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Prevalence of Latent Tuberculosis Infection (LTBI) among House Hold Contacts of Sudanese Patients with Pulmonary Tuberculosis in Eastern Sudan: Revisiting the Tuberculin Skin Test

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Abstract

A third of the world's population is latently infected with TB with an increased risk of developing active TB. Household contacts (HHCs) of pulmonary TB cases are at a greater risk of developing disease. Early identification and treatment of latent TB infected individuals may reduce progression to active TB. This study aimed to determine latent TB infections (LTBI) point prevalence among HHCs and community contacts (CCs) using Tuberculin Skin Test (TST) and whole blood IFN-γ release assay in an area of high TB prevalence. In a prospective, longitudinal and community-based study and following informed consent, 768 volunteers (HHCs n = 245; CCs n = 523) were enrolled. Tuberculin Skin Test (TST), whole blood stimulation with PPD and IFN-γ levels determination using ELISA were performed. Mean ages of HHCs and the CCs were not significantly different (HHCs 35.6 ± 15.7 and CCs 30.6 ± 11.7 years; $p = 0.99$), with a Male:Female ratio of 1:2. Mean recruitment TST indurations were 4.6 ± 5.5 mm and 2.8 ± 3 mm for HHCs and CCs respectively ($p = 0.000$). Follow-up (Day 614) mean TST indurations increased significantly to 9.1 ± 7.2 mm and 4.4 ± 3.2 mm for HHCs and CCs respectively ($p = 0.001$). Using TST indurations ≥ 10 mm, LTBI point prevalence for HHCs and CCs was significantly different (HHCs 461/1000 and 367/1000 individuals, $p = 0.03$). The mean IFN-γ levels for HHCs and CCs at recruitment day (Day 0) were 0.66 ± 0.17 IU/ml and 0.06 ± 0.04 respectively.

The mean of IFN-γ production levels dropped significantly at Day 614 for HHCs and CCs to 0.66 ± 0.15 IU/ml and 0.02 ± 0.02 respectively ($p = 0.03$) ($p = 0.00001$). Recruitment LTBI point prevalence using IFN-γ level ≥ 0.35 IU/ml for HHCs and CCs was 440/1000 and 203/1000 respectively ($p = 0.000000001$). No correlations between TST indurations and IFN-γ levels were detected among HHCs or CCs ($p > 0.05$). TST is a simple, efficient and cheap technique for LTBI diagnosis and triaging individuals for treatment.

Keywords

Latent TB Infections, Household Contacts, Community Contacts, TST

1. Introduction

Tuberculosis is an airborne disease of poverty that mostly affects young adults in developing countries [1] [2]. In 2009, WHO reported about 9.4 million new TB cases including 3.3 million women and1.1 million TB/HIV co-infected individuals [3]. Despite successful control of primary TB infection, some bacilli remain in a non-replicating or slowly replicating dormant state for the rest of the life of the individual [4]. Five to 10% of individuals with latent TB infection (LTBI) develop overt TB disease during their lifetime, mostly within 5 years of infection. The tuberculin skin test (TST) is one of the few tests that were first introduced in the 19th century [5]. TST introduced by Mantoux has been widely used as the screening test of choice to identify individuals with LTBI for more than a century [6]. TST has some limitations: low sensitivity in immune-compromised patients and a presumed cross-reactivity with hypersensitivity in BCG-vaccinated and nontuberculous mycobacteria (NTM) infections [7]. Recently, IFN-γ release assay (IGRA) which measures the production of IFN-γ in whole blood upon stimulation with specific mycobacterial antigens, has been introduced to diagnosed LTBI, but it is not currently routinely used [8] [9]. The usefulness of TST in determining LTBI point prevalence and the sensitivity and cost-effectiveness using mycobacteria PPD for stimulation in IGRA tests have recently been demonstrated by our group [2] [10]. TST and IGRA may allow triaging individuals for chemoprophylaxis to increase case detection and reduce the burden of active TB in low-resource countries [11]. This study aimed to determine the point prevalence of LTBI in an area with high TB prevalence in Eastern Sudan using TST and the IFN-γ release assay with PPD as stimulant.

