Asian Journal of Medicine and Health

20(11): 1-11, 2022; Article no.AJMAH.90923 ISSN: 2456-8414

The Prevalence of Tuberculosis among BCG Vaccinated Individuals at the North West Region of Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author DJH was the designer and supervisor of the work and wrote the first draft of the manuscript. Author DSK conceptualized the idea and was the main investigator and performed the statistical analysis. Author ATF contributed in developing idea and also participated in the drafting of the manuscript. Author WKR follow-up of the student during the work. Author NTF participate in the data analysis and preparation of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2022/v20i1130521

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/90923

> Received 08 June 2022 Accepted 17 August 2022 Published 20 August 2022

Original Research Article

ABSTRACT

Aims: This study focuses as it main objective to determine the effectiveness of early BCG vaccination in preventing tuberculosis.

Study Design: It was a cross sectional descriptive study. The study population being all early BCG vaccinated individuals.

Place and Duration of Study: This study was carried out from March to June 2017 at the Saint

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Elizabeth General Hospital Shisong, situated in Kumbo Town (Bui Division), North West region of Cameroon.

Methodology: It was based on the administration of a structured questionnaire to all early BCG vaccinated individuals. Biological data were obtained from sputum collected from participants. Sputum was analyzed using Xpert MTB/RIF assay which simultaneously detects MTB-complex and rifampin resistance strain by amplifying and identifying a MTB-complex specific sequence of the rpoB gene.

Results: This finding shows an overall prevalence of 13.08% on early BCG vaccinated individuals seeking medical care at the Saint Elizabeth General Hospital Shisong. The prevalence of TB in HIV positive subjects was 22.05%. The occurrence was high among HIV subjects not living on ART, 6 (33.33%) against 3 (13%) in HIV subject on ART. This study also showed that male patients 11 (15.94%) developed TB compared to female patients 3 (7.89%). The rate of infection, 9 (28.13%) occurring within the age range of > 28 - 38 associated to the development of TB with no correlation. Drinking habit was found to be associated with the development of TB with weak correlation and the occurrence of TB do not seem to be associated drinking duration.

Conclusion: These results show the necessity of the application of a suitable measure to prevent TB in adults, especially those at high risk of developing the disease irrespective of early childhood BCG vaccination status.

Keywords: Mycobacterium tuberculosis; BCG vaccine; HIV; North West Region of Cameroon.

1. INTRODUCTION

Tuberculosis (TB) is a contagious infectious disease with chronic evolution, widespread in the population, which, untreated or improperly treated, has a significant fatality. Developing countries face widespread tuberculosis infection - up to 95% of all cases and 98% of deaths, respectively [1]. It more commonly affects the adult population in their most productive years of life. thus having economic and social consequences [2]. In 2010, it was estimated that eight million new cases of TB occur each year and Africa has the highest incidence rate [3]. In the last two decades, there has been a progressive increase in the number of cases of tuberculosis across the globe due to increasing human immunodeficiency virus (HIV) incidence, low economic status of nations, migration and emergence of resistant strains of tuberculosis bacillus [4]. It has been reported in the literature that about one third of infected patient with IHV also have TB coinfection [5-9]. The means used to prevent and control TB include: improvement of socioeconomic conditions, early case finding and treatment, chemoprophylaxis, and vaccination [10]. Bacille Calmette-Guérin (BCG) vaccine is a live attenuated vaccines used against TB. It is derived from a strain of *Mycobacterium bovis* that was first attenuated by Calmette and Guérin at the Pasteur Institute in Lille, France [11]. This vaccine was introduced as one of the neonatal vaccines under the Cameroon National Expanded Program on Immunization. Despite the introduction of this

vaccine and the endless effort of the National TB Control Program (NTBCP) in Cameroon, the prevalence of tuberculosis (all forms included) is of 122 per 100,000 inhabitants [12]. Thus, the BCG vaccine remains ineffective in protecting against pulmonary TB in several settings [13-14]. This study focuses as it main objective to determine the effectiveness of early BCG vaccination in preventing TB, and how that effect is modified by the presence of exogenous and endogenous TB related risk factors.

2. MATERIALS AND METHODS

2.1 Study Area and Design

This study was carried out from March to June 2017 at the Saint Elizabeth General Hospital Shisong, situated in Kumbo Town (Bui Division), North West region of Cameroon. It was a cross sectional descriptive study. The study population being all early BCG vaccinated individuals. It was based on the administration of a structured questionnaire to all early BCG vaccinated individuals and, sputum samples collected from the eligible participants for the detection of MBTcomplex and Rifampin resistance strain.

