Available online www.unicrossjournals.com

UNICROSS JOURNAL OF SCIENCE AND TECHNOLOGY, UJOST

RESEARCH ARTICLE VOL. 2(1) MARCH, 2023 ISSN:2814-2241

Date Accepted: 31st March, 2023 Pages 61-71

GENEXPERT AND ZIEHL-NEELSON MICROSCOPY IN THE DETECTION OF TUBERCULOSIS IN SELECTED LOCAL GOVERNMENT AREAS IN CROSS RIVER STATE, NIGERIA.

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Abstract

In low income-high TB burden Countries including Nigeria, there is a need for rapid dual-model screening methods for early detection, prevention and treatment of TB infection. Determining the prevalence rate of TB in 3 (Calabar South (C.S), Ogoja (OG), Ikom (IK)) selected Local Government areas of Cross River State using GeneXpert (GXPT) and Acid-Fast Bacilli (AFB) Tuberculosis (TB) screening methods taking into consideration; the gender and age range was the outmost interest of this research. AFB-stained smear of 392 yielded 170 (43.4) TB +ve among which 25.8, 17.6 and 5.1 were prevalence rates of male, female and Tuberculosis Human Immunodeficiency Virus (TBHIV) co-infected. GXPT detected 162 (43.4); 25.8 male, 15.6 female in C.S. In OG, of 388 samples, AFB detected 157 (40.5);25% male, 15.5% female while 167 (43.0);26% male and 17% females, 7.2TBHIV co-infected were detected by GXPT. In IK, though lower (193) samples were analyzed, 89 (46.1); 22.8, 23.3 male and female screened by AFB while GXPT diagnosed 73 (37.8);16.6 and 21.2 % for male and female, 8.8 were TBHIV co-infected respectively. There was a significance different at P=0.05 in diagnosed TB among gender in relation to screening methods in OG. TB prevalence rate was higher 55.9, 60.0, and 53.4 % among participant of less < 38 years of age compare to 36.2, 40.0, and 30.6 % for their counterparts of age greater>39 years of age across the 3 experimented sites. Conclusively, the variations in TB detection parameters documented by the 2 screening methods encourages combination instead of single screening methods in TB detection. The results call for more emphatically awareness aim at discouraging the young people from involving in social activities that increases the risk of TB transmission.

Keywords: *Mycobacterium tuberculosis* (TB), Acid Fast Bacilli, GeneXpert, Calabar South (C.S), Ogoja(OG), Ikom (IK), Gender and Age Range.

1.0 **Introduction**

Tuberculosis (TB) is a lethal infectious disease caused by *Mycobacterium tuberculosis*. This infection affects the lungs including the respiratory organs of its victims. It is a contagious airborne disease transmitted when an infected person cough, talk, sneezes etc. TB is implicated in 1.7 billion, which is about 25% of the world's population out of which 1.5 million cases are fatal. This infection is present in every country with majority of its sufferers living in low income and middle-income countries especially in regions such as Sub-Saharan Africa and South East Asia [1, 2].

M. tuberculosis could develop latent TB infection (LTBI), this is a tuberculoid disease occurring as a result of granuloma formation. It works by creating a barrier around the pathogen in an effort to protect the host. LTBI has 5–10% chances of progressing to active TB under favorable condition. This poses a significant problem as LTBI individuals are potential future active TB cases, especially in countries that have high HIV burden.

Different challenges ranging from low specificity, sensitivity, false positive results, delay in result delivery etc. are major setback in early detection, prevention and treatment of TB in developing Countries [3]. According to Snow *et al.,* 2018 [4], historical data has proven that there is a drastically increase in risk of tuberculosis among adolescence and young adults. This is evident in habits such as; social and interactive contacts outside of house hold, migrants, refugees, homelessness, incarceration, diabetes, the use of tobacco among others as common among youth. Thus, this study undertakes to determine the prevalence rate of TB in selected local Government areas of Cross River State using GeneXpert (GXPT) and Ziehl-Neelson (AFB) taking into consideration prevalence rate in regards to gender and age range.

