates of *Mycobacterium tuberculosis* in
412 north India. *African J. of Microbiol. Res*. 2012;vol 6(15) 3696-99.

- 413 41. Parmasivan CN. An overview of drug resistant tuberculosis in India. Ind. J. T
414 uberc. 1998; :45:73-81.
- 415 42. Zenebe Y, Belay A, Wogahta T. Smear positive extrapulmonary tuberculosis
416 disease at university of Gondar hospital northwest Ethiopia. Bio Med Central. 2013;
417 VOL.6:21.
- 418 43. Oztop A, Unsal I, Gunay T. An analysis of adult patients with pulmonary and
419 extrapulmonary tuberculosis .Turk.J.Med Sci.2009;39:(5):725-32.
- 420 44. Noertjojo K, Tam CM, Chan SL. Extrapulmonary and pulmonary tuberculosis in
421 Hong Kong. The Int. J. Tuberc. Lung. Dis. 2002;6:879-86.
- 422 45. World health organization guidelines for tuberculosis diagnosis
423 <http://www.who.int/tb/research/retooling/en/>.

424 **ABBREVIATIONS**

- 425 **RNTCP** – Revised National Tuberculosis program
- 426 **NSP**- National Strategic Plan
- 427 **Tb**- Tuberculosis
- 428 **PTB**- Pulmonary tuberculosis
- 429 **EPTB** – Extrapulmonary tuberculosis
- 430 **BAL** - Broncho alveolar lavage
- 431 **MGIT**- Mycobacteria growth indicator tube
- 432 **LJ Media** – Lowenstein Jenson media
- 433 **ZN**- Ziehl Neelsen
- 434 **FNA** – Fine needle aspiration
- 435 **LNTB** – Lymph node tuberculosis
- 436 **SIRE KIT**– Streptomycin, Isoniazid, Rifampicin and Ethambutol.
- 437 **MDR** – Multi drug resistant
- 438 **WHO**- World Health Organization
- 439 **CDC** – Centers for Disease Control Atlanta.

440 **APPENDIX**
441 **TITLE**
442 **ABSTRACT**
443 **MANUSCRIPT**
444 **INTRODUCTION**
445 **AIM**
446 **MATERIALANDMETHODS**
447 **RESULTS**
448 **DISCUSSION**
449 **CONCLUSION**
450 **ACKNOWLEDGMENT**
451 **COMPETING INTEREST**
452 **AUTHORS CONTRIBUTION**
453 **ETHICAL CLEARANCE**
454 **REFERENCES**