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Prevalence of pulmonary tuberculosis and the associated clinical symptoms in Western Uganda

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Abstract

Pulmonary tuberculosis is a public health problem affecting over 5.8 million people worldwide per year. Burden of the disease varies across populations due to differences in biological and behavioral factors. In Western Uganda, the prevalence of pulmonary tuberculosis was not established and there was continued use of a biased clinical tuberculosis description guideline during treatment. Therefore, a cross-sectional study was conducted among people ≥12 years attending Buhinga hospital in Western Uganda from April to June 2019 to achieve the following specific objectives; (1)- Determine prevalence of pulmonary tuberculosis by age group and sex, 2-Assess the clinical symptoms associated with pulmonary tuberculosis amongst the participants. Participants were recruited by simple random sampling technique and standardized questionnaire were administered to obtain demographic and clinical data. 379 sputum specimens were collected and tested for *M. tuberculosis* using Gene X-pert and Classical Real Time PCR. Data was analyzed using SPSS software version 13. Prevalence of pulmonary tuberculosis by age group was highest in 20-29 years, high in 30-39 years and ≥50 years and least in 10-19 years old individuals. Females had a slightly higher prevalence of pulmonary tuberculosis than males. Youthful behavior of active participation in social activities and advanced age health associated factors contributed to the high prevalence of pulmonary tuberculosis amongst the participants. There was no significant relationship between pulmonary tuberculosis disease and demography; - age group and sex (P-value = 0.24). All pulmonary tuberculosis cases presented with persistent fevers, coughs for ≥ 2 weeks, night sweats and noticeable weight loss in the hierarchical order. The relationship between pulmonary tuberculosis disease and clinical symptoms; - persistent fevers, cough, noticeable weight loss and night sweats (P-values; <0.001, <0.001, 0.001 and <0.001 respectively) was significant. Age and clinical symptoms are important pulmonary tuberculosis control hotspots.

Keywords: PTB; Age group; Sex; Clinical symptoms; Prevalence

1 Introduction

Tuberculosis (TB) is caused by the bacillus *M. tuberculosis*, spread when people who are sick with TB expel bacteria into air. The disease typically affects lungs (Pulmonary TB/PTB) but can also affect other sites causing extra pulmonary TB. Despite the concerted TB control plan, the disease remains one of the world's biggest public health problems. Out of the global population of over 7 billion people, 2 billion people are estimated to be latently infected with TB [1]. In 2020, there were 5.8 million incident cases of TB and 1.3 million TB deaths Worldwide [2]. In 2019, 7.1 million TB incident

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cases were recorded (18% lower from 5.8 million in 2020) and 1.2 million TB deaths globally [3]. Infectious diseases do not affect age groups, males and females in the same way [4] and PTB is no exception. According to WHO TB (2019) report [3], the global male to female prevalence ratio is 1.34:1. This gender imbalance increases with age and may have implications in the management of TB. Two major hypotheses (behavior and physiologic) have been stated to explain sex bias in infectious diseases.

The behavior hypothesis relays gender-specific factors like social obligations, risk behaviors and life activities that influence exposure and transmission of *M. tuberculosis.* Males travel frequently, have more social contacts and spend more time in settings that may be favorable to PTB transmission such as bars, cinema halls or night clubs. Males also engage in professions such as mining which is associated with a higher risk of PTB infection [5, 6]. Conversely, house hold contact with an infected individual is a big PTB risk factor [7] in the developing countries [8]. In spite of men spending little time at the homes, they remain at a higher risk of acquiring TB from household contacts than women [9]. In high burden countries, smoking has been found to be frequent in men than women and a correlative analysis of cigarette smoking, sex and TB suggests that smoking might explain up to one-third of the gender bias observed in this setting [10]. Alcohol consumption is another risk factor for TB, and the prevalence in low income countries among men is higher than that among women. In summary, epidemiological or behavioral factors play an important role in PTB acquisition with a strong male bias. However, overall contribution of these behavioral factors is difficult to assess, therefore it is better for us to review the physiological hypothesis.

