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Distribution and antibiotic sensitivity pattern of *Mycobacterium tuberculosis* isolates from children, enrolled in a tertiary care hospital

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Abstract: The present study was planned to assess the distribution of tuberculosis in children and evaluate the antimycobacterial sensitivity pattern of *Mycobacterium tuberculosis* (MTB) isolates from pediatric patients. A total number of 1718 pediatric patients suspected of Mycobacterium tuberculosis were enrolled in the Institute of Child Health and Children's Hospital, Lahore during 2016-17. Out of 1718, only 710 different types of samples were tested for MTB. The samples were processed using bacteriology and GeneXpert along with the chest X-ray and clinical picture of the patients. The sensitivity pattern of Streptomycin, Isoniazid, Rifampicin and Ethambutol (SIRE) was determined using BACTEC MGIT 960. Total patients were divided into four groups including group A (birth to 12 months), B (1 to 5 years), C (6 to 10 years), and D (11 to 15 years). Out of 710, 106 (55 females and 51 males) were declared positive and 604 negative for tuberculosis. Out of 106 positive cases, 89 (83.96%) were sensitive to Rifampicin and 17 (16.04%) were resistant. Only, 04 (3.77%) were resistant to both Rifampicin and Isoniazid and declared as multidrug-resistant (MDR). It was concluded that children of age 11 to 15 years were more prone to MTB and a minimum percentage of MDR isolates was recorded in age group A (birth to 12 months).

Keywords: Childhood tuberculosis, mycobacterium tuberculosis, multidrug resistance, antibiotics sensitivity and resistance, tertiary care hospital.

INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* and is included in the top 10 leading causes of death in humans (Hershkovitz *et al*., 2015). Although, there are government-run programs to control tuberculosis in almost every country, the nationwide burden of TB remains the problem for the public (Chao *et al*., 2014). Every year, a global report on TB is published by World Health Organization (WHO) since 1997 which provides up to date and comprehensive assessment of TB over the world regarding TB epidemic, diagnosis, treatment and prevention. This report is prepared based on the country, regional, and global levels. Pakistan is among the top 20 countries where TB has gained a high and dangerous level with the highest number of TB incidents. There were 10 million patients of TB during 2017, including 1.0 million children, 3.2 million women, and 5.8 million men. Childhood TB is mainly caused by adult contact who is a victim of this infectious disease which may be present at home or school (Seddon *et al*., 2021). The TB transmission rate is gradually increasing in TB-endemic areas in the world, due to a large number of patients and delayed diagnosis. Furthermore, children are being affected in most of those regions where TB in adults is not controlled properly which reflects the ongoing transmission of TB in children (Marais, 2011). Two billion population of the world have been estimated for the infection of TB. Tuberculosis was detected in the Pre-dynastic Egyptian skeleton which was 5400 years old and was enough to prove it as an ancient disease.

TB can stimulate many pediatric diseases such as pneumonia, malnutrition, generalized viral, and bacterial infections. There is a need for an accurate diagnosis of TB in Children. Recent estimates suggest that 10-20% of the disease burdens are in the children less than 15 years of age in TB-endemic areas. The children of developing countries are the much higher victim of TB in comparison to developed countries (Marais and Schaaf, 2010). Five to eight percent of deaths of children have been reported in developing countries due to the infectious disease of TB (Kabra *et al*., 2004). In almost all parts of the world, the MDR-TB (Multidrug-resistant Tuberculosis) has been recorded. A small amount of sputum is produced by younger children, so microbiological confirmation is not a rule for the children suffering from TB and 10-15% of mucus samples show acid-fast bacilli (AFB) in

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approximately 70%TB patients but sputum cultures remain negative (Therese *et al*., 2012).