2. Materials & Methods

2.1. Scientific and Ethical Considerations

The study was scientifically reviewed and approved by the Ethics & Scientific Committees of the Institute of Endemic Diseases, University of Khartoum and the Ethics Committees of the Federal Ministry of Health, Khartoum. Written informed consents were obtained from all participants.

2.2. Study Population

Seven hundred and sixty eight individuals [HHCs n = 523; CCs n = 245] were recruited. Data was collected using a specially designed case record form (CRF) with sections for demographic, clinical and laboratory data. Index cases with smear positive TB and at least one House Hold Contact were enrolled. HHCs were included if they were: ≥10 years old, lived for ≥ six months at the same property as the respective index case, have no previous history of anti-tuberculous treatment and have signed an informed consent. Community Controls were enrolled if: age ≥10 years, have no history of tuberculosis in his/her household and without symptoms/signs of tuberculosis and have signed an informed consent.

2.3. Tuberculin Skin Testing (TST)

Tuberculin skin test (TST) was performed for all contacts by injecting 0.1 ml solution containing 5 tuberculin units (Razi Institute, Iran). The largest transverse diameters of indurations were measured 72 hours later using the ballpoint pen technique.

2.4. Whole Blood Stimulation and IFN-γ Release Assay

Five mls of whole blood were collected from all volunteers: three mls in lithium heparin tubes were alloquated into three tubes and stimulated with Phytoheamagglutinin (PHA) as positive control, PPD antigen (Spain diagnostics Ltd.) and no additive (negative control). Two mls in EDTA tubes were used for ESR measurement. The tubes were incubated for 24 hours at 37˚C in Cellestis incubator (Cellestis Ltd., Victoria, Australia) and the supernatant was collected after centrifugation and stored at −20˚C until assayed. IFN-γ in the supernatant was measured by ELISA (Koma Biotech, Seoul, South Korea) as per manufacturer instructions.

2.5. Statistical Analysis

Statistical analyses were performed using Epidemiological Information (Epi Info) software version 7.1.1.1. The levels of IFN-γcytokine and TST indurations between the HHCs and CCs were compared using student *t*-test and Chi-square tests. Pearson correlation test was used to correlate TST indurations and IFN-γ levels. *P* levels of <0.05 were considered significant.

3. Results (Tables 1-5)

A total of 768 consenting volunteers were recruited in the study; two hundred and forty five were HHCs for 87 index cases while five hundred and twenty three CCs were enrolled with a male: female ratio of 1:2. The mean ages of HHCs and the CCs were 35.6 ± 15.7 years and 30.6 ± 11.7 years respectively ($p = 0.99$). BCG vaccination as checked by the presence of scar was reported in a small percentage of volunteers (12.7%). The majority (>60%) of the study volunteers were from the indigenous tribes of Eastern Sudan [Bani Amir and Hadandawa]. The

Table 1. Baseline characteristics of the HHCs and CCs at Day 0 and Day 614 of follow up.

Variables	Total number of HHCs population (n = 245)	Total number of CCs population (n = 523)
Day 0 [Recruitment]:		
Total number screened	245	523
Age (mean ± SD)	35.6 ± 15.7	30.6 ± 11.7
Male: Female	1:2	1:2
ESR mm/first hour	54.4 ± 35.5	37.4 ± 35.9
TST (mean ± SD)	4.6 ± 5.5	2.8±3.7
IFN-γ Mean level IU/ml (mean ± SD)	0.66 ± 0.17	0.06 ± 0.04
Day 614 of Follow up:		
Number of volunteers followed up	196/245 (80%)	272/523 (52%)
Age (mean ± SD)	30.1 ± 11	36.1 ± 15.0
Male: Female	1:1.3	1:2
ESR mm/first hour	78.3 ± 33	42.5 ± 28.5
TST (mean ± SD)	9.1 ± 7.2*	4.4 ± 3.2
IFN-γ Mean level IU/ml (mean ± SD)	0.57 ± 0.152	0.019 ± 0.021**

*Highly significant difference in Screening and follow up TST indurations ($p < 0.001$). **Mean IFN-γ level IU/ml significantly reduced on Day 614 of follow up.