2.0 Materials and methods

a. Study Area

Cross River State is located within South-South geopolitical zone of Nigeria. The state was created from the eastern Region on the 27 May 1967. Its capital is Calabar, it borders to the north by Benue State, to the west by Ebonyi State and Abia State, and to the southwest by Akwa Ibom State while its eastern border forms part of the national border with Cameroon. Originally known as the South-Eastern State before being renamed in 1976, Cross River State formerly included the area that is now Akwa Ibom State, which became a distinct state in 1987.

It is often described as the tourism capital of Nigeria due to several initiatives implemented during the Duke Administration (1999-2007). This made the city the cleanest and environmentally friendly in Nigeria. Administratively, the city is divided into Calabar Municipal and Calabar South Local Government Areas. It occupies 21,019 km²with a population of 4,406,200, density of 209.6/km² [National Population Commission [NPC], 2022].

The State is composed of several ethnic groups including the Efiks, the Ejagham, Yakurr, Bahumono, Bette, Yala, Igede, Ukelle and the Bekwarra among others. The state is made up of eighteen (18) Local Government Areas. They are: Abi, Akamkpa, Akpabuyo, Bekwarra, Bakassi,

Biase, Boki, Calabar Municipal, Calabar South, Etung, Ikom, Obanliku, Obubra, Obudu, Odukpani, Ogoja, Yakuur and Yala.

b. Sample collection and preparation

I Smear microscopy

All sputum samples were collected at the spot into 60 mL universal container into an ice-cool pack and transported immediately to Infectious Disease Hospital (IDH) Calabar.

Sputum sample was divided into two equal portion, 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 wipe clean and placed it in a draining rack for air drying. Smeared slide was examined microscopically, using the 100x oil immersion objective for systematic scanning and affirmation of bacilli.

a. GeneXpert test

About 4mL 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 mouth universal cup containing the specimen. The container was shaken vigorously 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 code of the specimen cartridge scanned using the bar code scanner (Voyager CG 9540) and loaded into the GeneXpert machine. Sample identification (S-ID) was keyed in, create 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.

Human immunodeficiency (HIV) screening

The thumb of concerted individual was properly cleaned with 70% ethanol, lancet (pamoja.co.na) was used to prick the cleaned thumb for blood collection unto aAlare Determine ™sample pad (marked by the arrow symbol), few drops of chase buffer was applied and result read within 15 minutes One red visible bar on control window absence on the patients' window is interpreted as negative result and the latter is interpreted positive. No bar on both windows was regarded as invalid and repeated. All results after the stipulated time were not valid.

Data Analysis

The statistical analysis was done using Minitab version 17 for comparative (Chi square) and descriptive statistics. The significance difference at P = 0.005 in gender, age range in relation to TB screening methods was considered valid.

3.0 Results

A total of nine hundred and seventy-three (973) sputum samples were collected from concerted individuals. Out of which 392, 388 and 193 comprised of male and female collected were from Calabar South, Ogoja and Ikom respectively. Out of 392 collected from Calabar South, 51.5 % TB+ve were male, 33.2 % were female, 5.1 were TBHIV co-infected, 7.7 % were negative. TB detection based on screening methods reports 170 (43.4) TB positive out of 392, of which 25.8 % were male and 17.6 % were female detected by Ziehl-Neelson (AFB) method. GeneXpert (GXPT) detected 162 (43.4) TB positive out of 392, 25.8 % male, 15.6 % female. TB screening based on age range showed that participant of age less than <38 years were more TB positive 55.9 % compare to 36.2% recorded for individual greater than > 39 years of age. There was no significant different at *P*= 0.05 in gender and age range in relation to TB detection techniques (Table 1a and b).

Three hundred and eighty-eight (388) samples collected from Ogoja yielded 51% TB +ve for male, 32.5 % female, 7.2 TBHIV co-infected. Based on screening methods: AFB screened 157 (40.5) out of 388 samples, 25% male and 15.5 % female. GXPT screened 167 (43.0), 26 % male and 17% female. Evaluation based on age range reveals that in Ogoja community 60 % of TB +ve screened were people of less than < 38 years whereas 40 % were of age greater than > 38 years. The results showed significant different at *P= 0.05* in gender in relation to TB screening methods as screened variables based on age range showed association, this is evident in a lower P-value of 0.002 < 0.05(Table 2a and b).

