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Comparative Evaluation of Three Screening Methods (Conventional, Molecular and Immunological) in the Diagnosis of Overt and Latent TB Infection in Mixed Population in Cross River State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Challenges in TB diagnostic methods is the major global set back in the fight against TB infection, and the reason for increase in TB new cases baseline to 7.5 million in 2022. Investigating the efficiency and the sensitivity of combinational (Tuberculin Skin Test (TST), Acid Fast Bacilli (AFB),

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and GeneXpert (GXPT)) TB screening methods in the detection of overt, latent and HIV co-infection in mix population was the major focus of this study. Sampling areas were; Akamkpa (AKP), Calabar Municipal (CCM) and Ogoja all in Cross River State. Three hundred and ninety (390) individuals were enrolled in the exercise, 180 concerted for TST administration, of which 128 (77.11 %) were TST Positive (TST P+), 80 (62.5 %) males and 48 (37.5 %) females. All TST tested individuals 50 (27.78 %) in AKP came out positive for TST, 38 (21.11 %) males and 12 (6.67 %) females. In CCM, 20 (11.11 %) TST P+ were male, 19 (10.56 %) females and 5 (2.78 %) negative. Of the 34 (18.89 %) tested in OGO, 17 (9.44 %) were negative (16 (8.89 males and 1 (0.56 females), only 14 (7.78 %) were TST P+ for males, with no positive TST female among them. At *P* = 0.05, there was no significance different in TST positivity of males to females. There was a significance increase in the rate of TST P+ cases with induration diameter (ID) 5-0, 9-13 and >13 mm across the experimented locations. This was not applicable to ID 6-9, though had a higher mean value of 20.5 ± 8.6. The research report significance AFB positive data with a two tail value of 0.19** for age 39-48 and 0.22 * for age > 59 years compare to a younger age like 18-28 years. A repeated 2 in 4 AFBHIV co-infection at lower ID of 5.0 mm were observed with a higher mean score of 10.17 ± 5.8 in OGO. The research data also shows better TB detection output with the use of combinational (TST, AFB and GXPT) screening technique. In this study, GXPT was 82.8 % sensitive in detecting TB positive cases from the 128 (71.11 %) TST P+ individuals while sputum microscopy detected 64.1 % TB P+ cases from that same poll. Again GXPT was effective in diagnosing 50.5 % TB from 210 individuals that enrolled for AFB while sputum microscopy screened 39.1 % of that number. But, the combinational sensitivity synergy was 89.5 % efficient suggesting that, this method could serve as an improve TB screening method for detection of LTBI as well as HIV co-infection with lower induration diameter. It could also serve as a better management of positive cases and ensuring early commencement of treatment.

Keywords: Mycobacterium tuberculosis; overt and latent tb; afb sputum microscopy; tuberculin skin test (tst); genexpert tb screening technique; akamkpa, calabar municipal and Ogoja.

1. INTRODUCTION

Tuberculosis (TB) is a contagious and severe airborne disease caused by *Mycobacterium tuberculosis.* It is a gram positive, slow growing, non-motile, non-spore forming obligate aerobic bacterium belonging to the family *Mycobacteriaceae* [1]. This family is divided into *Mycobacterium tuberculosis* complex (MTB) where the causative agent of TB belongs, non-tuberculosis mycobacteria (NTM) and *mycobacterium leprae* (causative agent of leprosy). It primary target is the lungs, although other parts such as the kidney, spin and brain may be involved [2].

TB infection can be actively express when the causative agent causes clinical manifestations such as; persistent coughing, chest pain, coughing up blood, fatigue, weight loss, night sweat etc. and can spread from one person to another when an infected person cough, sneeze or talk. It can also be latent, this is when the bacterium remains in an inactive state but can becomes active under favorable environmental conditions [3-4].

One of the major WHO report in 2023 highlight significant development and challenges in the fight against TB infection. Enumerating that 7.5 million people newly diagnosed with TB in 2022 is the highest since the inception of global TB monitoring in 1995 surpassing the pre-COVID baseline of 7.1 million in 2019.