The physiological hypothesis points at biological differences between sexes that render one susceptible to disease. Biological explanations for the male bias in TB susceptibility include X-linked genetics and differences in immune responses, anatomy and nutrition status. The X-linked genetic factors play an important role as far as susceptibility of PTB disease is concerned. Nine genes are well understood to be associated with Mendelian susceptibility of *M. tuberculosis* disease of which 2 (IKBKB and CYBB) are X-linked and thus essentially only observed in males. The X-chromosome contains approximately 1100 genes majority of which are immunomodulatory compared to only 100 genes on the Y-chromosome [11]. The random inactivation of the X-chromosome prevents gene dosing effect in females hence chimera formation. As a result, females always express the beneficial X-linked polymorphisms inherited and are less vulnerable to deleterious mutations. On the contrary, males are at the mercy of the X-linked chromosome inherited from the mother. This is demonstrated by X-linked Toll-like receptor 8 gene polymorphisms which have been linked with susceptibility to TB particularly in male children [12, 13]. Additionally, females benefit from gene-dosing effects, where by an approximate 15% of X-linked genes escape silencing resulting into expression of certain proteins [14] and immune-modulatory miRNAs [15].

Uganda is one of the countries in the world with a high prevalence of TB disease estimated at 1,675 cases per 59,751 people representing 2.8% [16]. According to the University Research Council-USAID Defeat TB Project report, Kampala, Wakiso and Mukono (Kampala metropolitan area) are among the urban centers with the highest TB infection rates. In 2018, Wakiso had 5,108 people screened with 301 testing positive to TB, representing 6%, Mukono had 958 people tested with 62 (6%) confirmed TB positive while 14,605 people were tested in Kampala with 277 (1.9%) found infected with TB [16]. The burden of TB varies across districts but the variations are not being considered during routine assessment of the disease. This is evident in the Uganda's Ministry of Health National TB and Leprosy control Program guidelines where routine assessment of PTB clinical signs are standardized [17]. This standardization is a biased clinical assessment of the disease that cannot be applied in more diverse or less severely affected populations. In Western Uganda, the epidemiology of PTB and the segregated demographic data of sex and age were not established. Additionally, there were questions as to whether TB signs described in the past were still valid for the existing assessment of the disease. To obtain insight into matter, we carried out a study to determine prevalence of PTB disease by age group and sex and also assessed the clinical symptoms associated with PTB amongst the patient population at Fort Portal regional Referral Hospital in Kabarole district. The study was able to diagnose and enroll a number of patients on TB treatment hence reducing the risk of community PTB transmissions.

2 Material and methods

2.1 Study design and population set up

A cross-sectional study was designed to determine prevalence of PTB disease by age group and sex and also assess the clinical signs associated with PTB amongst the patient's ≥ 12 years who were attending outpatient department Fort Portal Regional Referral Hospital, Kabarole district. From April to June 2019, a total of 379 respondents were recruited into the study. Both demographic information and sputum specimens were obtained from the participants as shown in the study flow chart (*Figure 1).*