Although lesser number of samples 193 came from Ikom 39.4 were TB+ve male, 44.6 female and 8.8% TBHIV co-infection. AFB screened 89 (46.1), 22.8 % male and 23.3 % female. GXPT screened 16.6 % male and 21.2 % female. Again 53.4 % TB +ve screened were of lesser age group (<38 years) whereas 30.6 % were of greater > 39 years age category, 16 % were negative. The age range showed no relationship with the detection methods (Table 3a and b).

The different levels of screening methods were: single + (low), ++(medium) and +++(high) for AFB. PVL (positive very low), PL (positive low), PM (positive medium) and PH (positive high) for GXPT. Results based on severities, gender and locations shows inconsistencies. However, at the highest level +++ of AFB results in the 3 location (Calabar South (C.S), Ogoja (O.G), and Ikom (IK)) were: 12.5, 6.4 % for male and female in C.S, 11.9, 5.4 in OG, 7.3, 10.4 for IK. Figure 1a and sum total result in figure 1b shows a higher sever TB +ve trend in C.S followed by Ogoja and less severe in IK.

Variation was also observed in the screening levels of GXPT. The highest-level PH among male and female in C.S and OG were similar; 12.5 and 6.4 % but 6.7 and 8.8 % was recorded for male and female in IK (Fig 2a and b).

TBHIV co-infection was higher 45.9, 4.12 and 5.69 % in male across the 3 locations compared to 3.06, 3.09 and 3.11 for the female counterparts (fig 3a). Sum total reveals higher TBHIV infection in IK (8.8 %), followed by C.S (7.7 %) and lower 7.2 % in OG (fig 3b).

Table 1a showing AFB and GXPT (Range) Based on Gender for Calabar South

SEX	AFB (Range)				GXPT (Range)				
	+	++	+++	-VE	PL	PVL	PM	PH	HIV
MALE	15 (45.5)	37 (58.7)	49 (66.2)	5(27.8)	15 (46.9)	4 (33.3)	37 (66.1)	49 (66.2)	18 (60.0)
FEMALE	18 (54.5)	26(41.3)	25(33.8)	13(72.2)	17(53.1)	8(66.7)	19(33.9)	25(33.8)	12 (40.0)
	33	63	74	18	32	12	56	74	30

Key:AFB=acid fast bacilli, GXPT=GeneXpert, +=single positive, ++= medium positive, +++ high positive, -ve= negative, PL- positive low, PVL=positive very low, PM= positive medium, PH=positive high.

Table 1b: showing the level of AFB and GXPT Based on Age Rangein Calabar South

AGE BRACKET	AFB (Range)				GXPT (Range)				
	+	++	+++	-VE	PL	PVL	PM	PH	HIV
< 38 YRS	21(63.6)	31(50.0)	53(71.6)	4(66.7)	20(62.5)	11 (91.7)	24(42.9)	53(71.6)	17(56.7)
> 39 YRS	12 (36.4)	31 (50.0)	21(28.4)	2(33.3)	12(37.5)	1(8.33)	32(57.1)	21(28.4)	13(43.3)
	33	62	74	6	32	12	56	74	30

Table 2a showing AFB and GXPT (Range) According to Genderfor Ogoja

SEX	AFB (Range)				GXPT (Range)				
	+	++	+++	-VE	PL	PVL	PM	PH	HIV
MALE	10 (43.5)	41(61.2)	46 (68.7)	15(93.8)	15(40.5)	12(60.0)	37(66.1)	49(66.2)	16(57.1)
FEMALE	13(56.5)	26(38.8)	21(31.3)	1(6.25)	22(59.5)	8(40.0)	19(33.9)	25(33.8)	12(42.9)
	23	67	67	16	37	20	56	74	28

