DOI:10.24996/ijs.2018.59.2C.5

ISSN: 0067-2904

Evaluation of Case detection rates of Pulmonary Tuberculosis before and after adoption of GeneXpert MTB/RIF

Suhad Hadi Mohammed[*1], Mohanad Mohsin Ahmed[2], Ali Mahdi Al Mousawi[3]
[1]Kerbala University, Faculty of Applied Medical Sciences, Department of Clinical Laboratories, Kerbala, Iraq
[2]Kerbala University, Faculty of Medicine, Department of Microbiology, Kerbala, Iraq
[3]Kerbala University, Faculty of Medicine, Department of Community Medicine, Kerbala, Iraq

Abstract

Cities witnessing mass-gathering events, such as Kerbala-Iraq, are peculiar in respect to their needs in controlling tuberculosis. DOTS was implemented in Kerbala almost two decades ago. GeneXpert MTB/RIF assay is adopted in the hope to escalate case detection rates, however, its performance is not evaluated yet. Records of 3254 patients suspected to have pulmonary TB whom they referred to the chest and Respiratory illnesses center of Kerbala governorate were analyzed. The overall trends of TB detection rates showed declining pattern over the year before the adoption of GeneXpert MTB/RIF. In the year of adoption of Xpert MTB/RIF, the detection rates raised, then after declined again. The GeneXpert MTB/RIF has added 17.7% increase in detectionrates.

In conclusion: GeneXpertMTP/RIF usage had strengthen case detection rates especially for smear-negative cases.

Keywords: Tuberculosis, Genexpert, Case detection rates.

تقييم معدلات الكشف عن حالات مرض السل الرئوي قبل وبعد استخدام GeneXpert MTB/RIF

سهاد هادي محمد[*1]، مهند محسن احمد[2]، علي مهدي الموسوي[3]

[1]قسم التحليلات المرضية، كلية العلوم الطبية التطبيقية، جامعة كربلاء، كربلاء، العراق

[2]فرع الاحياء المجهرية، كلية الطب، جامعة كربلاء، كربلاء، العراق

[3]فرع طب المجتمع، كلية الطب، جامعة كربلاء، كربلاء ، العراق.

الخلاصة

أن المدن التي تشهد أحداثا أجتماعية مثل كربلاء – العراق ، تحتاج الى السيطرة على مرض السل بصورة خاصة. تم تطبيق استراتيجية (DOTS)من قبل منظمة الصحة العالمية في كربلاء قبل عقدين تقريبا. كما تم اعتماد الفحص با GeneXpert MTB/RIF أملا في زيادة معدلات اكتشاف الحالات، ومع ذلك، لم يتم تقييم أدائه حتى الان. تم تحليل بيانات 3254 مريضا يشتبه في إصابتهم بالسل الرئوي الذين سجلوا في مركز الامراض الصدرية وأمراض الجهاز التنفسي في محافظة كربلاء. وأظهرت النتائج العامة بأن هناك انخفاض في معدلات الكشف عن الاصابة بالسل قبل اعتماد GeneXpert MTB/RIF وفي السنة التي اعتمد فيها الفحص بGeneXpert MTB/RIF ، ارتفعت معدلات الكشف، ثم تراجعت مرة أخرى. وقد أضاف GeneXpert MTB/RIF زيادة بحوالي 17.7٪ في معدلات الكشف. وفي الاستنتاج، استخدام

*Email: Shm.med.school@gmail.com

GeneXpert MTB/RIF يعزز معدلات الكشف عن الحالات وخصوصا حالات المسحات السالبة بالفحص المجهري.

Introduction

Pulmonary Tuberculosis (PTB) presents as a serious public health problem worldwide. It is the second-highest cause of death among communicable diseases and according to the WHO, 1.8 million deaths and 10.4 million new PTB patients fell ill in 2015 globally [1].

Iraq is one of the countries in the Eastern Mediterranean region which accounts for 25% of the global TB burden in 2014. Its estimated that there are 20000 TB patients and 4000 deaths annually in Iraq which accounts 3% of the total number of cases in the region [1].