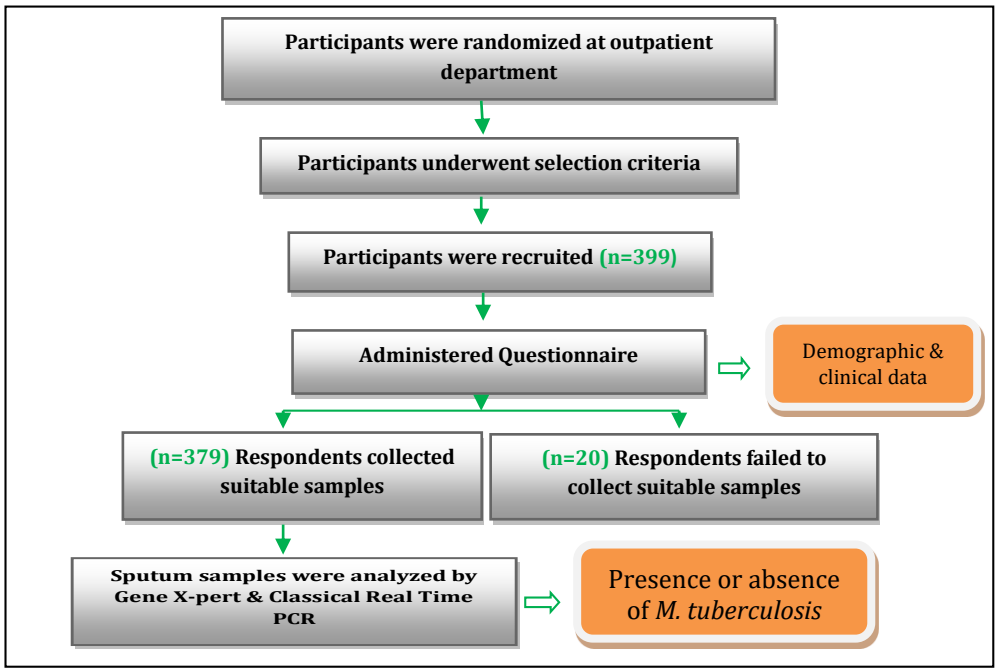

Figure 1 Flow chart summarizing the study protocols

2.2 Methods and laboratory analyses

5mls of each sputum sample were collected in falcon tubes. 2mls of each sputum sample were processed and immediately tested for *M. tuberculosis* by Gene X-pert method at Fort Portal Regional Referral Hospital. 2 to 3mls of the each remaining sputum sample was transferred from falcon tube to Norgen's sputum DNA collection, preservation and isolation kits [18] for *M. tuberculosis* Classical Real Time PCR analysis. To obtain accurate and reliable results, every sample was double tested using Gene X-pert and Classical Real Time PCR.

2.2.1 Gene X-pert

The assay [19] was carried out by the following way; 1ml of sputum sample was mixed with 2mls of Gene X-pert sample reagent, shaken several times, and incubated for 15 minutes at room temperature as per manufacturer's instructions. The mixture was transferred into the Gene X-pert cartridge and inserted into the Gene X-pert machine. Cycle thresholds of 5 *rpoB* gene probes automatically reported the presence of *M. tuberculosis* using the GeneX-pert Dx software, version 2.1. Gene X-pert pert assay semi- quantitatively estimates the concentration of bacilli as defined by the cycle threshold range.

2.2.2 Classical Real Time PCR

This technique involved two steps; DNA extraction and amplification

DNA extraction

DNA was extracted using Norgen's isolation kit, following manufacturer's recommendations, manufactured by Norgen Biotek Corporation, Canada.

DNA amplification

M. tuberculosis Classical Real Time PCR was performed with A BioRadiCycler IQ5 Thermal cycler using Applied Biosystems TB sequence detection system in the presence of SYBER -Green. Primers used were for IS6110 (Gene Bank No. X52471), designed from Primer Express Software, 2.0 (Biosystems), obtained from Applied Biosystems, Warrington, UK. The nucleotide sequences of the primers were: 5'- CCGAGGCAGGCATCCA-3' (position 1062 to 1077) and 5'-GATCGTCTCGGCTAGTGCATT-3' (position 1112 to 1132). The fluorescent dye used was Syber Green. The optimization of the real time PCR reaction was performed according to the manufacturer's instructions (Applied-Biosystems, user bulletin 2 applied to the SYBR-Green I core reagent protocol) but scaled down to 25 µl per reaction. The PCR conditions were standard (SYBR-Green I core reagent protocol) and all reagents were provided in the SYBR-Green I core reagent kit, including AmpliTaq-GOLD polymerase (Applied-Biosystems). PCR amplification was carried out in 30 µl 10 mMTris-

HCl buffer, pH 9.0, containing 50 mMKCl, 0.1% (v/v) Triton X-100, 1.5 mM MgCl2, 0.2 mM dNTPs, 5 µl of extracted DNA, 5 µM of each primer and 2 units *Taq*DNA polymerase. PCR conditions consisted of an initial denaturation step of 95°C for 5 minutes, followed by 24 cycles of 95°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute, with a final extension of 72°C for 10 minutes. PCR samples and controls were run in triplicate to ensure reproducibility of results. Quantification analysis of PCR products was performed using Bio-Rad IQ5 1.0 software.

