Detection of Serum IgG Antibodies against *Mycobacterium tuberculosis* among University Athletes in Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author IOO designed the study, performed the statistical analysis and wrote the protocol. Authors TIC, SAO and KCA managed the analyses of the study. Authors IOO and SAO managed the literature searches and wrote the first draft of the manuscript. Authors IOO and KCA supervised the whole study which, MUS used as part of her B.Sc. Project in the Department of Microbiology, University of Port Harcourt, Nigeria. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis, is a strictly aerobic bacterium that grows fastidiously and slow, which is among the top 10 causes of death globally and the leading cause from a single infectious agent (above HIV/AIDS). Among the deadly diseases ravaging the world, tuberculosis remains one of the commonest and deadliest. The objective of this study is to determine the prevalence of tuberculosis (TB) among the athletes of the University of Port Harcourt (UNIPORT), Rivers State, Nigeria.

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Study Design: Cross-sectional study.

Place and Duration of Study: Sports Institute, University of Port Harcourt (UNIPORT), Nigeria, between June 2012 and July 2015.

Methods: A total of 100 university athletes (51 males and 49 females, the age ranged from 15-47 years) was recruited for this study. Blood samples were collected from the athletes and analyzed for serum IgG antibodies against *Mycobacterium tuberculosis.* OneStep Tuberculosis (TB) rapid test was used for the detection of TB antibody (Isotypes IgG, IgM and IgA) in whole blood samples from the subjects. Commercially available ELISA based kits (manufactured by Dia.Pro, Milano, Italy) were also used.

Results: The seropositivity of TB among the athletes was 5.0%. The result of the study showed that male athletes had a higher prevalence of tuberculosis than their female counterpart (5.9% vs 4.1%). Subsequently, the age groups 15-25 years had a higher prevalence of tuberculosis (6.5%) compared to age groups 26 years and above (2.6%). A significant association existed between the athletes' ages and TB prevalence (p= 0.035) and none existed with gender (p=0.654).

Conclusion: This study confirms the presence of *Mycobacterium tuberculosis*-specific antibodies among university athletes. Strict surveillance of the diseases is highly recommended to curb its spread and the potential increase in Multidrug-resistance tuberculosis (MDR-TB) and Extensively drug-resistance Tuberculosis (XDR-TB) as it is highly contagious.

1. INTRODUCTION

Tuberculosis (TB) in humans, although curable is a chronic bacterial disease caused by *Mycobacterium tuberculosis* (MTB) which more often than not affects the lungs (pulmonary TB [PTB]), but can also attack other organs of the body as well, known as the extra-pulmonary TB [EPTB] [1]. Other infectious agents of Tuberculosis include *Mycobacterium bovis, M. africanum, M. microfti, M. canettii, M. caprae, M. pinnipedii, M. suricattae* and recently recognized *M. mungi* [1-2].

The bacteria can easily be spread from one person to another through aerosols. The inhalation of just a few of these germs can cause infection but does not necessarily mean everyone infected with TB becomes ill [3]. There are two TB-related conditions; latent TB infection (LTBI) (the individual is infectious, but doesn't fall ill) and active TB disease (individual becomes ill). TB disease becomes fatal if left untreated [4]. People living with HIV are 19 times more likely to develop active TB, while those with undernutrition are 3 times more at risk with 95% cases and death in developing countries. The risk is greater in individuals suffering from other conditions that impair the immune system [3].

Tuberculosis is indeed a global pandemic, killing someone approximately every 18 seconds — about 1.5 million in 2018 alone [5] with XDR-TB has been identified in more than 131 countries. TB mostly affects adults in their most productive years. However, all age groups are at risk. In 2018, an estimated 10 million people fell ill with tuberculosis (TB) worldwide, of which 1.5 million died including 251 000 people with HIV [3]. Nigeria ranked 6th among the eight countries that accounted for two-thirds of 87% new cases in the 30 high TB burden countries of the world, reported in 2018 [3]. An estimate of 1.5 million people died from TB in 2018 (including 251 000 people with HIV). TB is among the top 10 causes of death globally and the leading cause of a single infectious agent (above HIV/AIDS) [3]. A high burden of PTB was reported in Kaduna State with a prevalence rate of *M. bovis* (1.0%) and a 13.0% prevalence rate of *M. africanum* [6]. Similarly, Nwanta et al. [7] reported an overall prevalence rate of 37.9% MTB in Enugu state, Nigeria. Researchers from other West African Countries including Ghana (32.5%), Mali (25.9%), and Burkina Faso (40.5%) also reported the same trend [8-10].

To the best of our knowledge, studies on the prevalence of TB among athletes in Rivers State, Nigeria is scarce. This study becomes necessary to detect the presence of *Mycobacterium tuberculosis*-specific antibodies among University athletes in Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross-sectional study carried among athletes in the UNIPORT located in Choba

community of Obio-Akpor Local Government Area, Rivers State, Nigeria. It is among the several tertiary institutions in Rivers State, Nigeria. Its layout has three campuses which are located at Choba Park, Delta Park, and University Park (Abuja Park).

2.2 Study Population

A total of 100 athletes (51 males and 49 females) of the institution were enrolled in this study. It includes both males and female athletes of various ages ranging between 15-47 years with the exclusion of non-athletes and athletes of other surrounding institutions.

2.3 Blood Sample Collection and Processing

The sampling technique used for drawing the samples in this study was the convenience sampling technique. Venipuncture technique was used for blood collection. The blood sample was then collected aseptically into EDTA bottle and taken to the Virus Research Unit, Department of Microbiology, the UNIPORT for the laboratory analysis.