PTB has non-specific clinical features, thus diagnosis usually confirmed by laboratory testing. Conventional methods include Ziehl-Neelsen (ZN) staining of sputum samples and culturing of *Mycobacterium tuberculosis* (MTB) bacteria [2, 3] which is important for diagnosing false negative smears and testing for drug resistance [4]. Because culture results take weeks, several new rapid methods were developed during the last decade such as GeneXpert MTB/RIF and other molecular tests [5-7] hoping to improve case detection rates for better PTB control [8, 9].

The GeneXpert MTB/RIF assay is an automated real-time PCR for simultaneous detection of MTB diagnosis and rifampin resistance in less than 2 hours directly from sputum [10, 11]. It is nucleic acid amplification test that does not need specific pre-requites for its set-up and needs little technical training [12]. The GeneXpertMTB/RIF device was introduced by Cepheid in 2004, in 2010, WHO recommended its use for diagnosing PTB in patients with HIV as an initial diagnostic test. Then after the WHO recommendation was extended to test all individuals suspected to have PTB [13]. Since 2014, WHO has gained 3269 GeneXpert MTB/RIF devices for the public sectors in 108 of 145 countries[14]. In Kerbala, GeneXpertMTB/RIF was implemented science 2015.

The aim of the current study was to assess the impact of introducing GeneXpertMTB/RIF assay on detection rates for *Mycobacterium tuberculosis (MTB)* cases.

Materials and methods

The current study is a retrospective record-based study performed in the chest and Respiratory illnesses center of Kerbala governorate. Six years' data from 2010 through 2016 were analyzed. Data were reported using the criteria ofWHO/International Union of Tuberculosis and Lung Diseases (IUTALD) [15].

A total of 3254 subjects suspected to have PTB cases were included. Of which, there were 602 subjects have confirmed PTB by clinical features (which was considered the gold standard for PTB diagnosis) and laboratory finding based on ZN staining, and/ or Genexpert MTB/RIF tests. There were very few PTB cases that confirmed by culturing of the specimen because the center was not accredited to make culture until 2015. However, only 66 subjects were tested by culture technique. Thus the culturing results were not analyzed. The Extra-pulmonary TB subjects and patients who taking treatment for TB were excluded, The recorded data were analyzed using SPSS software version 20.0.

Results

In the current study, records of 3254 patients with signs of PTB whom they referred to the chest and Respiratory illnesses center of Kerbala governorate were analyzed. The studied population includes 1502 females and 1752 males. The study considered the clinical outcome as the gold standard for PTB diagnosis. Out of the 3254 tested subjects, a total of 602(18.5%) were confirmed to have PTB, the others were considered to be non-PTB. Among the PTB cases, 282 were females and 320 were males. The mean age± SD was (44.02 ± 17.23). Three sputum samples from each patient were tested by ZN staining. The number of smear-positive cases was 391 (65%), whereas 211 PTB cases were smear-negative, representing approximately 35% of the total PTB cases. Thus, smear positivity among all attendant to the center was 12.01%.

Among the 3254 referred cases, Genexpert MTB/RIF was available to test 616 cases, whereas, this technique was not available for test the other cases. Among the PTB cases, 110 were tested by Genexpert MTB/RIF, 83 (75.45%) were positive and the GeneXpert MTB/RIF positivity from all tested cases was 13.47% as shown in Table-1.

Table 1-Demographic data

Total attendant (2010-2016)	**3254**
Total PTB cases	**602**
Percentage of PTB cases	**602 /3254 = 18.5%**
% of smear positivity from total attendant	**391/ 3254 = 12.01%**
% of smear positivity from PTB case	**391/602=64.95 %**
Total number of cases tested by GeneXpert	**616**
Total number of PTB tested by GeneXpert	**110**
% of Genexpert positivity from total tested attendant	**83 /616 = 13.47%**
% of Genexpert positivity from Total tested PTB cases	**83/110= 75.45%**
Mean age of PTB patients	**44.02 ± 17.23**
Gender Female (%) **Number of cases tested by GeneXpert** **Number of positive cases by GeneXpert** **Male (%)** **Number of cases tested by GeneXpert** **Number of positive cases by GeneXpert**	**282 (46.84%)** **62** **50** **320 (53.15%)** **48** **33**