Despite the improvements, TB remained the second leading cause of death underscoring the ongoing severity of TB epidemic worldwide [5]. There is a call for re-strategized measures in TB screening/detection methods, employing a more rapid and combinational methods to mitigate public health set-back caused by resurgence TB infection [6-8]. Looking at the diagnosis, challenges of single diagnostic methods such as delay in result delivery, visiting hospital more than three times for a single test result, false positive cases as well as false negative cases results in inadequate case detection and treatment [9]. Hence the present study aimed evaluating a combinational diagnostic methods in TB screening in mixed population. The mix population here comprised; none infected, actively and latently infected individuals. This method is justified by its ability to screen both latent and active TB infection in apparently healthy and at risk individuals.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

A total number of three hundred and ninety (390) apparently healthy individuals participated in this exercise. One hundred and twenty each from both Akpabuyo and Calabar Municipal while one hundred and fifty was sampled from Ogoja making a total of three hundred and ninety (390) participants. In Akpabuyo, 43 females, 77 males, 82 males, 68 females in Ogoja and 69 females, 51 males in Calabar Municipal making a total number of 180 females , 210 males, all in Cross River State. The consent of individuals were sorted and permission obtained before participation in the exercise.

2.2 Administration of Tuberculin Skin Test (TST)

Since the research focuses more on investigating a more reliable TB detection strategy through the use of combinational screening strategy that has the ability of detecting latent state TB infection among the at risk group in a mix population, TST was considered an epidemiological tool for latent TB infection (LTBI) surveillance in the experimented environment (Akpabuyo, Calabar Municipal and Ogoja). A vial of purified protein derivative (PPD) usually 0.1 Tuberculin unit (TU) (Arkray Healthcare PVC. Ltd India)) was injected intradermal into the forearm of an individual. Previously exposed individual mounts an immune response to *M. tuberculosis* in the skin injected and inoculated with the bacterial proteins within 48-72 hours after injection. Diameter of (a palpable raised, hardened area) of ≥ 5 mm across the forearm perpendicular to the long axis in millimeters after the above-mentioned hours of inoculation was considered positive.

2.3 Sputum Smear Microscopy

All sputum samples from all experimental sites were collected at the spot into 60 mL universal container and carried in an ice-cool pack and transported immediately to Infectious Disease Hospital (IDH) Calabar. Sputum sample was divided into two equal part, one portion mixed with the help of applicator stick and evenly spread over a central area of about 10-20 mm on the slide using a continuous rotational movement. The prepared slide was placed on a dryer with smeared surface upwards, and air dry for about 30 minutes. The slide was heat fixed, allowed to cooled before the addition of carbol fuchsin stain. The smear was heated until vapor begins to rise (i.e., about 60°C) and allowed for 5 minutes. The stain was washed off with a running clean tap water.

Smear was decolorized with 3 % v/v acid alcohol for 2-5 minutes until the smear was sufficiently decolorized, the slide was again washed and excess water tipped off before counterstaining. Slide was flooded with malachite green stain for 1-2 minutes before washing. Thereafter, the back of the slide was wiped clean and allowed to dry by placing on a draining rack to air dry. The Smeared slide was examined microscopically, using the X-100 oil immersion objective for systematic scanning and affirmation of bacilli.

2.4 Gene Xpert Test

About 4 mL of Xpert MTR/RIF sample reagent (LOT 0047C470) was added to 2 mL of the second portion of the sputum (2:1V/V). A paper towel soaked in hypochloride acid (HCL) was used to shake the wide mount universal cup containing the specimen. The container was shaken vigorously and repeatedly for 10-20 times. The shaking was done twice before incubating for 15 minutes. At the expiration of the incubation period, a 2 mL Pasteur pipette was used to aspirate 2 mL of liquid portion of the sample and loaded on the cartridge port slowly to minimized aerosol. The lid of the cartridge was closed, the bar coad of the specimen cartridge scanned using the bar coad scanner (Voyager CG 9540) and loaded into the GeneXpert machine. Sample identification (S-ID) was keyed in, and start test command instructions selected on the computer monitor attached to the GeneXpert machine to begin operation. Positive sample takes 1h: 25 minutes while negative result takes 1h: 14 minutes to get ready.