Table 2b: showing AFB and GXPT Levels Based on Age Range for Ogoja

AGE BRACKE	AFB (Range)				GXPT (Range)				
	+	++	+++	-VE	PL	PVL	PM	PH	HIV

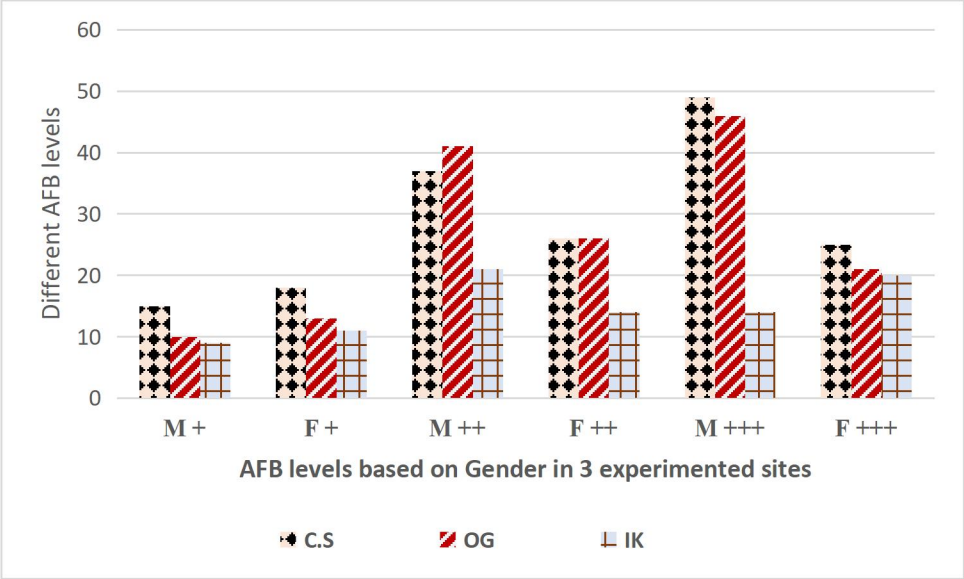
T									
< 38 YRS	16(69.6)	35(52.2)	46(68.7)	14(87.5)	20(54.1)	11(55.0)	24(42.9)	53(71.6)	15(53.6)
> 39 YRS	7(30.4)	32(47.8)	21(31.3)	2(12.5)	17(45.9)	9(45.0)	32(57.1)	21(28.4)	13(46.4)
	23	67	67	16	37	20	56	74	28

Table 3a: Showing AFB and GXPT Levels According to Gender for Ikom

SEX	AFB (Range)				GXPT (Range)				
	+	++	+++	-VE	PL	PVL	PM	PH	HIV
MALE	9(45.0)	21(60.0)	14(41.2)	5(83.3)	3(37.5)	4(50.0)	16(45.7)	13(43.3)	11(64.7)
FEMALE	11(55.0)	14(40.0)	20(58.8)	1(16.7)	5(62.5)	4(50.0)	19(54.3)	17(56.7)	6(35.3)
	20	35	34	6	8	8	35	30	17

Table 3b: Showing AFB and GXPT Levels According to Age Range for Ikom

AGE BRACKET	AFB (Range)				GXPT (Range)				
	+	++	+++	-VE	PL	PVL	PM	PH	HIV
< 38 YRS	13(65.0)	14(40.0)	27(79.4)	4(66.7)	4(50.0)	7(87.5)	20(57.1)	25(83.3)	10(58.8)
> 39 YRS	7(35.0)	21(60.0)	7(20.6)	2(33.3)	4(50.0)	1(12.5)	15(42.9)	5(16.7)	7(41.2)
	20	35	34	6	8	8	35	30	17



Key: C.S=Calabar South, OG=Ogoja, IK=Ikom, M+=Male single AFB positive, F+= single AFB positive, ++ medium positive and +++ high positive.

Figure1a: Showing higher level (+++) of AFB detection among males in IK and OG compare to females.