As indicated by WHO the diagnosis of childhood TB is usually based on chest radiography, tuberculin skin test (TST), clinical features, and exposure history. During the curing of MDR TB, the extensive drug-resistant TB (XDR-TB) patients were 8.5% in 2017. The extremely drug-resistant and multidrug-resistant strains are involved in the development and appearance of TB resistant drugs (Violence *et al*., 2009). Inadequate treatment, invalid prescription, non-availability of time for treatment, insufficient drugs administration is the cause of drug-resistant TB. However, the success in cure is very low in extremely drug-resistant TB. In the present study, we are reporting distribution and antibiotic sensitivity patterns of *Mycobacterium tuberculosis* isolates from children enrolled in a tertiary care hospital of Pakistan.

MATERIALS AND METHODS

Study Area

A prospective and observational study was performed for the incidence of Mycobacterium tuberculosis (M. tuberculosis) from the hospitalized and OPD patients coming in the TB clinic at Children's Hospital and Institute of Child Health, Ferozepur Road, Lahore.

Study Population

Pediatric patients suffering from MTB who visited The Children's Hospital and Institute of Child Health,(CH & ICH) Ferozepur Road, Lahore from January 2016 to December 2017 were taken as the population of the study. The total number of patients in both years (2016 and 2017) was 1718 ((male=828 and female=890) who visited the CH & ICH, Lahore. The patients were divided into four age groups including Group A (Birth to 12 Months), B (1 to 5 Years), C (6 to 10 Years), and D (11 to 15 Years).

Sampling

Early morning sputum for three consecutive days or Gastric aspirate or Bronchial wash of the patients of suspected Tuberculosis was collected. Other samples taken were CSF, Lymph node, Pleural fluid, Pericardial effusion, Ascetic fluid and Pus for extra pulmonary TB patients.

Diagnosis

Diagnosis of the MTB was done by using the following methods such as Radiology, Clinical picture, Bacteriology, Gene Xpert, PCR, etc. In the present study, standard anterior-posterior and lateral view X-rays were done and read by two independent experts (Marais *et al*., 2006). Clinical parameters of all patients were documented. Signs and symptoms were chronic cough (persistent cough with or without wheezing for more than 21 days), fever (body temperature >38°C for 14 days and other common causes such as pneumonia were excluded), failure to thrive, feelings of sickness, fatigue, weakness, weight loss and/or night sweats. Ziehl-Neelsen staining was used for the early diagnosis of TB. The samples were confirmed by polymerase chain reaction (PCR) using species-specific primers (Mukherjee *et al*., 2012). Samples showing an amplicon size of 123 bps were considered as positive. The Gene Xpert Dx system (Cepheid, Sunnyvale, CA) was also used for antibiotic sensitivity testing along with BACTEC MGIT 960 for the detection of MDR-MTB isolates.

Ethical approval

All the experimental work has been approved by the Institutional Review Board (IRB)/Ethical Committee of The Children's Hospital & The Institute of Child Health, Lahore under the reference number 02/123 /15.

STATISTICAL ANALYSIS

Data was imported to SPSS version 21 from excel spread sheet and analyzed by using this software. Age and sex were summarized into means and percentages. Two-tailed z-test was also used. P-Values of <0.05 were considered statistically significant.

RESULTS

The percentage of female patients was recorded higher in comparison to male patients as shown in fig. 1. The maximum percentage of TB patients was observed in group C and minimum in group A as presented in fig. 2. Out of a total of 1718, there were 116 (6.75%) TB patients in January for both years (2016 & 2017). The month of July showed a higher percentage of patients in both years as shown in fig. 3. Type wise division was made for TB patients for the years 2016 and 2017. Extra Pulmonary Tuberculosis showed a higher percentage of patients in both years as shown in fig. 4. A total of 569 (Male=267 and Female=302) patients were found involved in Pulmonary TB. The female TB patients in group C were found in a high percentage in 2016 & 2017 registered for pulmonary TB. A total of 230 (Male=134 and Female=186) patients were found involved in Abdominal TB. The female TB patients in group D were found in a higher percentage according to gender-wise distribution. A total of 239 (Male=111 and Female=128) patients were found involved in Lymph Node TB in these two years. The high percentage of Lymph Node TB patients was observed in group C and according to gender-wise female patients in the group C were found in a high percentage. A total of 169 (Male=82 and Female=87) patients were found involved in Spine TB in the years 2016-17. The high percentage of Spine TB patients was recorded in group C and Gender wise, male patients in Group C were found in high percentage. A

total of 342 samples were tested through the smear method in the year 2016. Out of total samples, 32 were positive and 310 were negative. However, through Gene Xpert, 53 were declared positive and 289 as negative.