2.5 Human Immuno-Deficiency Virus (HIV) Screening

A prolonged state of HIV is responsible for the development of acquired immunodeficiency syndrome (AIDs). HIV or AIDs is characterized by immune depression and a proliferating platform for TB infection. This screening was employed in this study to detect the number of participant that were Serology-positive and or co-infected with TB for immediate commencement of treatment and evaluation of TB co-infection.

The thumb was properly cleaned with 70 % ethanol, lancet (pamoja.co.na) was used to prick the thumb for blood collection unto a sample pad (marked by the arrow symbol), few drops of chase buffer was applied and result read after or within 15 minutes.

One red visible bar on control window absence on the patients' window was interpreted as negative result and the latter was interpreted positive. No bar on both windows was regarded as invalid and repeated. All results after the stipulated time were not considered.

2.6 Data Analysis

The data collected were analyzed using SPSS version 20 for Descriptive statistics, unpaired T-test for comparison of the mean range of TST positive, AFB positive, and GXPT and HIV positive cases across the experimented locations. Minitab 17 software statistical package assisted in determining the correlation between the level of significance considered at 90% confidence intervals with a P-value of < 0.05 or α-0.05.

3. RESULTS

A total of 390 individuals were enrolled in this study, of this, 180 presented themselves for the TST administration. 128 (71.11 %) (128/180 X 100/1)) were TST positive, 80 (62.5 %) males and 48 (37.5%) females in all experimented location.

All TST tested 50 (27.78) individuals in AKP responded positively to PPD in TST with 38 (21.11 %) positive males and 12 (6.67%) positive females. In CCM, 44 (24.44%) candidate were given TST and 20 (11.11%) were TST P+ males while 19 (10.56 %) females, 2 (1.11 %) and 3 (1.67%) were TST negative males and females. Thirty four (18.89%) apparently healthy individuals in OGO who received TST, 17 (9.44 %) were negative, 16 (8.89%) males, 1 (0.56%) females. 14(7.78%) was the TST P+ value for males, there was no TST P+ among the females counterpart. AKP had the highest number 50 (27.78%) of TST P+ cases followed by CCM 39 (21.67%) and 14 (7.78%) for OGO (Fig. 1) There was no significant difference at P = .05, (0.08 n.s) in the positivity of males to females participants (Table 1).

Significant difference was observed in the number of TST positive cases at induration diameter (ID) of 5.0, 9-13 and >13 across experimented locations, this was not applicable to positive cases with ID range of 6-9 (mm), though had the highest mean value of 20.5 ± 8.6 (Tables 2 & 3).

The level of AFB positivity was significant within age 39-48 and > 59 with a two tail value of 0.19** and 0.22* compared to 18-28 and 29-38 age bracket (Table 4).

3.1 TST AFB, HIV-Co-infection in Akamkpa (AKP)

Two (1.11%) of 4 AFB Positive individuals were co-infected with HIV at lower ID of 5.0. 1 (0.56 %) of 10 (4.76 %) AFB positive at ID of 9-13 were serology positive. There were no AFB HIV co-infection at ID 6-9 and > 13 mm (Table 5).

3.2 TST AFB, HIV-Co-infection in Calabar Municipal (CCM)

In CCM, sputum microscopy TB screening method detected 2 (0.95%) of 4 (2.22%) TST P+ individuals which were all serology positive at ID of 5.0 mm. At ID 6-9 mm, 31 individuals were TST Positive, AFB screened 10 (4.76%) and 2 (1.11%) serology P+ cases. Again ID > 13 mm had 3 (1.67%) TST P+ which were all detected by sputum microscopy with 1 (1.11%) serology co-infection (Table 6).