Table 2. TST indurations, IFN-γ level and ESR in the study population at Day 0 and Day 614 of follow up.

Variables	House-Hold Contacts (n = 245)	Community Contacts (n = 523)	*p* value
TST (mean ± SD):			
Day 0 [Screening]	4.6 ± 5.5	2.8 ± 3.7	0.000**
Day 614 [follow up]	9.1 ± 7.2	4.4 ± 3.2	0.001**
IFN-γ Mean level IU/ml (mean ± SD):			
Day 0	0.66 ± 0.17	0.06 ± 0.36	0.001**
Day 614	0.57 ± 0.152	0.02 ± 0.22	0.000**
ESR mm/first hour: (mean ± SD)			
Day 0 [Screening]:	37.4 ± 35	42.5 ± 28.5	0.03*

Continuous variables are expressed as means ± SD. **Significant difference **Highly significant differences.*

Table 3. TST indurations and IFN-γ production on Day 0 and Day 614 in House-Hold Contacts (HHCs) in different age groups.

Age groups	10 - 20	21 - 30	31 - 40	41 - 50	≥51
TST (mean ± SD):					
Day 0	3.9 ± 2.2	4.5 ± 5.5	5.5 ± 5.7	5.3 ± 7.4	3.4 ± 1.2
Day 614	4.5 ± 3.0	4.8 ± 5.6	7.5 ± 5.3	7.9 ± 6.5	5.7 ± 7.0
IFN-γ IU/ml (mean ± SD):					
Day 0	0.018 ± 0.02	0.21 ± 0.03	0.43 ± 0.30	0 .37 ± 0.29	0.290 ± 05
Day 614	0.010 ± 0.15	0.161 ± 0.12	0.21 ± 0.37**	0.39 ± 0.2	0.55 ± 56**

*/**statistically significant differences.

Table 4. TST indurations and IFN-γ production levels on Day 0 and Day 614 in different Community Contacts (CCs) age groups.

Age group	10 - 20	21 - 30	31 - 40	41 - 50	≥51
TST (mean ± SD):					
Day 0	3.1 ± 4.3	3.6 ± 4.9	4.5 ± 5.8	4.6 ± 7.4	2.4 ± 1.2
Day 614	3.7 ± 6.0	4.8 ± 5.6	3.5 ± 4.3	3.5 ± 4.3	5.7 ± 7.0*
IFN-γ IU/ml (mean ± SD):					
Day 0	0.005 ± 0.02	0.02 ± 0.03	0.02 ± 0.03	0.023 ± 0.130	0.09 ± 0.05
Day 614	0.004 ± 0.03	0.11 ± 0.015	0.02 ± 0.124	0.020 ± 0.112	0.21 ± 0.25*

*Statistically significant differences.

Table 5. LTBI point prevalence using TST indurations (≥10 mm) & IFN-γ production levels (≥0.35 IU/ml) in the study population at recruitment [Day 0].

	LTBI Point prevalence		
	Total Study population (n = 768)	**HHCs (n = 245)**	**CCs (n = 523)**
LTBI Point prevalence using TST induration ≥ 10 mm:		**461/1000 individuals**	**367/1000 individuals**
LTBI Point prevalence using IFN-γ Mean level ≥ 0.35 IU/ml:		**440/1000 individuals**	**203/1000 individuals**

overall recruitment day [D0] mean TST induration was 4.7 ± 5 mm, while it was 4.6 ± 5.5 mm and 2.8 ± 3.7for HHCs [n = 245] and CCs [n = 523] respectively (p = 0.000). The mean TST induration on Day 614 follow-up was 9.1 ± 7.2 mm for HHCs [n = 196/245; 80%] while it was 4.4 ± 3.2 for CCs [n = 272/523 (52%) (p = 0.001). TST indurations increased in HHCs in the age groups 31 - 40 and 41 - 50 compared to those in 10 - 20, 21 - 30 and ≥51 age groups on D0, with similar increase in these groups at Day 614 but with slight reduction in the ≥51 age group. Similar results were reported for CCs but with significant increases in ≥51 age group on Day 614 (p = 0.001). Using TST induration of ≥ 10 mm, the LTBI point prevalence for HHCs and CCs was 461/1000 and 367/1000 individuals respectively (p = 0.03).