2.2 Study Population

All early BCG vaccinated individuals seeking medical help at the Saint Elizabeth General Hospital Shisong integrated day care Centre.

2.3 Selection Criteria

2.3.1 Eligibility criteria

Individuals with visible BCG vaccination scar presenting with: signs and symptoms of TB, TB test request by Doctors on consultation, people at risks of developing TB disease irrespective of ethnic group seeking medical help at the hospital.

2.3.2 Inclusion criteria

All patients that fulfilled the eligibility criteria, sign the consent form as a volunteer, with no payment involvement and also accept to participate in the study

2.3.3 Exclusion criteria

All patients that didn't fulfilled the eligibility criteria, refusal to participate in the study.

2.3.4 Sample size calculation

The minimum acceptable sample size was 4 as calculated using Lorenz formula [15]. But to make the study more credible, sample size was set at 107.

 $N = (p)(q)(Z)^2/d^2$

Where N = sample size, Z = the normal distribution value for which a = 0.05 P = prevalence in the level of country [16]. d = degree of precision (level of acceptable error), q = 1 - p. Using Z = 1,96 p = 266 / 100000, d = 0.05. N = 4

2.4 Method of Data Collection

Questionnaires were administered to participants whose consent has been obtained and sputum samples collected in sterile scraw-cap container from.

2.4.1 Data collection technique

Consent forms and questionnaires were administered to participants. The consent forms were to explain the aim, objectives and procedures of study to the participants in order to obtain their consents. The questionnaires that addresses: the socio-demographic characteristics of participants; participant's knowledge concerning tuberculosis; factors influencing contact with tuberculosis; sign / symptoms of tuberculosis; participant's medical antecedents with respect to tuberculosis and laboratory findings on sputum collected.

2.4.2 Sputum samples collection

Each participant was to go away from other people either outside or beside an open window before collecting the specimen. This was to help protect other people from contracting the pathogens when they cough. The sterile screwcap containers were not to be open until patients are ready to use. Participants had to gargle with distilled water to rinse their mouths. They had to take deep breaths through their mouths and cough up mucus from deep in their chests. They had to open the jar and hold it close to the mouth, spit the mucus into the jar, without putting any outside the jar, and screw the container so it wouldn't leak. After collection, the sample was labeled and transported immediately to the laboratory for analysis. The analysis was carried out using the GeneXpert device to detect the Mycobacterium tuberculosis complex and the rifampin-resistant strain.

2.5 Transport and Conservation of Sputum Samples

Sputum samples after collection were labeled and transported immediately to the laboratory for analysis. Unanalyzed samples were stored at 2 to 8°C before processing whenever possible.

2.6 Laboratory Analysis of Specimens for Detection of MBT/RIF Resistance

Detection of MBT/RIF resistance has been made by the Xpert MTB/RIF Assay. The standard operating procedure of the method was clearly previously described by the laboratory.

2.6.1 Principle of the Xpert MTB/RIF Assay procedure

The Xpert MTB/RIF Assay is an automated *in vitro* diagnostic test using nested real-time PCR for the qualitative detection of MTB-complex and RIF resistance. The primers in this test amplify a portion of the *rpoB* gene containing the 81 base pair core region. The probes are designed to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

2.7 Statistical Analysis

Descriptive statistical analysis of data was done with the help of Epi info 7.2.0 program and Excel 2007. Data analysis were done firstly by calculating frequencies and medians and results presented in the form of tables.

3. RESULTS

3.1 Sociodemographic Characteristics of the Study Population

A total of 107 participants were recruited in this study coming from North West Region, South West Region and West Region of Cameroon. Out of this number, North West recorded the highest with 91 (85.05%), West Region with 12 (11.21%) and South West Region having 4 (3.74%) of the total population studied.

3.2 Distribution of the Study Population According to Sex and Age

Within a total of 107 participants who took part in this study, 69 (64.50%) male and 38 (35.50%) female. This population was of ages 18 and above. The ages were grouped in five groups; = 18, > 18 - 28, > 28 - 38, > 38 - 48 and > 48 with > 28 - 38 years scoring the highest frequency 32 (29.90%). The distribution of the studied population according to sex and age is shown in Table 1.