Table 1. Distribution of TST positive and Negative cases for Male and Female in All Experimented Locations (N= 180)

	Male			**Female**		
	No. tested	**TST positive**	**TST negative**	**TST positive**	**TST negative**	**Total**
Akpabuyo (AKP)	50	38 (21.11)	0 (0.00)	12 (6.67)	0(0.00)	50(27.78)
Calabar Municipal (CCM)	44	20 (11.11)	2 (1.11)	19 (10.56)	3 (1.67)	44 (24.44)
Ogoja (OGO)	34	14 (7.78)	16 (8.89)	0 (0.00)	1 (0.56)	34 (18.89)
Total	**128**	**72 (40.00**	**18 (10.0)**	**31 (17.22)**	**4 (2.22)**	**128 (71.11)**

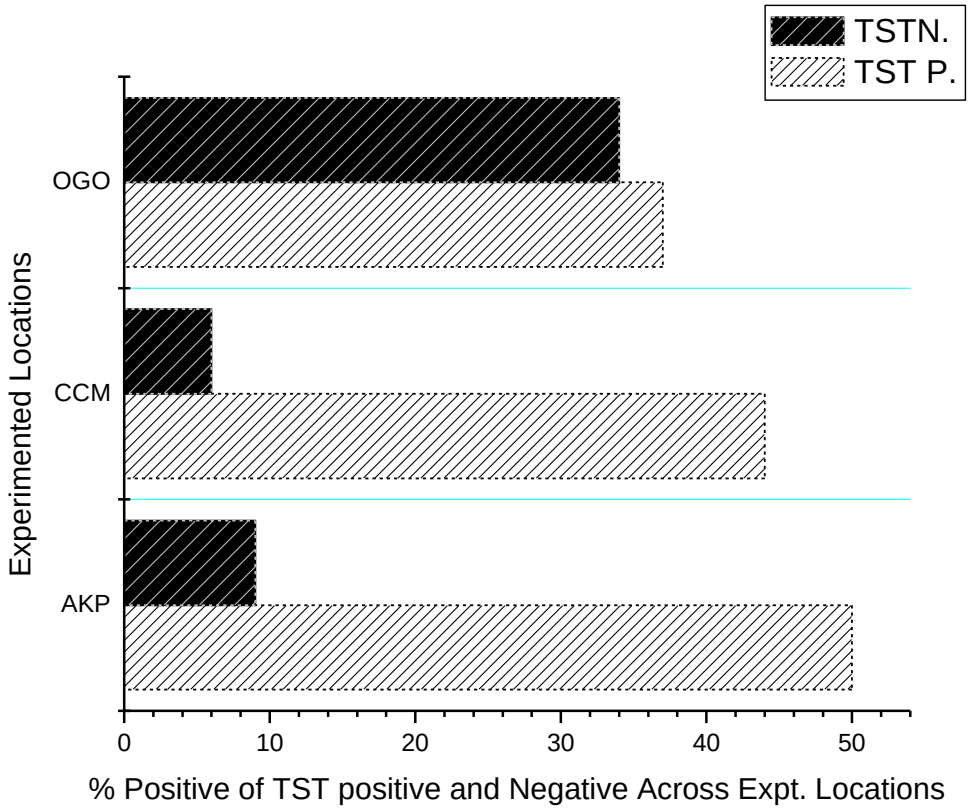

Fig. 1. Sum-total of the Distribution of Positive and Negative TST Cases in the Three Experimented Locations

Table 2. Induration Diameter and no. of TST Positives Case According Experimented Locations

Induration Diameter (Range)	AKP	CCM	OGO	P-Value (< 0.05)	Significance
5.0	8 (4.44)	4 (2.22)	13 (7.22)	0.18	**
6-9	22 (12.22)	31 (17.22)	10 (5.56)	0.09	n.s
9-13	12 (6.67)	6 (3.33)	1 (0.56)	0.2	*
>13 mm	8(4.44)	3 (1.67)	10 (5.56)	0.1	**
Total	**50 (27.78)**	**44 (24.44)**	**34(18.89)**		

Table 3. Induration Range, Mean value ± Standard Deviation of TST Positive Cases Based on Experimented Sites