By comparison of the GeneXpert MTB/RIF results and ZN staining technique, the study revealed that all of the smear-positive cases were positive by GeneXpert MTB/RIF. Whereas, 13 of smear-negative PTB cases were positive by GeneXpertMTB/RIF as shown in Table-2, This means GeneXpert MTB/RIF has added 17.7% (70 detected by smear microscopy compared to 83 detected by GeneXpert MTB/RIF relative increase in the rapid TB case detectionrate.

Table 2-Comparison of ZN smear and GeneXpert MTB/RIF results

PTB Cases	Genexpert		Total
ZN staining	Positive	Negative	
Smear-Positive	70	0	70
Smear-Negative	13	27	40
Total	83	27	110

Regarding Gender, of 602 PTB patients 46.84% were female and 53.15% were male. Smear positivity for female cases was (188/282=66.66%) and for male cases was (203/320=63.43%). GeneXpertMTB/RIF test result revealed that among 282 cases of females, there were 62 cases were tested by GeneXpertMTB/RIF and the positivity was (50/62=80.64%). For the male's cases, 48 cases were tested by GeneXpertMTB/RIF and the positivity was (33/48=68.75%). The smear-negative GeneXpertMTB/RIF positive cases were (13), female cases were higher than males, 9 and 4, respectively.

Concerning Age of the patients, the higher proportion of PTB cases were occurring in the age group (15-24) years (121/602=20.09%) and 72.25% of cases occur in age groups (15-54) years. In females, the higher proportion of PTB occur in the younger reproductive age group (15-24). Whereas the higher proportion of male TB patients occurs in older age group (35-44). There was a significant difference between Age and Sex in PTB patients, Table-3

Table 3-Age and Gender distribution among PTB patients

Age group	N. of TB(%)	Female(%)	Male(%)
5-14	11 (1.82)	9 (81.81)	2 (18.18)
15-24	121 (20.09)	75 (61.98)	46 (38.01)
25-34	118 (19.6)	50 (42.37)	68 (57.62)
35-44	96 (15.9)	25 (26.04)	71(73.95)
45-54	100 (16.61)	47 (47)	53 (53)
55-64	89 (14.78)	47 (52.8)	42 (47.19)
≥ 65	67 (11.12)	29 (43.28)	38 (56.71)
Total	602	282	320
P- value	0.000		

Incidence of PTB cases

As shown in Figure-1, there was declining trend in the PTB incidence over the period 2010-2014. In 2015 there was an increase in the incidence of PTB cases and then after there was another decrease. Interestingly, the number of attendants to the center has clearly higher in the 2015 and 2016 than other studies years as shown in Table-4.

Table 4-Distribution of PTB cases through years (2010-2016)

year	Total Attendant	Total TB cases	Smear positive (%)	Male	Female
2010	**507**	**121**	**73 (60.33)**	**43**	**30**
2011	**395**	**91**	**59 (64.83)**	**33**	**26**
2012	**401**	**87**	**53 (60.91)**	**26**	**27**
2013	**271**	**78**	**42 (53.84)**	**19**	**23**
2014	**336**	**62**	**56 (90.32)**	**28**	**28**
2015	**652**	**85**	**59 (69.41)**	**34**	**25**
2016	**692**	**78**	**49 (62.82)**	**20**	**29**
total	**3254**	**602**	**391**	**203**	**188**