The overall D0 mean IFN-γ level was 0.085 ± 0.45 IU/ml compared to 0.217 ± 0.87 IU/ml at Day 614. D0 mean IFN-γ production levels were 0.66 ± 0.17 IU/ml and 0.06 ± 0.04 for HHCs and CCs respectivelyitdropped significantly for CCs at Day 614 [HHCs 0.57 ± 0.15 IU/ml; **CCs 0.02 ± 0.02**; (p = 0.03). IFN-γ release was significantly high in 41 - 50 and ≥51 age groups for HHCs on D0. IFN-γ production levels [D614] dropped in the age groups 10 - 20, 21 - 30 and 31 - 40 and continued to increase in the age groups 41 - 50 and ≥51 (p = 0.001). Mean IFN-γ Levels showed significant variable levels on D0 and D614 in different age groups. Using IFN-γ of ≥0.35 IU/ml, the point prevalence was calculated for HHCs and CCs as 440/1000 and 203/1000 individuals respectively (p = 0.000000001).

The mean ESR at D0 was slightly higher for CCs compared to HHCs (p = 0.03).

4. Discussion

Tackling the problem of Latent TB infections (LTBI) with early diagnosis and selective treatment especially among house hold contacts of patients with smear positive pulmonary can help control the disease and help reduce the risk of late presentation. TST remains the most commonly used test for LTBI diagnosis to initiate preventive treatment, this is justified by the fact that *in vivo* tests identified the presence of a number of cytokines and chemokines in TST reaction site; these include IL-4, IFN-γ, TNF-α, IL-10, IL-12, so TST indurations might capture an immune response in *M tuberculosis*-infected individuals that could be missed by the IFN-γ release assay [2] [10] [12]. Recently, Shakak and colleagues demonstrated that the reactivity of TST in duration of ≥10 mm is as good as IFN-γ release assay (IGRA) in diagnosing LTBI. In addition, it has an advantage over IFN-γ release assay since it can detect patients with progressing disease that will be missed by IGRA tests. These patients usually have evolving immune responses that predominantly exhibit Th2 cytokine patterns with secretion of large amounts of IL-10 with no or minimum IFN-γ secretion [10]. Our results showed that LTBI point prevalence reported by TST (induarion ≥10 mm) were not markedly different from those reported by IFN-γ release assays and were higher among HHCs compared to CCs as reported previously [1] [10] [13] [14]. In addition, this study reported a higher LTBI point prevalence among HHCs and CCs compared to those reported from central Sudan [2]. This is not totally surprising, since Eastern Sudan is known to be more endemic for *Mycobacterial* diseases. The comparatively high LBTI point prevalence among CCs could further point to the high prevalence of TB in communities of Eastern Sudan. In this study, TST LTBI point prevalences were significantly higher than those reported by IGRA; this could probably mean that TST could be more sensitive than IGRA in picking up LTBI infections in area with high TB prevalence. The fact that females constituted the majority of the study volunteers, could be explained by the simple fact that females and children do not get equal share of nutrition and access to treatment in most of the communities in Eastern Sudan. Our results showed that increasing age is accompanied by increasing IFN-γ levels in agreement with previous studies [15].

5. Conclusion

TST in duration ≥10 is a simple, cheap and accurate test for the diagnosis of LTBI especially in areas of high TB prevalence. Point prevalence of LTBI reported by TST was higher than reported by IFN-γ release assay.

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Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contributions

SAO, EAGK conceived and wrote the study proposal, prepared the necessary scientific and ethics approvals and contributed to volunteers' recruitment. WSES, AMM were involved in volunteers' recruitment, care and follow-up, participated in manuscript writing and revision. All authors read and approved the final manuscript.

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Abbreviations

LTBI	Latent TB Infection
HHCs	House Hold Contacts
CCs	Community Contacts
TST	Tuberculin Skin Test
IFN-γ	Interferon-gamma
IGRA	Interferon Gamma Release Assay