Induration Diameter (Range)	AKP	CCM	OGO	Mean value
5.0	8	4	13	8.33 ± 2.6
6-9	22	31	10	20.5 ± 8.6 **
0-13	12	6	1	7.33 ± 2.4
>13 mm	8	3	10	7.00 ± 2.1
Total	**50**	**44**	**34**	

Table 4. AFB positive Cases in AKP, CCM and OGO Based on Age Bracket (N = 210)

Age Bracket (years)	AKP	CCM	OGO	Total
18- 28	6 (2.85)	0 (0.00)	10 (4.76)	16 (7.62)
29-38	12 (5.71)	8 (3.81)	6 (2.85)	26 (12.38)
39-48	8 (3.81)	0 (0.00)	18 (8.57)	26 (12. 38)
>59 (yrs.)	4 (14.29)	2 (0.95)	8 (3.81)	14 (6.67)
Total	**30 (14.29)**	**10 (4.76)**	**42 (20.0**	**82 (39.05)**

Table 5. Distribution of TST, AFB and HIV positive Cases Based on Induration Diameter in AKP

Induration Range (mm)	No. of TST positive Cases (n=180)	No. of AFB positive Cases (n=210)	No. of HIV positive Cases
5.0	8 (4.44)	4 (1.90)	2 (1.11)
6-9	22 (12.22)	12 (5.71)	0 (0.00)
10-13	12 (6.67)	10 (4.76)	1 (0.56)
>13 (mm)	8 (4.44)	8 (3.81)	0 (0.00)
Total	**50 (27.78)**	**34 (16.19)**	**3 (1.67)**

Table 6. Distribution of TST, AFB and HIV positive Cases Based on Induration Diameter in CCM

Induration Range (mm)	No. of TST positive Cases (n=180)	No. of AFB positive Cases (n=210)	No. of HIV positive Cases
5.0	4 (2.22)	2 (0.95)	4 (2.22)
6-9	31 (17.22)	10 (4.76)	2(1.11)
10-13	6 (3.33)	5 (2.38)	0 (0.00)
>13 (mm)	3 (1.67)	3 (1.43)	1(1.11)
Total	**44 (24.44)**	**20 (9.52)**	**7 (3.87)**

Table 7. Distribution of TST, AFB and HIV positive Cases Based on Induration Diameter in OGO

Induration Range (mm)	No. of TST positive Cases (n=180)	No. of AFB positive Cases (n=210)	No. of HIV positive Cases
5.0	13 (7.22)	8 (3.80)	2 (1.11)
6-9	10 (5.56)	3 (1.43)	1(0.56)
10-13	4 (2.22)	3 (1.43	0 (0,00)
>13 (mm)	37 (5.56)	28 (13.8)	0 (0.00)
Total	**37 (20.56)**	**42 (20.0)**	**3 (1.67)**

3.3 TST AFB, HIV-Co-infection in Ogoja (OGO)

The total number of TST P+ cases at ID 5.0 were 13 (7.22 %), 8 (3.80) were captured by sputum microscopy screening technique with 2 (1.11%) HIV co-infection. Another single serology 1 (0.56 %) P+ case was detected among the 3 (1.43%) AFB P+ cases screened from 10 (5.56%) TST P+ cases with ID of 6-9 mm. There was no AFB HIV co-infection among the TST AFB P+ cases within ID 10-13 and > 13 mm.

The total mean score of all TSTAFB and HIV co-infections was higher 10.17 ± 5.8 at ID of 6-9 mm in OGO compared to other locations (Table 8).

3.4 Sensitivity of TST, AFB and GXPT in TB Detection

In AKP, the total number of TST P+ were 50 (27.78%), of this number, microscopy screened 34 (16.19%) as AFB P+ while GXPT technique detected 48 (22.86%) as GXPT P+. Forty four (24.44%) were TST P+ in CCM, sputum microscopy captured 20 (9.9.52%) and 19 (9.05%) were screened P+ by GXPT technique. Five individuals that were TST P+ were undetected by either AFB or GXPT as TB P+. Again in OGO 42 (35.56%) TST were screened TB P+ by sputum microscopy and 39 (18.57 %) by GXPT TB screening technique (Table 9).