3.3 Marital Status of the Study Population

Out of the 107 people who were recruited in the study, they were 83 maried, 20 single, 1 divorce and 3 widow / widower.

3.4 Education Level of Study Population

Educational level of the studied population was divided into: no formal education, primary, secondary and tertiary. Within 107 participants, 12 (11.20%) had attended university, 43 (40.20%) secondary, 46 (43.00%) primary and 6 (5.60%) no formal education. The highest populated group were of primary education.

3.5 Occupation of Study Population

Distributing the studied population according to their respective domain of occupation, it was observe that out of the 107 participants who took part in this study, farmers were the most represented group with 70 (65.30%), businessmen 14, (13%), drivers 6 (5.60%), student 5 (4.70%), pastor 3 (2.80%), builder 3 (2.80%), nurse 2 (1.90%) and 1 (0.90%) each for electrician, soldier, hair dresser, and teacher.

3.6 Distribution of Study Population with Respect to TB Related Risks Factors

3.6.1 Distribution of study population with respect to HIV status

With respect to HIV status of the study population, 65 (60.75%) participants were known to be HIV negative, 40 (37.38%) HIV positive and 2 (1.87%) of unknown HIV status. Within the 40 (37.38%) known HIV positive, 22 were on antiretroviral therapy (ART). The duration on ART was divided into; four ranges in years: > 0 - 3; 8 people, > 3 - 6; 8 people, > 6 - 9; 4 people, > 9 - 12; 1 person and > 12; 1 person.

3.6.2 Distribution of study population with respect to smoking habit and duration

In a total of 107 who took part in this study 94 (87.90%) were known to be none smokers and 12 (12.10%) were smokers. Duration on smoking was divided into four ranges in years > 0-10; had 2 people, > 10 - 20; 7 people, > 20 - 30; 2 people > 30; 1 person with majority of the people falling within age range > 10 - 20.

3.6.3 Distribution of the study population with respect to drinking habit and duration

In a total of 107 who took part in this study 74 (69.16%) known not to drink alcohol and 33 (30.84%) do not. Duration on drinking was divided into four ranges in years > 0 - 10; 21 people, > 10 - 20; 7 people, > 20 - 30; 4 people > 30; 1 person with majority of the people falling within > 0 - 10 range.

3.6.4 Distribution of the study population with respect to contact with a TB case and number of person per room for the past six months

In a total of 107 participants who took part in this study, 103 (96.30%) have never live with a TB patient and 4 (3.70%) were known to have live with a TB patient. Out of 107, 64 people were known to be living 2 per room, 26; 1 per room, 16; 3 per room and 1 person living 4 per room for the past six months.

3.7 Sample Appearance and MBT/RIF Assay

Sample collected were classify into 3 categories base on macroscopic appearance as mucous purulent (MP), bloody (BL) and salivary (SAL). 88 (83.00%) of the sample collected were MP, 14 (13.20%) SAL and 4 (3.8%). In a total of 107 sample collected, 14 detect MBT complex with 3 rifampin resistance strain.

3.7.1 Socio-demographic determinant factors for occurrence of TB in the study population

These results are shown in Table 2. Stratifying age, educational level, sex and marital status with MBT/RIF assay, MBT complex was not detected within the age ranges = 18 and > 18 -28. 9 (28.13%) people had tuberculosis, within the age range > 28 - 38, 1 (3.23%) person in the range > 38 - 48 and 4 (26.67%) people within the > 48 age range with the > 28 - 38 age range being the most infected age group. Base on educational level, primary education recorded the highest rate with 10 (21.74%) people infected with MBT complex, followed by secondary with 3 (6.98%) people and 1 (16.67%) person with no formal education. No infection was detected at the tertiary level of education. By sex, infected was detected in 11 (15.94%) men and 3 (7.89%) in women. 12 (14.46%) of the MBT complex was detected within the married people, 1 (5.00%) in singles tatus and 1 (33.33%) infection detected in widow/widowers. No infection was detected in divorce status.