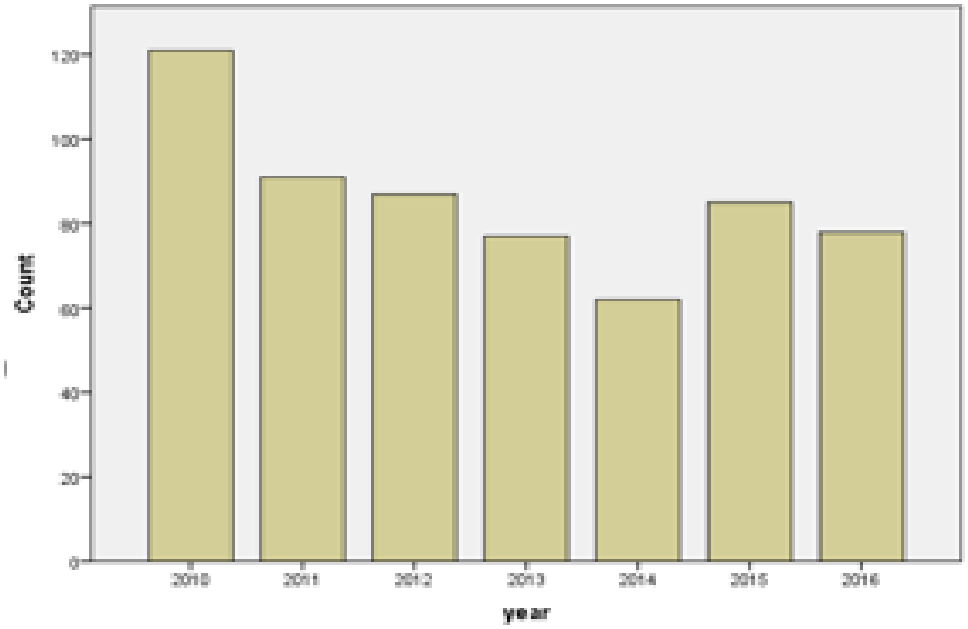

Figure 1-Incidence of PTB cases through six years.

Discussion

TB considered a serious public health problem worldwide.The current number of PTB cases that occur globally each year (9.4 million) is greater than that occur at any time in history. Over 90% of TB cases occur in low and middle-income countries [16]. Early PTB diagnosis is critical to disrupt bacterial transmission and subsequently improve disease control. PTB has non-specific clinical features, thus diagnosis usually confirmed by laboratory testing. Conventional laboratory methods used to diagnose PTB include Ziehl-Neelsen (ZN) staining of sputum samples and culturing of bacteria. Smear staining is specific, inexpensive, and could be done in basic laboratories. However, its insensitive, false positive results may occur. In addition to that, smear staining cannot be used to test drug resistance profile which is critical for starting treatment of MDR-TB patients [2].

Smear-positive patients are responsible for disease transmission. However, Its reported that smear-negative patients are responsible for approximately 17% of transmission which was neglected [4].
Culturing of MTB is important for diagnosing false negative smears and testing for drug resistance but it takes weeks. Therefore, several new rapid methods were developed like GeneXpert MTB/RIF for detection of MTB and rifampin resistance in less than 2 hours directly from sputumto allow rapid and accurate disease diagnosis hoping to decrease PTB incidence rates [16], and improve case detection

rates thus described as a potential "game changer" for PTB control [17]. One of the most important advantages of GeneXpert MTB/RIF device is that the reagents that used has anti tubercle activity, thus MTB will be inactivated *in vitro*. Subsequently, it can be used as a rapid diagnostic test as its biosafety risk are limited [12].

To the best of our knowledge, the current study is the first study in Kerbala aiming to investigate the impact of GeneXpert MTB/RIF usage (which is implemented since 2015) in improving case detection rates.

A total of 3254 patients with suspected PTB were evaluated by ZN staining technique and clinical outcome (which was considered the gold standard in the diagnosis of PTB). Some of the cases (616) were evaluated by Genexpert MTB/RIF. Total 602 (18.5%) were confirmed as PTB which reflect that the disease is of major clinical significance in Kerbala during the period 2010-2016. Lower percentage had been reported in previous studies [18]. This differences in PTB incidence percentage might be attributed to the knowledge level of people about the disease.

