MOLECULAR DIAGNOSIS OF URINARY *Mycobacterium tuberculosis* AMONG PATIENTS ATTENDING UROLOGY CLINIC IN AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA

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ABSTRACT

Background: Tuberculosis, a communicable disease with significant morbidity and mortality. It is still among the top killers of infectious diseases; *Mycobacterium tuberculosis* is a successful pathogen that has evolved several mechanisms to manipulate the host immune response.

Objective of the study: The objective of this study was to determinethe prevalence of urinary tuberculosis among patients attending urology clinic in Aminu Kano Teaching Hospital, Kano, Nigeria.

Materials and Methods: A prospective cross-sectional laboratory based study thatinvolved the use of questionnaire and consent/assent form prior to sample collection. Xpert MTB/Rif assay was used to analyze the urine samples.

Results: The results of this study shows that, out of 71 samples analyzed along with positive control (H37RV) and molecular grade water was used as negative control. The results showed that, 2 (2.8 %) were from urine samples. Data generated were analyzed using descriptive statistics and results were presented in tables and charts.

Conclusion: The study confirmed the presence of urinary tuberculosis in the study area, with prevalence of 2.8%. The pattern of tuberculosis was susceptible *Mycobacterium tuberculosis.* Therefore, there is need to introduce a routine screening of urinary tuberculosis among patients presenting sign and symptoms of urinary tract infection using Gene Xpert. This will be achieved through the engagement and enlightenment of the clinicians, strengthening the laboratory capacity for diagnosis and make the services available and accessible to the patients who need them.

Keywords: Mycobacterium tuberculosis, Extra-Pulmonary Tuberculosis, Prevalence and Gene Xpert.

INTRODUCTION

Tuberculosis (TB) remains a major public health problem with 8.6 million new cases, including 0.8 million urinary tuberculosis and 1.3 million deaths globally (Al-Hajoj *et al.,* 2013).Urinary tuberculosis is among the extra-pulmonary tuberculosis, which refers to TB in part of the body other than the lung, is known to affect virtually every part of the body, however, lymph nodes and the pleura are the most common site (Al-Muhsen, 2010). Although, active pulmonary TB is transmissible by droplet spread by coughing, extra-pulmonary TB is thought to result from haematogenous spread from an initial lung infection and is not infectious (Al-Muhsen, 2010).

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Urinary TB can occur alone or together with pulmonary TB (Alonzo and Pepe, 2009).Urinary TB is especially common in people living with HIV and adults (Al-Otaibi and El Hazmi, 2010). Therefore, it is much more common in countries with a high HIV prevalence and in countries where a lot of people are infected with TB (Al-Otaibi and El Hazmi2010).

Urinary tuberculosis is a disease of public health concern in many countries including Nigeria. Latent tuberculosis persists in over a billion individuals' worldwide (Alrabiaah *et al.,* 2012). In addition, the emergence of multi drug-resistant tuberculosis (MDRTB) is of great concern (Asghar *et al.,* 2008). In 2012, WHO stated that the incidence of urinary tuberculosis was 0.31 % implying 50 % increase in the incidence rates in 2011, with 10 % death among the patients with extra-pulmonary tuberculosis was reported by WHO (Awoyemi *et al.,* 2012). Drug-Resistance tuberculosis among follow-up tuberculosis patients in Kano State, Nigeria, was reported to be 4.7% prevalence (Mohammad *et al.,* 2017).The Nigerian Federal Ministry of Heath reported that Nigeria has incidence of about 399,000 cases of all forms of tuberculosis annually and 10% TB prevalence, ranking the fourth largest TB burden in the world and largest in Africa (Banada *et al.,* 2010). Tuberculosis spread through the air when infectious people cough, spit, talk or sneeze; untreated person with active tuberculosis can infect between10 to 15 people every year (Mohammad *et al.,* 2017). Tuberculosis usually kills a person by gradually creating eating holes in the lungs, thereby moving to other organ of the body causing extra-pulmonary tuberculosis (Barnes P.F., 1997). People infected with *Mycobacterium tuberculosis* have 10% lifetime risk of falling ill with TB (Banada *et al*., 2010; Blackemore *et al.*, 2010).However, persons with compromised immune systems, such as people with human immunodeficiency virus (HIV), malnutrition, diabetes mellitus or people who use tobacco, have a much higher risk of becoming ill with TB (Alonzo and Pepe, 2009; Blackemore *et al*., 2010). The deadly synergisms between TB and HIV with the emergence of drug resistance have complicated the control of TB (Al-Muhsen, 2010; Blackemore *et al*., 2010). Generally, a rising proportion of urinary tuberculosis has been reported from different countries (Blackemore *et al*., 2010; Cailhol *et al.,* 2005). It is thought that the reason for higher rates of urinary TB in people living with HIV is that their immune systems cannot respond as strongly to a TB infection, and are not able to contain the TB bacilli (Chijioke *et al.,* 2018).