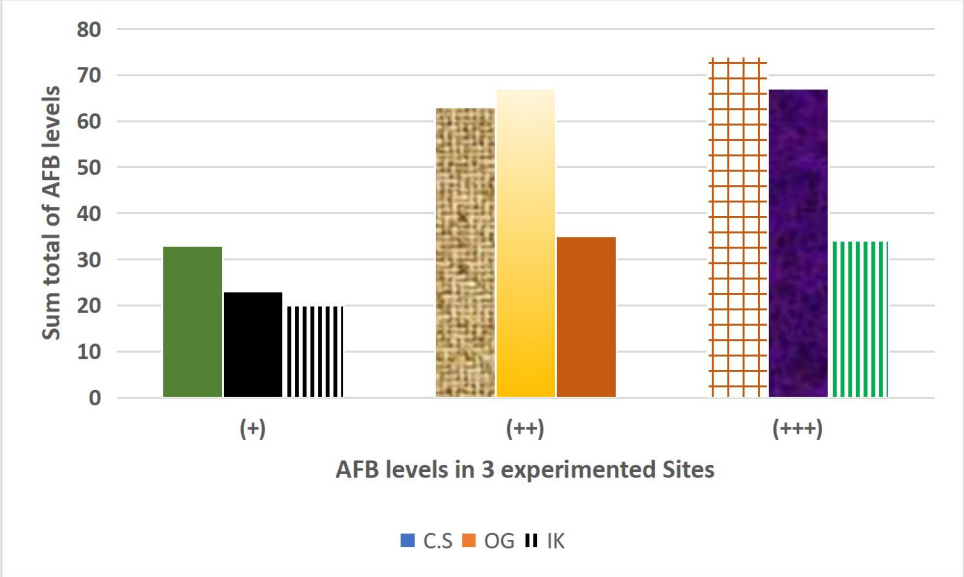
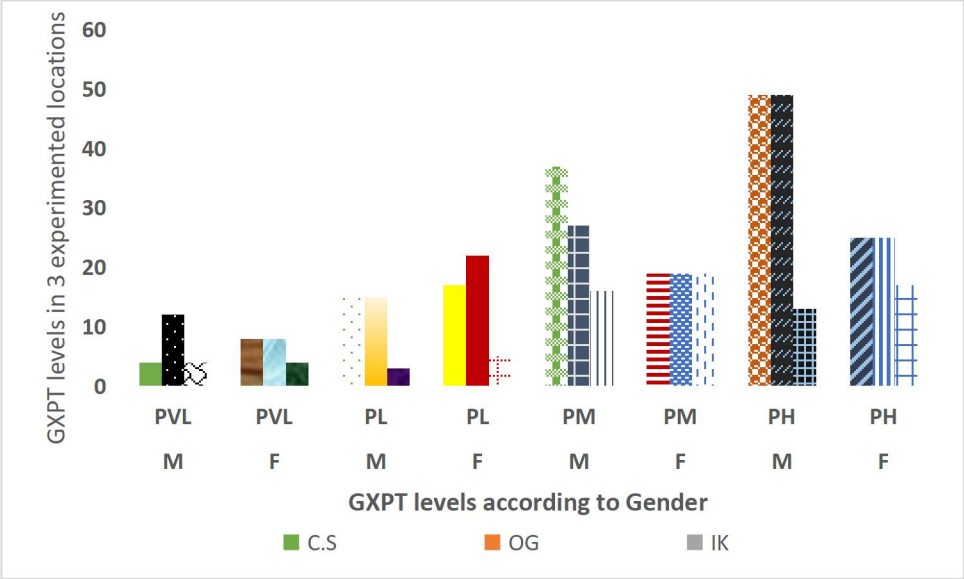


Figure1b: Shows higher severity (+++) of AFB screening in C.S followed by OG and Ik



Key: PVl=positive very low, PL=positive low, PM=positive medium. PH=positive high, M=male and F=female.

Figure2a: GXPT TB at PH and PM level was higher in C.S, OG and PM.