2.3 Data analysis

All data was analyzed using descriptive and analytical statistics

3 Results

A total of 399 participants were recruited for the study and 379 (95%) respondents were able to produce suitable sputum specimens. 14 and 6 respondents produced unsuitable (salivary) samples and no samples respectively. Of the 379 respondents, 205 (54%) were males while 174 (46%) were females. 38 (10%), 68 (17.9%), 208 (54.9%) and 65 (17.2%) of the participants were under age groups; 10-19 years, 20-29 years, 30-39 years and ≥50 years respectively. All PTB cases were new (undetected by the health system) with no history of anti-TB treatment.

3.1 Prevalence of PTB disease in Western Uganda

According to the study, Prevalence of PTB refers to the proportion of study participants who tested positive for PTB on both Gene X-pert and Classical Real Time PCR assays and expressed as a percentage. Therefore, overall prevalence of PTB in western Uganda was 2.6% (10 out of 379), which is lower than Uganda's TB prevalence of 2.8% [16].

3.2 Prevalence of PTB disease by age group and sex

Table 1 Prevalence of PTB by age group of the respondents

Age group of respondents (years)	PTB prevalence, n (%)
10-19	0 (0.0)
20-29	4 (5.9)
30-49	4 (1.9)
≥50	2 (3.1)
P-value	0.24

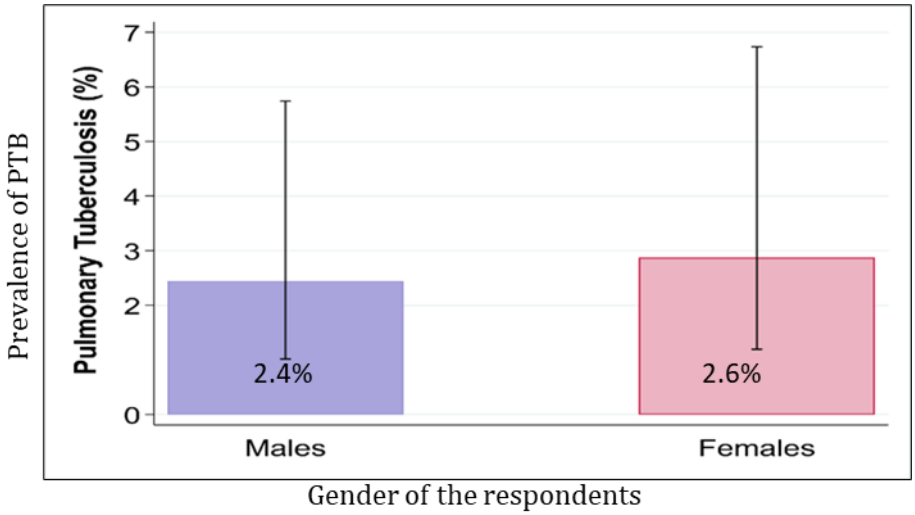

Figure 2 Prevalence of PTB by sex of respondents

Prevalence of PTB by age group was 0 (0.0%) in 10-19 years, 4 (5.9%) in 20-29 years, 4 (1.9%) in 30-39 years, 2 (3.1%) in ≥50 years old individuals *(Table 1)*. Prevalence of PTB by sex was 5 (2.4%) in males and 5 (2.9%) in females *(Figure*

2). The prevalence amongst females was highest (2/ 8.7%) in age group ≥50 years old and amongst males was highest (3/ 7.0%) in 20-29 years old individuals *(Figure 3).* There was no significant relationship between PTB disease and demography; - age group, sex and PTB disease (p = 0.24)