3.7.2 Clinical and immunological factors for occurrence of TB among the study population

The results of the clinical and immunological factors for occurrence of TB among the study population are shown in Table 3. Base on clinical and immunological determinant factors, 9 (22.50%) MBT complex infection was detected within known HIV positive status and 5 (7.60%) detected in known HIV negative. No infection was detected in the unknown HIV status group of the study population. With respect to ART, 6 (33.33%) of MBT complex infection was detected in the group not on ART and 3 (13.64%) was detected in the group on ART. Within those on ART, 2 (25.00%) was detected within the age range of > 3 - 6 years on ART, 1 (12.25%) within the age range > 0 - 3 years on ART and no

infection detected within the > 6 - 9, > 9 - 12 and > 12 age ranges on ART.

3.7.3 Host and environmental determinant factors for occurrence of TB among the study population

These results are shown in Table 4. Stratifying host and environmental determinant factors with MBT/RIF assay, 5 (41.66%) people were infected amongst smokers. The age range of > 10 - 20recorded 4 (80.00%) people with MBT complex infection out of the 5 people infected followed by the > 30 age range with 1 (20.00%) person infected and none detected in the other age ranges. Within the population known to drink alcohol, 3 (24.24%) and 3 (37.50%) people within the age ranges > 0-10 and > 10 - 20 respectively were infected. 1 (12.50%) person each for > 0 -10 and > 0 - 10 age ranges. Within four participants with history of contact with TB case, 1 (25.00%) was infected. Base on number of people per room, 8 (57.14%) people with infection was detected in people living 2 per room, 3(21.42%) in those living 3 per room, 2 (14.28%) in those living 1 per room and 1 (7.14%) of infection detected in those living 4 per room.

4. DISCUSSION

In 2011, 8.7 million active TB cases were reported worldwide, from which 1.1 million (13%) had HIV infection. Almost 79% of HIV infections were located in Africa [17-21]. This study focuses on determining the prevalence of tuberculosis on early BCG vaccination individuals, and how it is influence by the presence of TB related risk factors in patients assessing health care at Saint Elizabeth General Hospital The results obtained from this study could contribute to the management and control of tuberculosis and HIV. The presence of tuberculosis was confirmed by detecting the bacterial genetic materials in raw sputum using Xpert MTB/RIF assay. The Xpert MTB/RIF Assay simultaneously detects MTB-complex and RIF resistance by amplifying a MTB-complex specific sequence of the rpoB gene. Finding from this study shows that out of a total of 107 participants who took part in this study, 14 was diagnose positive with tuberculosis with 03 resistant strain to rifampicin, one of the first line drug against TB. This finding shows an overall prevalence of 13.08% on early BCG vaccinated individuals seeking medical care at the said Hospital. This high prevalence could be attributed to several parameters: potential

Indicator	Frequencies	Percentages	
	Sex		
Male	69	64.50	
Female	38	35.50	
Total	107	100.00	
	Age		
=18	01	00.90	
> 18 - 28	28	26.20	
> 28 - 38	32	29.90	
>38 - 48	31	29.00	
>48	15	14.00	
Total	107	100.00	

Table 1. Sex and age distribution of the study population

Table 2. Socio-demographic determinant factors of TB in the study of population

Variables	Frequency	Percentage	MBT	MBT not	R^2	P-
	(N)	(%)	detected	detected		value
			[N (%)]	[N (%)]		
Age					0.02	0.12
=18	01	00.90	00 (0.00)	01 (100.00)		
>18 - 28	28	26.20	00 (0.00)	28 (100.00)		
> 28 - 38	32	29.90	09 (28.13)	23 (17.88)		
>38 - 48	31	29.00	01 (3.23)	30 (96.78)		
> 48	15	14.00	04 (26.67)	11 (73.33)		
Total	107	100.00	14 (13.08)	93 (86.29)		
Education level					0.05	0.02
No formal education	06	05.60	01 (16.67)	05 (83.33)		
Primary	46	43.00	10 (21.74)	36 (78.26)		
Secondary	43	40.20	03 (6.98)	40 (93.02)		
Tertiary	12	11.20	00 (0.00)	12 (100.00)		
Total	107	100.00	14 (13.08)	93 (86.92)		
Sex					0.01	0.24
Male	69	64.49	11 (15.94)	58 (84.06)		
Female	38	35.51	03 (7.89)	35 (92.11)		
Total	107	100.00	14 (13.08)	93 (86.92)		
Marital Status					0.00	0.10
Married	83	77.57	12 (14.46)	71 (85.54)		
Single	20	18.69	01 (5.00)	19 (95.00)		
Divorce	01	00.93	00 (0.00)	01 (100.00)		
Widow / widower	03	02.80	01 (33.33)	02 (66.67)		
Total	107	100.00	14(13.08)	93 (86.92)		