Out of 3254, 391 were smear-positive and among the tested cases by Genexpert MTB/RIF (616), 83 were positive. Thus the smear positivity from all cases was 12.01% and GeneXpert MTB/RIF positivity was 13.47%. Among PTB cases (602), the smear positivity was approximately 65%. And among the tested PTB cases by Genexpert-MTB/RIF (110), 83 (75.45%) were GeneXpert MTB/RIF positive while the other were GeneXpert MTB/RIF negative. Comparable results were reported by Munir *et al* [19] who found that the smear-positivity was 67.5% and Genexpert MTB/RIF positivity was 77.4%.

GeneXpert MTB/RIF results were compared against smear staining technique. The results showed that 70 cases were positive in both acid fast bacilli staining and Genexpert MTB/RIF, 27 were negative in both, while 13 cases were smear-negative and Genexpert MTB/RIF positive.

Thus Genexpert MTB/RIF could add approximately 12%(13/110) additional positive cases which were considered negative cases by ZN staining. This might possibly due to the higher specificity and sensitivity of GeneXpert MTB/RIF as compared to ZN staining because low bacterial counts on smear are more difficult to find during microscopic examination compared to higher bacterial counts and subsequently, are more likely to be missed [20]. Therefore, subjects whom considered smear-negative and at the same time still harbor and discharge MTB would continue suffering from disease and can transmit the bacteria to the community. Additionally, using of Genexpert MTB/RIF can reduce the number of sputum samples required by ZN staining technique in addition to its ability to increase case detection rates. Interestingly, the presence of high proportion (70) of PTB cases which were positive in both ZN and Genexpert tests might possibly reflect the high quality of ZN staining technique in health care centers as a preliminary diagnostic test in resource limited area.

The study revealed that the Mean age of patients was 44.02 ± 17.23 years. The mean age of female was 39.45 and males 42.29. The higher incidence of PTB cases were seen among males (53.15%) as compared with females (46.84%). Table-1. Male to female ratio was1.13. The WHO had reported that the global ratio is 1.85 [21]. A higher proportion of PTB incidence among men were reported in several previous studies [22-24]. Opposite results were reported in other previous studies [25, 26].

The exact reason behind gender imbalance is still unknown but it might possibly due to the interaction of several contributing factors including biological, epidemiological, and social factors. The impact of estradiol sexual hormone on disease acquisition which acts as macrophage activator through IFN-gamma and thus enhances cell mediated immune response and subsequently the females were less susceptible to TB infection [27]. Additionally, there are evidences showed that sever form of PTB disease (such as cavitary lesions) occur in male more than female [28]. Another suggested reasons include the bad quality of sputum sample, less access to health care centers and social stigma and the influence of disease on marital status make females ignore the disease which affects the case detection rates and more unreported cases among females [29]. Smear positivity for female cases was (188/282=66.66%) and male cases was (203/320=63.43%). The current results disagreed with **Chinnakali P.** *et al*., [30] who reported that smear positivity rates in males were significantly higher than that of females.

Interestingly, GeneXpert MTB/RIF test result revealed that among 282 cases of females there were 62 cases were tested by GeneXpert MTB/RIF and the positivity was (50/62=80.64%). For the male's cases, 48 cases were tested by GeneXpert MTB/RIF and the positivity was (33/48=68.75%), Table-1.

The smear negative GeneXpert MTB/RIF positive cases (13), female cases were higher than males, 9 and 4, respectively. This might possibly due to bad quality of sputum samples collected from female patients and considered by ZN as false-negative cases but with GeneXpert MTB/RIF as positive cases the higher proportion of PTB cases were occurring in the age group (15-24) years and 72.25% of cases occur in age groups (15-54) years. In females, the higher proportion of PTB occurs in the younger reproductive age group (15-24). Whereas the higher proportion of male TB patients occur in older age group (35-44). There was a significant difference between Age and Sex in PTB patients, Table-3. This results were comparable with other studies which indicated that most TB cases occur in the most productive age [31]. These could be explained by the fact that younger individuals more active and communicable than elderly.