The TB detection level of GXPT technique in AKP was 98 % sensitive, followed by OGO and CCM being the least. TB screening level of sputum Microscopy was however, higher than GXPT in OGO and CCM (Fig. 2).

4. DISCUSSION

The present study evaluates the combinational synergy of three (TST, AFB and GXPT) TB screening methods in the detection of TB in apparently healthy population within Cross River State. Higher percentage of TST P+ 80 (62.5 %) males to 48 (37.5 %) females was observed. AKP had the highest 38 (21.11 %) TST P+ cases compared to CCM and OGO respectively. There is no gender related interpretation to the level of TST positivity though in previous studies, TB and LTBI were predominant in the male population because it is suggested that males are more likely to undertake risky behaviors such as ignoring the recommended preventive measures against TB. The working principles of TST is immunological based. Thus, the immune response of an individual to purified protein derivative (PPD) irrespective of gender is activated in response to either previously vaccinated with BCG, recent exposure to MTB-infection, underlying or undetected infections with *Mycobacteriaceae'* family. The concentration of the above mentioned factors in a given locality plays a major role in the positivity outcome of TST investigation as reported by Lou et al. [10] in an experiment to determine the prevalence of positive Tuberculin skin test (TST) and associated factors among medical students at Makerere University, Uganda.

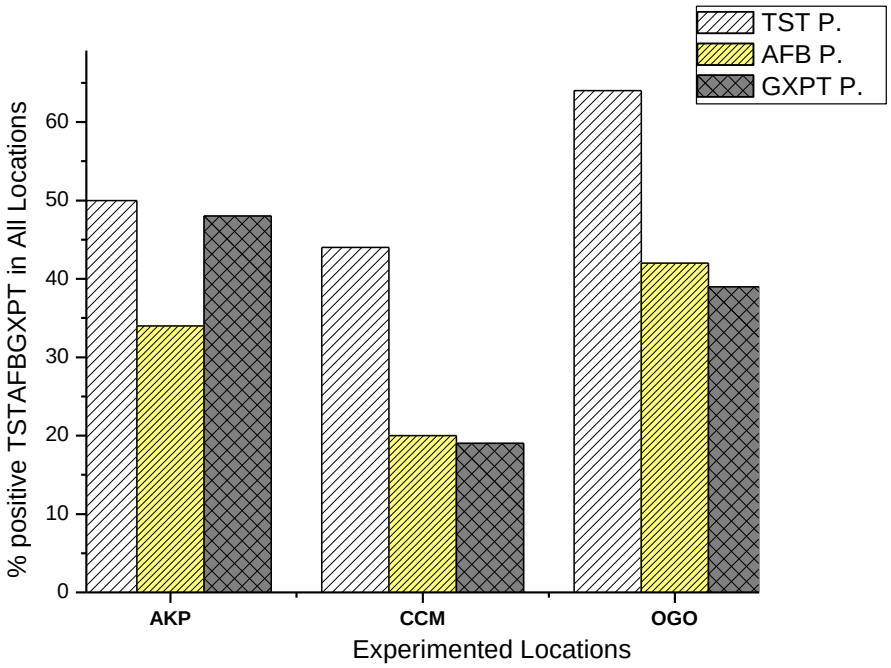

Fig. 2. The positivity outcome of AFB GXPT from TST Positive Cases in All Experimented Locations

Table 8. Mean value ± Standard Deviation of (TST, AFB and HIV positive) Cases across the Experimented Sites

Induration Diameter	AKP	CCM	0GO
5.0	3.055 ± 1.8	2.889 ± 1.6	5.508 ± 2.9
6-9	12.16 ± 7.0	17.04 ± 0.9	10.17 ± 5.8 **
9-13	8.62 ±± 4.0	3.055 ± 1.8	5.033 ± 2.9
>13 m	**5.29 ± 3.0**	**3.6056 ± 2.0**	**5.1316 ± 2.9**