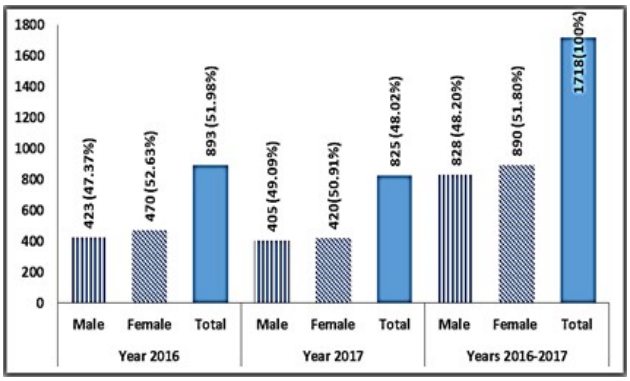

Fig. 1: Gender wise distribution of pediatric patients

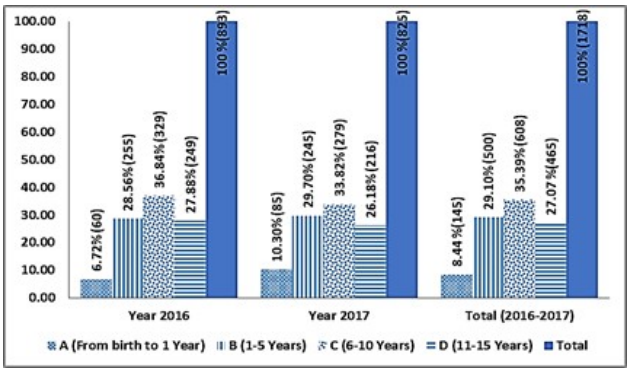

Fig. 2: Age-wise distribution of pediatric patients registered for suspected TB in Children's Hospital, Lahore during the years 2016 and 2017

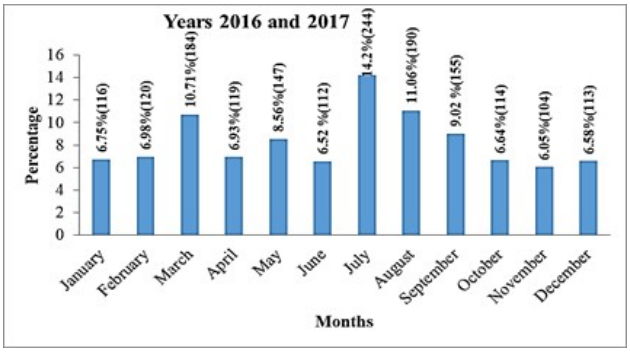

Fig. 3: Month-wise distribution of pediatric patients registered for suspected TB in Children's Hospital, Lahore during 2016 and 2017

In the same way, a total of 368 samples were tested through the smear method in the year 2017. Out of total samples, 30 were positive and 338 were declared negative. However, through Gene Xpert, 53 were declared positive and 289 as negative. Overall, a total of 710 samples were tested through the smear method and GeneXpert in the years 2016-17. Out of total samples, 62 were positive and 648 were negative when tested by the smear method. However, through Gene Xpert, 106 were declared positive and 604 as negative. There was a significant difference between Smear and Gene Xpert results as P-Value was noted 0.0003 or <0.05 obtained through a two-tailed Z test.