2.4 Serological Analysis of Tuberculosis

OneStep Tuberculosis (TB) RapiCard[TM] InstaTest (ref 118779-1-44) by Cortez Diagnostics, Inc (21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA). The tuberculosis rapid test is a quick and easy test that qualitatively detects antibodies to tuberculosis (isotypes IgG, IgA and IgM) present in the blood, plasma or serum of humans working by the principles of chromatographic immunoassay. The principle of this test works using the combination of antigens and a specific antibody binding protein conjugated to the solid membrane surface of the cassette. Whole blood specimens were used in this study following the manufacturer's directions. The samples were also analyzed for *Mycobacterium tuberculosis*-specific IgG antibodies using the commercially available ELISA kit (MTB IgG manufactured by DIA.PRO Diagnostic Bioprobes, Milano, Italy). An automated washer (Biotek ELx 50, USA) was used to was the microplates for 5 cycles. A spectrophotometric plate reader (Biotek ELx808i, USA) at an absorbance of 450-630 nm was used to measure the colour intensity of the coloured reaction. Every stage of the ELISA process was done following the manufacturer's instructions. Test results were interpreted as samples

showing an OD 450nm value lower than the Cut-Off value were considered negative for anti-MTB IgG. Samples showing an OD450nm value higher than the Cut-Off value were considered positive for anti-MTB IgG. Also, a quantification of the IgG content in arbU/ml is possible for those samples that show an OD450 nm higher than the Cut-Off (or S/C0 > 1); this provides the possibility for the clinician to follow up the immunological status of the tuberculosis patient.

2.5 Data Analysis

The seroprevalence was calculated. Chi-square test was used to establish relationships between demographic factors and TB prevalence using Microsoft Excel spreadsheet (Microsoft Corporation). Significance level was set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

The following results were obtained from the study carried out at the UNIPORT, Department of Microbiology. The subjects were grouped into 51 males and 49 females. Their age ranges from 15 years to 47 years. The following tables further explain the results observed in this study.

3.1.1 Prevalence of tuberculosis concerning sex

The prevalence of tuberculosis with sex distribution is shown in Table 1. The highest prevalence of 3 (5.9%) from the males while 2 (4.1%) was the prevalence for females.

Table 1. Prevalence of tuberculosis concerning sex

Sex	No tested	No. positive for TB (%)	p-value
Male	51	3 (5.9)	
Female	49	2 (4.1)	
Total	100	5 (5.0)	0.654

3.1.2 Prevalence of tuberculosis concerning age

The TB prevalence concerning the athletes' age group is shown in Table 2. The highest prevalence of 4(6.5%) was recorded among 15-25 years while 1(2.6%) was recorded among 26 years and above.

Table 2. Prevalence of tuberculosis among athletes concerning age

Age (years)	No tested	No. positive for TB (%)	P-value
15-25	61	4 (6.5)	
26 & above	39	1 (2.6)	
Total	100 (100)	5 (5.0)	0.035

3.2 Discussion

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex (MTBC), continues to be among the major killer diseases worldwide, especially in developing countries despite considerable progress in diagnosis and treatment [11-16]. In the diagnosis of tuberculosis and the follow-up of infected patients, ELISA for antibodies may be useful to provide information on the immunological status of the patient, in addition to Nucleic Acid Tests (or NATs) able to determine the presence of the *Mycobacterium tuberculosis* itself [11-16].

This study which was carried out to determine the prevalence of TB from the University athletes recorded a prevalence of 5.0% from a sample size of 100 subjects. This is incomparable with 21.6% that Nwachukwu and Peter [17] reported in Abia State, Nigeria, but slightly lower than 10.3% Azuonwu et al. [18] reported in Bayelsa State, Nigeria. The disparity of the prevalence can be associated with the low sample size of this study furthermore the number of athletes in the university is scantily populated when compared side by side the previous study carried out.

The prevalence of the athletes based on their gender was 5.9% for males and 4.1% for females. There was no significant association between the gender of the athletes and TB prevalence with a p value of 0.65. The disease is not prominent to a particular gender than the other. This was supported by the findings of Azuonwu et al. [18] who reported a high prevalence of 55.7% among females than the males. Although Okonko et al. [19] and Jumbo et al. [20] also reported a higher TB prevalence among the males in their various studies.

Athletes within ages 15-25 were most common among the age groups involved in this study. They also had the highest TB prevalence of 6.5%. A similar study among adolescents and young adults in Kenya and Botswana reported the ages of 12-18 and 10-19 respectively as having a much higher prevalence than adults [21-22]. In history, adolescents and young adults between ages 12-24 years have been noted to be at a higher risk of tuberculosis infection produced by *M. tuberculosis* [23]. No significant association occurred between the ages of the athletes and TB prevalence with a p-value of 0.035 (p<0.05). TB is prominent in young people as they have a much wider range of social contacts outside of the household.

4. CONCLUSION

Result of the study which showed that the prevalence of Tuberculosis among these athletes is 5.0% suggests that TB can be found among the simulated healthy individual or group of people. This also proves the known fact that it is easily spread and not all infected individuals become physically ill. We recommend frequent surveillance of both new and old TB cases to curb a potential increase in Multidrug-resistance Tuberculosis (MDR-TB) and Extensively drug-resistance Tuberculosis (XDR-TB).

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the University Research Ethics committee of University of Port Harcourt Teaching Hospital (UPTH) and Rivers State University Teaching Hospital (RSUTH) and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki. Ethical approval was sort from the Health Research and Ethics Committee of the University of Port Harcourt, Nigeria. The gender and age (15-47 years) of the athletes was obtained as the demographic factors.

of Miss Mbet-Obong Udo Sampson (MUS) in collecting these samples, which she used as part of her B.Sc. Project in the Department of Microbiology, University of Port Harcourt, Nigeria. The authors are grateful to the University athletes for their willingness to be part of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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