Urinary tuberculosis receives less interest than pulmonary tuberculosis because of its low infectious potential (NBLCP, 2014).However, it is considered as a serious clinical problem because of the diagnostic challenges encountered and the propensity to cause high morbidity and mortality (Barnes, 1997; Cailhol *et al*., 2005; Chijoke and Aderibigbe, 2018). A recent study from Brazil also highlighted the lack of diagnostic capabilities as a major reason for late diagnosis and management of extra pulmonary tuberculosis (Al-Hajoj*et al.,* 2013; Banada*et al.,* 2010; Chijioke*et al.,*2018;). One third of the world population is infected with tuberculosis, 10% of infected individuals develop tuberculosis in their life time and therefore, early diagnosis of active tuberculosis remains an elusive challenge (WHO, 2011; FMOHN, 2016). Diagnosis of urinary tuberculosis continues to be a challenge in developing countries with poor resources like Nigeria. Data regarding urinary tuberculosis (TB) in the study area were lacking, despite the occasional screening (Al-Muhsen *et al.,*2010; Mattelelli *et al.,* 2012; Mohammad *et al.,* 2017). Despite the increase in the prevalence and death, identification of urinary tuberculosis using conventional method remain a problem, due to time consuming, low sensitivity and specificity compared to the molecular approach (Asghar *et al.,* 2008; Mohammad *et al.,* 2017).

Molecular method (using Gene xpert) can be used to identify sensitive *Mycobacterium tuberculosis* and drug resistance within two hours to achieve possible turnaround time (Alrabiaah *et al.,* 2012; Chandir *et al.,* 2010). The Xpert MTB/RIF assay method has been endorsed by the WHO in December 2010 as a replacement for sputum smear microscopy (Blackemore *et al.,* 2010). By using MTB/RIF assay for, the time required for the identification of Mycobacteria has been shortened and it should be encouraged to be a tool for prompt diagnosis of EPTB (Al-Muhsen, 2010; Cailhol *et al.,* 2005).The aim of this study was to determine the prevalence of urinary tuberculosis among patients attending urology clinic in Aminu Kano Teaching Hospital, Kano, Nigeria.

MATERIALS AND METHODS

The research work was conducted at Aminu Kano Teaching Hospital, Kano, Kano State, Nigeria. Kano is one of the most populous State in Nigeria with over 15 million residents projected population. Nigeria has the third highest number of tuberculosis cases in the world (NTBLCP, 2014).This study is a cross-sectional prospective laboratory based study conducted on consented and assented participants attending Aminu Kano Teaching Hospital, Kano, Nigeria; that showed sign and symptom of urinary tuberculosis attending urology clinic; within a period of six month. Ethical approval to carry out this study was obtained from ethics committee of Aminu Kano Teaching Hospital, Kano, Nigeria; (certificate number: NHREC/21/08/2008/AKTH/EC/2546), this study was performed in accordance with declaration of Helsinki. Questionnaire was administered to obtained socio demographic data, other factors associated with extra-pulmonary tuberculosis and pattern of extra-pulmonary tuberculosis from consented participants in the study area.

The sample size in this study was calculated according to the formula described by Cochrane (1963) and Glenn (2010).