Variables	Frequency	Percentage	MBT	MBT not	R^2	P- Value
	(N)	(%)	detected	detected		
			[N (%)]	[N (%)]		
HIV status					0.05	0.02
Negative	65	60.75	05 (7.60)	60 (92.31)		
Positive	40	37.38	09 (22.50)	31 (77.50)		
Unknown	02	01.87	00 (0.00)	02 (100.00)		
Total	107	100.00	14 (13.08)	93 (86.92)		
Subjects					00.02	0.48
on ART						
No	18	45 .00	06 (33.33)	12 (66.67)		
Yes	22	55.00	03 (13.64)	19 (86.36)		
Total	40	100.00	09 (22.50)	31 (77.50)		
Duration						
on ART						
>0-3	08	36.36	01 (12.50)	07 (78.50)	0.02	0.48
>3-6	08	36.36	02 (25.00)	06 (75.00)		
>6-9	04	18.18	00 (00.00)	04 (100.00)		
>9-12	01	04.54	00 (00.00)	01 (100.00)		
>12	01	04.54	00 (00.00)	01 (100.00)		
Total	22	100.00	03 (13.63)	19 (86.36)		

Table 3. Clinical and immunological factor of TB stratify by MBT/RIF assay of the study population

Table 4. Host and environmental determinant factor for occurrence of TB among the studypopulation

Variables	Frequency	Percentage	MBT	MBT not	R^2	P-
	(N)	(%)	detected	detected		value
			[N (%)]	[N (%)]		
Subject					0.08	0.04
smoking						
NO	95	87.78	09 (9.47)	86(90.53)		
YES	12	11.21	05 (41.66)	07(58.33)		
TOTAL	107	100.00	14 (13.08)	93(86.92)		
Smoking					0.11	0.30
duration						
(year)						
>0-10	02	16.66	00 (0.00)	02(100.00)		
>10-20	07	58.30	04 (57.14)	03(42.88)		
>20-30	02	16.70	00 (00.00)	02(100.00)		
>30	01	08.30	01 (100.00)	00(00.00)		
Total	12	100.00	05 (41.66)	07(58.33)		
Subject					0.04	0.03
drinking						
No	74	69.16	06 (08.11)	68(91.89)		
Yes	33	30.84	08(24.24)	25(75.75)		
Total	107	100.00	14(13.08)	93(86.92)		
Drinking						
duration					0.12	0.05
(years)						
>0-10	21	63.60	03(14.29)	18 (85.71)		
>10-20	07	21.20	03(42.86)	04 (57.43)		
>20-30	04	12.12	01(25.00)	03 (75.00)		
>30	01	03.03	01(100.00)	00 (00.00)		

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Variables	Frequency	Percentage	MBT	MBT not	R^2	P-
	(N)	(%)	detected	detected		value
			[N (%)]	[N (%)]		
TOTAL	33	100.00	08(24.24)	25 (75.76)		
Subject in contact with TB case					0.00	0.48
No	103	96.30	13(12.62	90 (85.38)		
Yes	04	03.70	01(25.00)	03 (75.00)		
Total	107	100.00	14(13.08)	93 (86.92)		
Number of persons/room					0.03	0.08
1	26	24.29	02(7.70)	24 (92.31)		
2	64	59.80	08(12.50)	56 (87.50)		
3	16	15.00	03(18.75)	13 (81.25)		
4	01	00.93	01(100.00)	00 (0.00)		
Total	107	100.00	14(13.08	93 (86.92)		