Table 9. Sensitivity of TB Screening of TST, AFB and GXPT TB Screening Techniques across Experimented Locations

Induration Diameter (mm)		5.0	6-9	9-13	>13	Total
AKP	TST	8 (4.44)	22 (12.22)	12 (6.67)	8 (4.44)	50 (27.78)
	AFB	4 (1.91)	12 (5.71)	10 (4.76)	8 ***(3.81)	34 (16.19)
	GXP	7 **(3.33)	22*** (10.47)	11 **(5.24)	8 ***(3.81)	48 (22.86)
CCM	TST	4 (2.22)	31 (17.22)	6 (3.33)	3(1.67)	44 (24.44)
	AFB	2 *(0.95)	10 (4.76)	5 (2.38)	3***(1.43)	20 (9.52)
	GXP	2* (0.95)	10 (4.76)	4 (1.90)	3 ***(1.43)	19 (9.05)
OGO	TST	13 (7.22)	10 (5.55)	1 (0.56)	10(5.56)	34 (18.89)
	AFB	8 (3.81)	3 (1.43)	3* (1.43)	28 (13.33)	42 (20.0)
	GXP	8 (3.81)	3 (1.43)	3* (1.43)	25 (11.90)	39(18.57)

The significant difference at *P* =0.05 in the prevalence of AFB positive cases observed within the age bracket 39-48 and > 59 years of age has epidemiological related facts. This age group (39-48 y) comprises of young youths with high level of social interactions which increases their exposure to TB environment. At this age, there is decline in immune system which increases the risks of reactivation of latent TB infection. Social behavioral pattern and lifestyle of smoking and alcoholic consumption, high prevalence of diabetes, HIV, lack of access to health care due to high level of work commitment are some of the common demographic risks that increases the TB prevalence rate in this group of people as reported by Chen et al. [11].

The results of the study also reports high prevalence of infection at lower induration diameter (ID) of 5.0 mm across all experimented locations. There were sparely variations in the distribution of AFB-HIV co-infection in other ID (mm). HIV virus induced immune suppression by attacking and destroying the $CD4^+$ T lymphocyte cells, critical for immune responses. This automatically impaired the host ability to mount strong immune reaction to PPD in TST screening test. The adjustment threshold of 5 mm increases the sensitivity of TST in detecting latent TB in HIV-positive individual enhancing early intervention and treatment [12].

The research data also shows better TB detection output with the use of combinational (TST, AFB and GXPT) screening technique. In this study, GXPT was 82.8% sensitive in detecting TB positive cases from the 128 (71.11 %) TST P+ individuals while sputum microscopy detected 64.1% TB P+ cases from that same poll. Again GXPT was effective in diagnosing 50.5% TB from 210 individuals that enrolled for AFB while sputum microscopy screened 39.1% of that number, making a combinational sensitivity synergy of 89.6% TB detection. This is justify by the above combinational sensitivity synergy in TB detection output.

More awareness is required to better inform the public on the risk factors of latent tuberculosis in order to contain its spread and allow for a tuberculosis-free society.

5. CONCLUSION

The research report showed high prevalence of TST P+ cases among males compare to females with Akamkpa having the highest positive results 38(21.11). There was a significant difference at *P* =0.05 in the prevalence of AFB positive cases observed within the age bracket 39-48 and > 59 years of age due to a decline in the immune system caused by aging factors, which in turns increases the risks of reactivation of latent TB infection. AFBHIV-co-infection at lower induration diameter (mm) across experimented locations was also reported. The efficiency and sensitivity of TB detection from TST P+ poll was higher 82.8 % in GXPT compared to 64.1 % in sputum microscopy. Again GXPT screened 50.5 % TB from 210 individuals enrolled for AFB while sputum microscopy detected 39.1 % from the same number. But, the combinational sensitivity synergy was 89.5 % efficient. Data of the study suggest that, this method could serve as an improve TB screening method for better management of TB and ensuring proper treatment of positive cases.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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