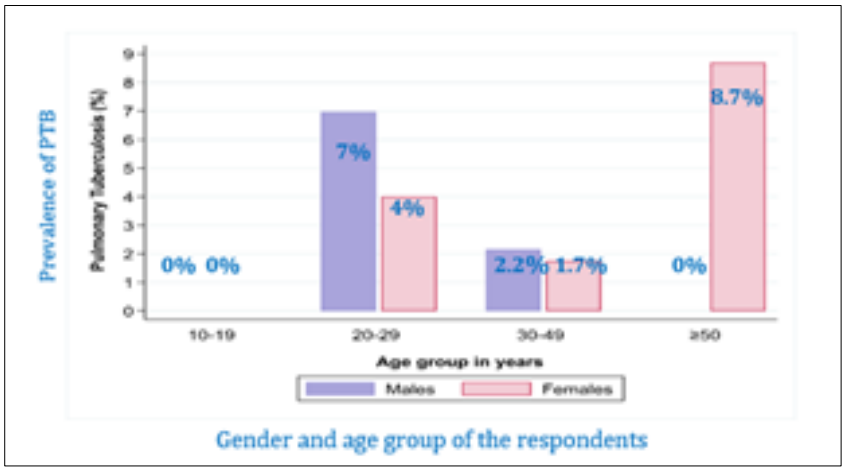

Figure 3 Prevalence of PTB by both age group and sex of the respondents

3.3 Clinical symptoms associated with PTB disease

Out of 379 study participants, 73 had at least one symptom representing 19%. 31 (8.2%) of the participants had noticeable weight loss, 23 (6.1%) had night sweats for ≥ 3 weeks, 11 (2.9%) had cough for ≥ 2 weeks and 8 (2.1%) had persistent fever. 10 of the 379 participants tested positive for PTB of which 10% had one symptom (cough) while 90% had cough and at least one other symptom (night sweats, weight loss or fever). 100% (8 out of 8) of the participants who had fever tested positive for PTB, 90.9% (10 out of 11) of the participants who had cough for ≥ 2 weeks tested positive for PTB, 21.7% (5 out of 23) participants who had night sweats for ≥ 3 weeks tested positive for PTB and 16.1% (5 out of 31) who had noticeable weight loss were diagnosed with PTB. The relationship between PTB disease and clinical symptoms; - cough, persistent fevers, noticeable weight loss and night sweats (P values; <0.001, <0.001, 0.001 and <0.001 respectively) was significant.

4 Discussion

Prevalence of PTB disease in populations can vary due to demographic differences like sex and age. This study analyzed the prevalence of PTB amongst the population in Western Uganda and it has been revealed that prevalence of PTB was highest in age group 20-29 years old. This age bracket comprises of the youth whose behavior and active participation in social activities predisposes them to TB infection. The youth are fond of traveling frequently, have more social contacts and spend more time in settings that may be favorable to PTB transmission such as bars, cinema halls or night clubs. Conversely, No PTB case was recorded in age group 10-19 years old. This age bracket is composed of teens whose movements are controlled by either their home families or study educational institutions. The restricted movement trend was visible amongst the study participants where by teens accounted for the least recruitment. For the advanced age groups (30-39 years and ≥50 years old), the high prevalence of PTB was most likely influenced by two factors. Firstly, majority of the enrolled study participants were under the said brackets and this increased chances of the diagnosed PTB cases. Secondly, the age brackets consisted of the elderly with high possibilities of having other morbidities that predispose them to PTB disease. Our results differ from the WHO TB (2019) report [3] that shows increased chances of developing TB amongst people of advanced ages. Hence, a more quantitative study should be carried out in a larger population to elaborate more on the role of age specific factors in PTB disease. Analysis by sex showed that; prevalence of PTB was slightly higher in females than males despite men dominating the study. Females have less travels, social contacts and have more protective immunomodulatory genes on the X-chromosome [11], so we would expect them to have fewer PTB cases than males. The most probable reasons for the higher PTB bias in females were the age associated factors like immunosuppression and underlying medical conditions given the fact that most of the cases observed were amongst the advanced age group (≥ 50 years old). On other hand, males of 20-29 years old had the highest number of PTB cases. This trend was linked to the active behavior and social activities associated with this youthful male age bracket. The study found out no significant relationship between PTB disease and demography; - age group and sex. Our study population was small to give satisfactory evidence therefore we recommend a wider study into the age factor that we believe plays an important role in the development of PTB disease.