classification of BCG vaccination status by the presence of the scar, reactivation of latent infection during adulthood, rapid progression of primary infection or the reinfection. In the context of the study, it was difficult to directly attribute this elevation to one of these parameters. The pathogenic mechanisms of tuberculosis imply different immune responses, so the high prevalence of TB in early BCG vaccinated individuals could vary related to each of these parameters [22-26]. The prevalence of TB in HIV positive subjects was 22.05%. This prevalence was similar to 22% recorded in a similar study carried out in Cali and Medellin cities of Colombia among people living HIV [27]. The occurrence was high among HIV subjects not living on ART, 6 (33.33%) against 3 (13%) in HIV subject on ART. Similar results were obtained by Musa et al. in Nigeria [28]. Statistical analysis show an association with correlation between ART and occurrence of TB ($r^2 = 0.06$, P = 0.14). The high prevalence amongst HIV positive patients could be due to the fact that this study was conducted in an HIV treatment centre with many HIV positive patients, 40 (37.38%) to 65 (60.75%) none HIV patients who come for ART refill. This could also be due to the National HIV treatment policy where patients before ART initiation must be tested and treated for TB two week if diagnose positive with TB before ART is initiated to avoid combine toxicity of drugs. In this study, history of contact to MBT complex was statistically insignificant associated with TB (r^2 = 0.00, p-value = 0.48). This was consistent with that of a similar study conducted in Addis Ababa [29]. This study showed that male patients 11 (15.94%) developed TB compared to female patients 3 (7.89%). This may be attributed to the fact that men were the most represented group in

this study 69 (64.49%) compare to 38 (35.51%) female. It could also be due to the fact that men often migrate to towns in search of jobs where they live with friends or live in TB high incidence areas. Marital status was not significantly $(r^2 =$ 0.00, p-value = 0.10) associated with TB, inconsistence with similar studies in Ethopia and west Africa [30]. This may be due to high diversity in the marital status of the study population; married 83 (77.57%), single 20 (18.69%). Marital status is one of the factors whose impact on HIV infection has always been studied but contradictory findings have been reported. Some parameters that can influence this factor include a lack of economic independence . lack of formal education, the use of contraceptives, low knowledge about HIV transmission and prevention [31-32]. This study show high rate of infection, 9 (28.13%) occurring within the age range of > 28 - 38 associated to the development of TB with no correlation ($r^2 =$ 0.02, p-value = 0.12). At this age group, in the search of employment, young people are more active and move from rural to urban areas which are areas with a high incidence of tuberculosis. Smoking habit was found not to be associate with the development of TB (p-value=0.00, r^2 = 0.08), consistence with a similar case control study in Gambia [33], but inconsistence with that of a clinic-based case-control study in West Africa [34]. This could be due to the low prevalence of smoking in the study population or could also be due to a social desirability bias whereby smokers denied their smoking status. There was an association with weak correlation between smoking duration and the development of occurrence of TB in the study population (P =0.05, $r^2 = 0.11$). The smoking duration range of > 10 - 20 record the highest infection within Hubert et al.; AJMAH, 20(11): 1-11, 2022; Article no.AJMAH.90923

smokers 4 (80.00%). Drinking habit was found to be associated with the development of TB with weak correlation (P = 0.03, $r^2 = 0.04$) and the occurrence of TB do not seem to be associated drinking duration (P = 0.05, $r^2 = 0.12$). This may be attributed to the irregularity of drinking in the study population. Educational level was found to be associated with the development of TB in this study population (p-value = 0.02, $r^2 = 0.05$), inconsistence with that of a similar study from South West Ethiopia [35]. The population of this study consisted of farmers, businessmen, drivers, students, pastors, builder, nurses, electrician, soldier, hairdresser and teacher. Each of these professions could influence pulmonary immunity to a greater or lesser extent [36-38].

5. CONCLUSION

These results show the necessity of the application of a suitable measure to prevent TB in adults, especially those at high risk of developing the disease irrespective of early childhood BCG vaccination status. Furthermore, the results of this study show that HIV infection seems to abrogate the protective effect of BCG.

CONSENT AND ETHICAL APPROVAL

The protocol was submitted to the National Ethical Committee for Human Research for an Ethical Clearance. The participants of this research have some potential risks linked to this study which include: violation of autonomy, rupture of confidentiality on the private data with regards to participants. exploitation of participants and inequality in risk/benefit ratio and also rupture of social equilibrium in community. Such potential risks were minimized by administering informed consent to participants before administering questionnaire, respecting participants. autonomy of Required the information has been encrypted and data has been restricted to all unauthorized persons by physical and electronic barriers. Authorizations were obtained from the hospital administrative authority. This study is benefit to the society as the results obtained will improve on the measures that are put in place to fight tuberculosis.

FUNDING DECLARATION

The authors received no financial support for the research, authorship, and/or publication of this article.

ACKNOWLEDGEMENTS

Overwhelming gratitude to the Director of ST. Elizabeth General Hospital Shisong Sr Kinyuy Mary Aldrine, for the acceptance and authorisation of this study to be carried out in the said Hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/90923