The current study demonstrated that there has been declining trend in the PTB incidence over the period 2010-2014. In 2015 there was an increase in the incidence of PTB cases and then after there was another decrease. This might possibly due to the usage of GeneXpert MTB/RIF in the diagnosis of PTB which had been indorsed after 2014 and had an impact on PTB control and prevention of transmission. The WHO stated that there is a decline in the incidence rates and mortality rates attributed to PTB but at the same time this decline is very slowly [16].

Conclusion

The implementation of Genexpert in the diagnosis of PTB could strengthen PTB case finding for especially, those patients who had sputum smear-negative.

References

1. World health Organization. **2016**. Global tuberculosis report 2016.
2. Nathanson E, Nunn P, Uplekar M, Floyd K, Jaramillo E, Lönnroth K, Weil D, Raviglione M. **2010**. MDR tuberculosis—critical steps for prevention and control. *New England Journal of Medicine*, **363**(11):1050-1058.
3. Cox H, Kebede Y, Allamuratova S, Ismailov G, Davletmuratova Z, Byrnes G, Stone C, Niemann S, Rüsch-Gerdes S. and Blok L. **2006.**Tuberculosis recurrence and mortality after successful treatment: impact of drug resistance. *PLoS medicine*, **3**(10): e384.
4. Mostaza J, García N, Fernández S, Bahamonde A, Fuentes M, Palomo M. **2007.** Analysis and predictor of delays in the suspicion and treatment among hospitalized patients with pulmonary tuberculosis. In: *Anales de medicina interna (Madrid, Spain: 1984)*, 478-483.
5. Chaudhary M, Gupta S, Khare S. and Lal S. **2010.** Diagnosis of tuberculosis in an era of HIV pandemic: a review of current status and future prospects. *Indian journal of medical microbiology*, **28**(4): 281.
6. Weinstein, MP. and Wilson, ML. **2011.** Recent advances in the laboratory detection of Mycobacterium tuberculosis complex and drug resistance. *Clinical infectious diseases*, **52**(11): 1350-1355.
7. Pai, M., Minion, J., Sohn, H., Zwerling, A. and Perkins, MD. **2009.** Novel and improved technologies for tuberculosis diagnosis: progress and challenges. *Clinics in chest medicine*, **30**(4): 701-716.
8. World health Organization, Initiative ST, **2010.** Treatment of tuberculosis: guidelines: World Health Organization.
9. Raviglione, M., Marais, B., Floyd, K., Lönnroth, K., Getahun, H., Migliori, GB., Harries, AD., Nunn, P., Lienhardt, C. and Graham, S. **2012.** Scaling up interventions to achieve global tuberculosis control: progress and new developments. *The Lancet*, **379**(9829): 1902-1913.
10. Zeka, AN., Tasbakan, S. and Cavusoglu, C. **2011.** Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. *Journal of clinical microbiology*, **49**(12): 4138-4141.
11. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R. and Rustomjee, R. **2010.** Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine*, **363**(11): 1005-1015.
12. Sharma, SK., Kohli, M., Yadav, RN., Chaubey, J., Bhasin, D., Sreenivas, V., Sharma, R. and Singh, BK. **2015.**Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. *PloS one*, **10**(10): e0141011.

13. World Health Organization, **2013**. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MT.

14. Zumla A, George A, Sharma V, Herbert RHN, Oxley, A. and Oliver, M. **2015.**The WHO 2014 global tuberculosis report—further to go. *The Lancet Global Health*, **3**(1): e10-e12.

15. Munir, MK., Anwar, N., Iqbal, R., Shabbir, I. and Nosheen, S. **2011.** Diagnosis of tuberculosis: molecular versus conventional method. *Pakistan Journal of Medical Research*, **50**(2): 50.

16. Lawn, SD. and Nicol, MP. **2011**. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future microbiology*, **6**(9): 1067-1082.