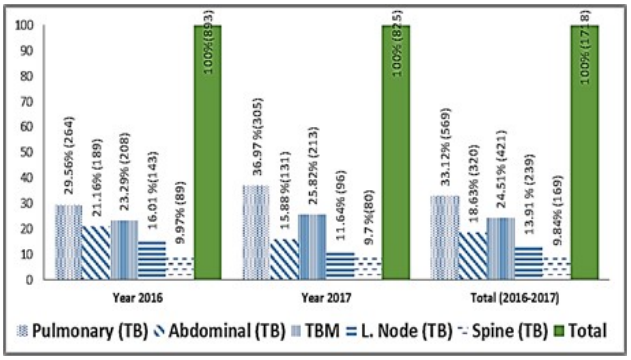

Fig. 4: Type wise distribution of pediatric patients registered for suspected TB in Children's Hospital, Lahore during 2016 and 2017

Table 1: Characteristics of pediatric patients confirmed for tuberculosis (n=106 out of 710 cases tested).

Characteristic	No. of Patients	No. of Positive Cases
Gender		
Male	361	51
Female	349	55
Age of Children		
A (birth to 1year)	47	03
B (1 to 5 years)	136	11
C (6 to 10 years)	268	37
D (11 to 15 years)	259	55
Types of Tuberculosis		
A. Pulmonary	440	68
B. Extrapulmonary	270	38
Sensitivity against Rifampicin		
Sensitive		89
Resistant		17
Sensitivity against SIRE		
Streptomycin	17	17
Isoniazid	17	13
Rifampicin	17	0
Ethambutol	17	17
*MDR Cases		
Resistant to Rifampicin and Isoniazid	4	

*MDR: Multi-Drug Resistant; SIRE: Streptomycin, Isoniazid, Rifampicin, and Ethambutol

DISCUSSION

TB is an infectious leading health problem over the world. It has become an alarming threat to public health especially in developing countries. In the present study gender-wise comparison showed a higher percentage of female patients in both years. The results of the study

supported the previous results (Satyanarayana *et al*., 2010), who recorded 1074 patients in total for TB out of which the ratio of females was recorded high 651 (61%) in comparison to male patients of childhood TB. The present study was carried out at Lahore in the years 2016-2017 and results showed an agreement with the study carried out in Karachi during the years 2008-2010 (Batra *et al*., 2012) that TB was recorded higher in female patients (2.3%) in comparison to males (1.2%). Furthermore, the results of another study indicated that female patients were higher (61.7%) than males (38.3%) The higher infection of TB in females in the present study was also supported by other studies (Ayaz *et al*., 2012), where female TB Patients (33.33%) showed high prevalence in comparison to male patients (30.66%). The high percentage of TB in female patients was due to the close contact of the child with the household. Another report (Laghari *et al*., 2018) has supported the present study where a high percentage of female TB patients of childhood TB was 1199/2167 (55.3%).

The children from the age group of 6 to 10 years may have frequent contact with outside places as well as in house contact. The results of the study were similar to another study (Shah and Uppuluri, 2010) where the clinical profile of tuberculosis in children was reported and the high percentage (46.9%) of TB patients belonged to the age of 5 to 10 years. However, Sarma *et al.* (Sharma *et al*., 2008) reported 1098 childhood TB patients and recorded the highest number of patients in the 11-14 years age group. The age group of 5 to 12 years showed a high percentage of TB 479/1212 (39.5%) in the study of Wu *et al*., whereas other age groups were <1 year, 1 to 4 years, 13 to 18 years were 210 (17.3%), 349 (28.8%) and 174 (14.4%) respectively (Wu *et al*., 2012).

The results of this study showed a close relationship with the results of Ayaz *et al.,* (Ayaz *et al*., 2012) that 17/49 (37.77%) highest prevalence of TB was observed in July. However, in May and June, the prevalence was 34.61% and 25% respectively, reflecting the high prevalence of tuberculosis in spring and summer. The increase in the number of patients of tuberculosis in summer might be due to incensement in outdoor activities, humidity, etc. The results of the present study were very close to the study conducted in Northern India, as the peaked tuberculosis was diagnosed between April and June.