$n = [z^2 p (1- p)] \div [d^2]$

Where: n = number of samples

z = statistical level of confidence at 95 % = 1.96

p = prevalence = 4.92 (0.0492)

d = allowable error of 5 % (0.05)

$n = [(1.96)^2 \times 0.0492 (1- 0.0492)] \div [(0.05)^2] = 71$

Therefore, seventy one (71) samples from suspected patients with sign and symptoms of urinary tuberculosis, those that agreed signed and consented or assented to participate in the study were collected under aseptic condition.

MTB/RIF ASSAY (PCR TEST)

Urine samples were collected in a sterile gallon from consented / assented participants for 24 hours; the urine sample deposit was spun, supernatant was also decanted, 1ml of the sample (ratio of 2:1). The mixture was incubated at room temperature for fifteen (15) minutes, votexed and also re-incubated at room temperature for another 5minutes. Using sterile pasture pipette, 2mls of the mixture was pipetted and dispensed in to the cartridge. Cartridges were labeled with sample ID on the left side of cartridges or affixing a pre-label paper. Touching the sterile transfer pipette was avoided. The front of the pipette was wrapped at the bulb-end of pipette. Using the sterile transfer pipette provided in the Xpert/Rif kit (Cepheid, Sunnyvale, California USA), the liquefied sample was drawn into the transfer pipette until the meniscus of pipette is above the minimum mark (2 ml). The pipette was carefully put into the paper/plastic cover. The transfer pipette was discarded into bio-hazard waste bin. The cartridge lid was closed. The lid snaps were firmly placed (as shown in Fig 1) and team loaded the Gene Xpert machine (Cepheid, Sunnyvale, California USA) (figure 2) and run for 1hour 55minutes. Only computer results were printed automatically.

RESULTS

The socio-demographic characteristic of studied population of TB patients was obtained. Out of 71 studied participants, more than 40% of the patients were aged between 21 and 40 years, mostof them were males 60.6 %. About 33.8 % of the patients had a tertiary education, 31.0 % had a secondary education and 21.1 % had no formal education. Also, 56.3 % were married, 9.9 %widowed, 23.9 % were single and 9.9 % were divorced (Table 1)

Table 1: Socio-Demographic Characteristics of Studied Participants

Characteristics	Males	Females	Frequency (%)
Age			
≤20	2(2.8)	1(1.4)	3(4.2)
21 – 30	19(26.8)	5(5.0)	24(33.8)
31 - 40	10(14.1)	10(14.1)	20(28.2)
41 – 50	8(11.3)	6(8.5)	14(19.8)
51 – 60	5(7.0)	2(2.8)	7(9.8)
>60	1(1.4)	2(2.8)	3(4.2)
Marital status			
Single	13(18.3)	4(5.6)	17(23.9)
Married	25(35.2)	15(21.1)	40(56.3)
Widowed	4(5.6)	3(4.2)	7(9.9)
Divorced	3(4.2)	4(5.6)	7(9.9)
Educational status			
Non-formal	6(12.7)	9(12.7)	15(21.1)
Primary	394.2)	7(9.9)	10(14.1)
Secondary	16(22.5)	6(8.5)	22(31.0)
Tertiary	20(28.2)	4(5.6)	24(33.8)
Occupation			
Civil servants	23(32.4)	2(2.8)	25(35.2)
Farmers	5(7.0)	2(2.8)	7(9.9)
Business men/women	10(14.1)	6(8.5)	16(22.5)
House wives	0(0.0)	10(14.1)	10(14.1)
Students	5(7.0)	5(7.0)	10(14.1)
Unemployed	2(2.8)	1(1.4)	3(4.20)
HIV status			
HIV Negative	43(60.6)	28(39.4)	71(10.0)
HIV Positive	0(0.0)	0(0.0)	0(0.0)

Note: HIV = Human Immunodeficiency Virus

Pattern of urinary tuberculosis

Pattern of urinary tuberculosis include: drug-resistance tuberculosis (DR-TB) and sensitive MTB. In this study, patients were found to have sensitive MTB (Table 2).