17. Evans CA. **2011.**GeneXpert—a game-changer for tuberculosis control?*PLoS medicine*, **8**(7): e1001064.

18. Ali, H., Zeynudin, A., Mekonnen, A., Abera, S. and Ali, S. **2012.** Smear positive pulmonary tuberculosis (PTB) prevalence amongst patients at Agaro teaching health center, south west Ethiopia. *Ethiopian journal of health sciences*, **22**(1): 71-76.

19. Munir, MK., Rehman, S., Aasim, M., Iqbal, R. and Saeed, S. **2015.** Comparison of Ziehl Neelsen Microscopy with GeneXpert for Detection of Mycobacterium Tuberculosis. *IOSR J Dent Med Sci*, **14**(11): 56-60.

20. Ramsay, A., Bonnet, M., Gagnidze, L., Githui, W., Varaine, F. and Guerin, P. **2009**. Sputum, sex and scanty smears: new case definition may reduce sex disparities in smear-positive tuberculosis. *The International Journal of Tuberculosis and Lung Disease*, **13**(5): 613-619.

21. World health Organization, **2009.** Global tuberculosis control: a short update to the 2009 report.

22. Thorson, A., Hoa, N., Long, N., Allebeck, P. and Diwan, V. **2004.** Do women with tuberculosis have a lower likelihood of getting diagnosed?: Prevalence and case detection of sputum smear positive pulmonary TB, a population-based study from Vietnam. *Journal of clinical epidemiology*, **57**(4): 398-402.

23. Crampin, A., Glynn, J., Floyd, S., Malema, S., Mwinuka, V., Ngwira, B., Mwaungulu, F., Warndorff, D. and Fine, P. **2004.**Tuberculosis and gender: exploring the patterns in a case control study in Malawi. *The international journal of tuberculosis and lung disease*, **8**(2): 194-203.

24. Nsubuga, P., Johnson, JL., Okwera, A., Mugerwa, RD., Ellner, JJ. and Whalen, CC. **2002.** Gender and HIV-associated pulmonary tuberculosis: presentation and outcome at one year after beginning antituberculosis treatment in Uganda. *BMC pulmonary medicine*, **2**(1): 4.

25. Codlin, AJ., Khowaja, S., Chen, Z., Rahbar, MH., Qadeer, E., Ara, I., McCormick, JB., Fisher-Hoch, SP. and Khan, AJ. **2011.**Gender differences in tuberculosis notification in Pakistan. *The American journal of tropical medicine and hygiene*, **85**(3): 514-517.

26. Onifade, DA., Bayer, AM., Montoya, R., Haro, M., Alva, J., Franco, J., Sosa, R., Valiente, B., Valera, E. and Ford, CM. **2010.** Gender-related factors influencing tuberculosis control in shantytowns: a qualitative study. *BMC Public Health*, **10**(1): 381.

27. Neyrolles, O. and Quintana-Murci L. **2009.** Sexual inequality in tuberculosis. *PLoS medicine*, **6**(12): e1000199.

28. Thorson, A., Hoang Long, N., Olof Larsson, L. **2007.** Chest X-ray findings in relation to gender and symptoms: a study of patients with smear positive tuberculosis in Vietnam. *Scandinavian journal of infectious diseases*, **39**(1): 33-37.

29. Uwizey, C., De Serres, G., Gilca, R., Schwartzman, K. and Gasana, M. **2011**.Tuberculosis may be underestimated in Rwandan women. *The International Journal of Tuberculosis and Lung Disease*, **15**(6): 776-781.

30. Chinnakali, P., Selvaraj, K., Thekkur, P., Ramasamy, G., Thulasingam, M., Vasudevan, K. **2014.** Age and Sex differences in sputum smear microscopy results for acid fast bacilli in a tertiary care centre, south India. *Journal of Respiratory Medicine*, **2014**.

31. Munir, MK., Anwer, N., Iqbal, R., Nosheen, S., Rehman, S. and Salam, AA. **2014.** Comparison of Genotype MTBDRplus Testing Kit with conventional method for Drug Susceptibility Testing of Isoniazid and Rifampicin in Tuberculosis Patients. *Pakistan Journal of Medical Research*, **53**(2): 25.