Overall, a higher percentage of 1149 (66.88%) was counted for Extra pulmonary TB and indicated a difference with the other study (Sanches *et al*., 2015) in which out of 386 patients, pulmonary TB 260 (67.4%) was higher in percentage in comparison to Extra pulmonary TB 126 (32.6%). This may be due to the fact that in tertiary care hospital mostly complicated cases are referred. The results of this study can be compared with that of Safdar *et al.* (2010) where a total of 920 cases of childhood TB were registered in three districts of Pakistan. Out of a total of 920, 610 cases were Pulmonary, 202 cases of extra Pulmonary, and 108 cases remained unclassified (Safdar *et al*., 2010). In the study of Maltezouet.el. (Maltezou *et al*., 2000), 102 total children of Extra pulmonary TB were diagnosed and among them lymphadenitis (48), pleural effusion (27), meningitis (16), skeletal TB (5), miliary TB (3), abdominal (2) and pericarditis (1) cases were found. The school children showed a high percentage of meningitis and lymphadenitis. However, the study showed a close relationship with the study in Beijing children hospital (Wu *et al*., 2012), as out of total 1212 patients, TB types were recorded Pulmonary TB 557/1212 (46%) and extra pulmonary 655/1212 (54%). However, out of total 655 extra pulmonary TB patients TBM, DTB, Abdominal TB, Joint TB, TBL and others were 254, 205, 88, 61, 32 and 15 respectively. The results of the present study were in association with previous studies because it was also conducted in tertiary care hospital where complicated cases or the cases which were not diagnosed in primary and secondary health care setups were referred. Besides, pulmonary tuberculosis is easily diagnosed and can be treated in primary and secondary care hospitals.

The present study showed close relation with the results of Khan *et al*. (Khan *et al*., 2018), who reported a 40-50% sensitivity of Gne Xpert more than LED-FM microscopy (Abdelwahab *et al*., 2020). Furthermore, the present study showed agreement with the results where Gene Xpert was a more effective technique in comparison to the ZN technique as detection rate was 62.1% and 41.6% for Gene Xpert and ZN respectively (Chinedum *et al*., 2017). Out of 106/710 positive patients for MTB tested through Gene Xpert, 17 patients were resistant to Rifampicin. Only 04/106 (3.77%) patients were resistant to both Rifampicin and Isoniazid as tested in BACTEC MGIT 960. The results of the present study showed an agreement with the results of Javid *et al*., studied in 2016, as MDR-TB cases were noted at 4.3% in Pakistan. The results of the study also showed a close relationship with the results of the study of Tayyab *et al.,* that MDR-TB patients among various TB types were observed 80% resistance to rifampicin at the highest level.

CONCLUSION

It was concluded that children of age 11 to 15 years were more prone to MTB and MDR- MTB cases were 3.77%.

REFERENCES

Abdelwahab MS, Abdel-Aal FH and Galal SM (2020) The use of GeneXpert Mycobacterium tuberculosis/ rifampicin assay in diagnosis of pulmonary tuberculosis in children. *Curr. Med. Res. Pract.,* **1**(3): 327-331.

Ayaz S, Nosheen T, Khan S, Khan SN, Rubab L and Akhtar M (2012). Pulmonary tuberculosis: Still prevalent in human in Peshawar, Khyber Pakhtunkhwa, Pakistan. *Pak. J. Life. Sci.,* **10**(1): 39-41.

Batra S, Ayaz A, Murtaza A, Ahmad S, Hasan R and Pfau R (2012). Childhood tuberculosis in household contacts of newly diagnosed TB patients. *PLosOne,* **7**(7): e40880.

Chao HH, Guo CH, Huang CB, Chen PC, Li HC, Hsiung DY and Chou YK (2014) Arsenic, cadmium, lead and aluminium concentrations in human milk at early stages of lactation. *Pediatr. Neonatol.,* **55**(2): 127-134.

Chinedum O, Emwiomwan A, Ifeanyi O and Babayi A (2017). Comparative analysis of Ziehl-Neelsen and genexpert techniques for the diagnosis of tuberculosis in human immuno-deficiency virus positive patients in Benin City. *Ann. Clin. Lab. Res.,* **5**(4): 1-6.