Table 2: Results obtained according to study population (n=71)

Gender	Number of samples screened	Indeterminate	MTB Detected
Female	26	0	1
Male	45	0	1
Total	**71**	**0**	**2**

Note: MTB = *Mycobacterium tuberculosis*

DISCUSSION

The Xpert MTB/RIF (PCR) assay shows that, all samples were analyzed along with positive control (H37RV) and negative control (Molecular grade water) respectively. As a result of some specific symptoms of extra-pulmonary tuberculosis, the diagnosis of this disease became a major challenge in resource limited setting like Nigeria (Sandgren *et al., et al.,* 2010).

Due to short coming of conventional methods, Xpert MTB/RIF assay became the next option for extra pulmonary specimens like urine (Range *et al.,* 2018). The study confirmed 2 (2.4) positive cases of urinary tuberculosis among studied participants, according to WHO, extra pulmonary specimens account for 10% of tuberculosis cases in South East, Nigeria also urogenital tuberculosis is responsible for 30 to 40% of all extra pulmonary tuberculosis in the area(WHO, 2013; Salami and Katipi 2006). It is known to be a secondary manifestation of the disease, as in most other form of urinary tuberculosis, it originated from pulmonary TB which focuses elsewhere in the body (WHO, 2011). The miliary TB formed subsequent dissemination from the dormant stage for years and then begins to spread to other part of the body (WHO, 2011). In this study males had 1.4% prevalence and females had 1.4%, which is lower than the work conducted in Ilorin North Central, Nigeria which got 9.5% prevalence (FMOHN, 2016).Though this could be as a result of smaller sample size in our study. Also in early eighties, in Lagos, South Western, Nigeria, 14% prevalence of urinary tuberculosis was obtained, a value higher than our findings (Banada *et al.,* 2010; Sandgren *et al.,* 2010).

The study conducted by Awoyemi in Ibadan, Nigeria, stated that, sensitivity of Gene Xpert as diagnostic tool depends on its limit of detection (Banada *et al.,* 2010). The MTB/RIF assay was reported to identify MTB at 131 CFU/ml of the specimen (Bruchfeld *et al.,* 2002). It was reported that, the results of sensitivity of Gene Xpert might have been enhanced by the detection of remnant DNA from dead bacterial cells, which makes it to have advantages over culture and microscopy (Salami and Katipi, 2006). A study conducted in USA, reported that Gene Xpert assay, exhibits better sensitivity in the diagnosis of MTB and Rif resistant MTB from the urine specimens (FMOHN, 2016; Helb *et al.,* 2010; Mohammad *et al.,* 2017).WHO, reported that, in light of the same digested condition used in the treatment procedure for both sputum and urine samples, due to over exposure to the extreme alkaline environment of tubercle bacilli in the urine samples, which is responsible for inactivating a percentage of MTB, thereby resulting in the low recovery rate by conventional methods (Salami and Katipi, 2006). The hypothesis in this procedure appears to be suitable for sputum, but too rigorous for urine (NTBLCP, (2014); Onipede *et al.,* 1999. Despite an excellent technology for detection of urinary tuberculosis from urine specimen, the major issue facing this assay is the high cost compared to AFB smear microscopy. Although urgent detection of extra-pulmonary tuberculosis in this study also supports the use of Gene Xpert MTB/MTBRIF assay for diagnosis of suspected patients with EPTB; however, this finding should be treated with caution because of the small sample size. Nevertheless; the presence of extra-pulmonary tuberculosis in the study area with prevalence of 4.2 % has highlighted the need for further studies.

CONCLUSION

The prevalence of extra pulmonary tuberculosis is in existence in the studied area with prevalence of2.8%, this is a serious public health concern. Which constitutes a major public health problem that required urgent attention. Therefore, it is advised that patients with symptoms of extra-pulmonary tuberculosis should be investigated using MTB/RIF Assay and all suspected patients with urinary tuberculosis should have access to services so that appropriate treatment can be given to the patients from the outset so as to prevent the amplification of resistance.

Conflicts of interest

All authors declared that, there is no conflict of interest.

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