The study found out an association between PTB disease and clinical symptoms. All PTB positive cases presented with clinical manifestations of persistent fevers, coughs for ≥ 2 weeks, night sweats and noticeable weight loss in the hierarchical order. All patients who had fever were diagnosed with PTB making the symptom an important indicator of the disease. During PTB disease, the interaction between *M. tuberculosis* or endogenous pyrogens (cytokines) such as interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-α with the organum vasculosum of the lamina terminalis (OVTL) leads to production of fever. The OVTL is highly vascular and lacks a blood brain barrier, permitting it to be stimulated by *M. tuberculosis* and other pyrogenic substances [20]. Its stimulation leads to increased synthesis of prostaglandin which acts on the hypothalamus resulting into an increase in body temperature [20]. Although short term fevers may be beneficial, prolonged fevers tend to extract a metabolic cost with depletion of muscle mass and essential nutrients leading to malnutrition that ultimately weakens the immune system [21]. We also observed those participants who tested positive for PTB without fever had cough and any other symptom (night sweats or noticeable weight loss). Meanwhile, more than half of the participants with fever also had night sweats. When the human body is responding to cytokines induced by *M. tuberculosis*, the hypothalamus resets body temperature to higher level for a while. Later, body temperature returns to normal, and extra heat is lost by sweating. This shows that prediction of presumptive PTB requires more than one clinical symptom.

Cough was observed in almost 90% of the participants who tested positive for PTB. We also found out that 10% of the non-coughing patients who tested negative for the disease lacked other PTB clinical symptoms. In people with PTB disease, coughing reflex is stimulated by *M. tuberculosis* organisms that are predominantly transmitted via cough, initiated by respiratory tract nociceptive neurons [22]. The cell wall of *M. tuberculosis* consists of a complex lipid called sulfolipid-1 that stimulates nociceptive receptors to produce inflammatory neural peptides that induce coughing [23]. There was a positive correlation between clinical symptoms and PTB with cough and fever being good predicators of presumptive PTB disease. Hence cough and fever are possible targets that can be explored for future better TB diagnostic innovations.

5 Conclusion

In Western Uganda, we found out that prevalence of PTB by age group was highest in 20-29 years, high in 30-39 years and ≥50 years and least in 10-19 years old individuals. Youthful behavior and active participation in social activities were factors that contributed to the highest PTB prevalence amongst the 20-19 years old. Females had a slightly higher prevalence of PTB than males due to health challenges associated with advanced age of ≥50 years. There was no relationship between PTB disease and demography; - age group and sex. All PTB cases presented with persistent fevers, coughs for ≥ 2 weeks, night sweats and noticeable weight loss in the hierarchical order. There was significant relationship between PTB disease and clinical symptoms; - cough, persistent fevers, noticeable weight loss and night sweats. Cough and fever are important future PTB diagnostic possibilities.

Recommendations

The study recommends the following; 1- A wider population study into the age factor that is believed to play an important role in the development of PTB disease. 2-Studies onto the cough and fever to explore possible biomarkers, which can be used to develop rapid, and easy to use screening kits for PTB disease.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

Ethical clearance was sought from the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, the Research Ethics Committee of Mbarara University and the Uganda National Council of Science and Technology. Written permission was obtained from Kabarole district Directorate of Health services and Buhinga hospital.

Statement of informed consent

Informed consent was obtained from all the respondents who participated in the study. All their information was kept confidential.

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