Hershkovitz I, Donoghue HD, Minnikin DE, May H, Lee OY-C, Feldman M, Galili E, Spigelman M, Rothschild BM and Bar-Gal GK. (2015) Tuberculosis origin: The Neolithic scenario. *Tuberculosis,* **95**(S): S122-S126.

Kabra S, Lodha R and Seth V (2004) Some current concepts on childhood tuberculosis. *Indian J. Med. Res.,***120**(4): 387-397.

Khan AS, Ali S, Khan MT, Ahmed S, Khattak Y, Irfan M and Sajjad W (2018) Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan. *Braz. J. Microbiol.,* **49**(4): 909-913.

Laghari M, Sulaiman SAS, Khan AH and Memon N (2018) Epidemiology of tuberculosis and treatment outcomes among children in Pakistan: A 5 year retrospective study. *Peer J.,* **6**(1): e5253.

Maltezou H, Spyridis P and Kafetzis D (2000). Extra-pulmonary tuberculosis in children. *Arch. Dis. Child,* **83**(4): 342-346.

Marais B, Hesseling A, Gie R, Schaaf H and Beyers N (2006). The burden of childhood tuberculosis and the accuracy of community-based surveillance data. *Int. J. Tuberc. Lung Dis.,***10**(3): 259-263.

Marais BJ (2011). Childhood tuberculosis: Epidemiology and natural history of disease. *Indian J. Pediatr,* **78**(3): 321-327.

Marais BJ and Schaaf HS (2010). Childhood tuberculosis: an emerging and previously neglected problem. *Infect. Dis. Clin,* **24**(3): 727-749.

Mukherjee A, Lodha R and Kabra S (2012). Recent advances in diagnosis of tuberculosis. *Pediatr. Infect. Dis,* **4**(2): 45-50.

Safdar N, Hinderaker S, Baloch N, Enarson D, Khan M and Morkve O (2010). Diagnosis and outcome of childhood tuberculosis: Implementing public health policy in three districts of Pakistan. *Int. J. Tuberc Lung Dis.,* **14**(7): 872-877.

Sanches I, Carvalho A and Duarte R (2015). Who are the patients with extrapulmonary tuberculosis? *Rev. Port. Pneumol. (English Edition),* **21**(2): 90-93.

Satyanarayana S, Shivashankar R, Vashist RP, Chauhan LS, Chadha SS, Dewan PK, Wares F, Sahu S, Singh V and Wilson NC (2010). Characteristics and programme-defined treatment outcomes among childhood tuberculosis (TB) patients under the national TB programme in Delhi. *PLosOne,* **5**(10): e13338.

Seddon JA, Johnson S, Palmer M, van Der Zalm MM, Lopez-Varela E, Hughes J and Schaaf HS (2021). Multidrug-resistant tuberculosis in children and adolescents: current strategies for prevention and treatment. *Expert. Rev. Respir Med.,* **15**(2):221-237.

Shah I and Uppuluri R (2010). Clinical profile of abdominal tuberculosis in children. *Indian J. Med. Sci.,* **64**(5): 204-209.

Sharma S, Sarin R, Khalid U, Singla N, Sharma P and Behera D (2008). The DOTS strategy for treatment of paediatric pulmonary tuberculosis in South Delhi, India. *Int. J. Tuberc. Lung Dis,* **11**(12): 74-80.

Therese KL, Gayathri R, Balasubramanian S, Natrajan S and Madhavan H (2012). Phenotypic and genotypic characteristics of drug resistance in *Mycobacterium tuberculosis* isolates from pediatric population of Chennai, India. *Indian J. Med. Microbiol,* **30**(4): 411-417

Violence WHODo, Prevention I, Violence WHO, Prevention I and Organization WH (2009). Global status report on road safety: time for action: WHO.

Wu XR, Yin QQ, Jiao AX, Xu BP, Sun L, Jiao WW, Xiao J, Miao Q, Shen C and Liu F (2012). Pediatric tuberculosis at Beijing Children's Hospital: 2002-2010. *Pediatrics,* **130**(6): e1433-e1440.