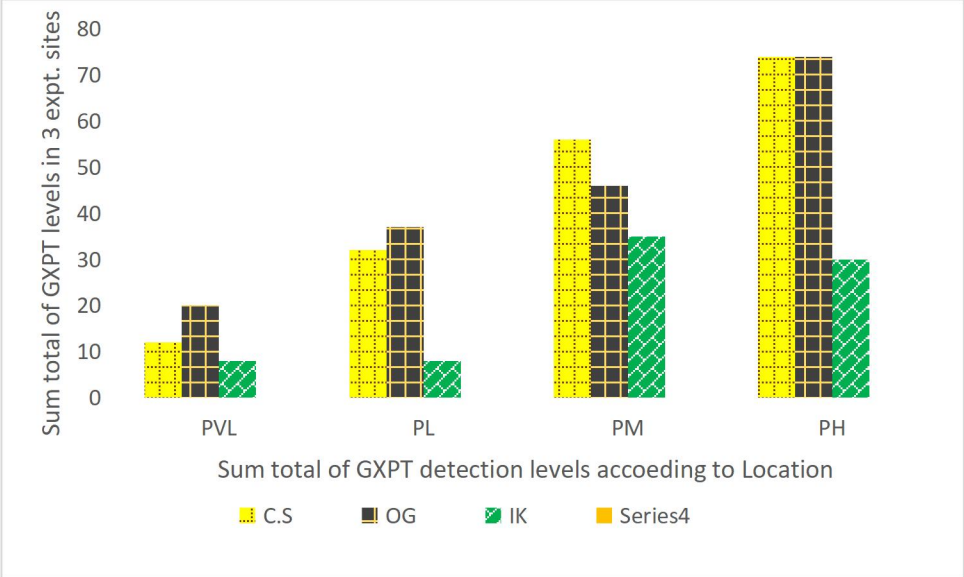


Figure 2b: Similar GXPT PH detection level in C.S and OG.

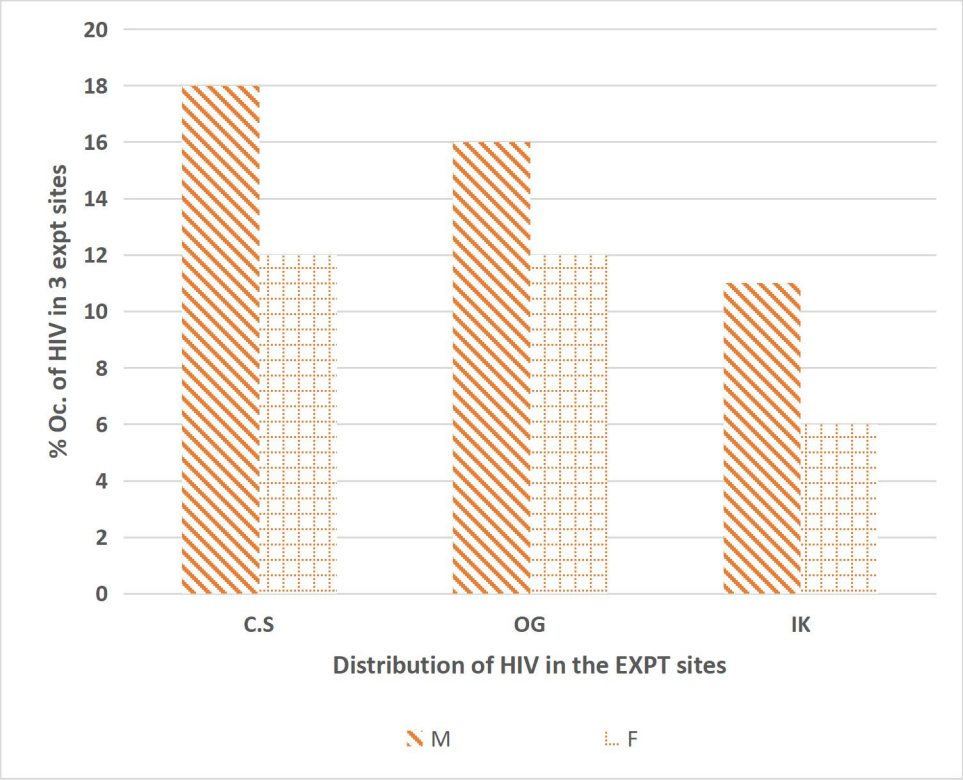


Figure 3a: Higher TBHIV co-infection among males compare to females across all experimented sites.

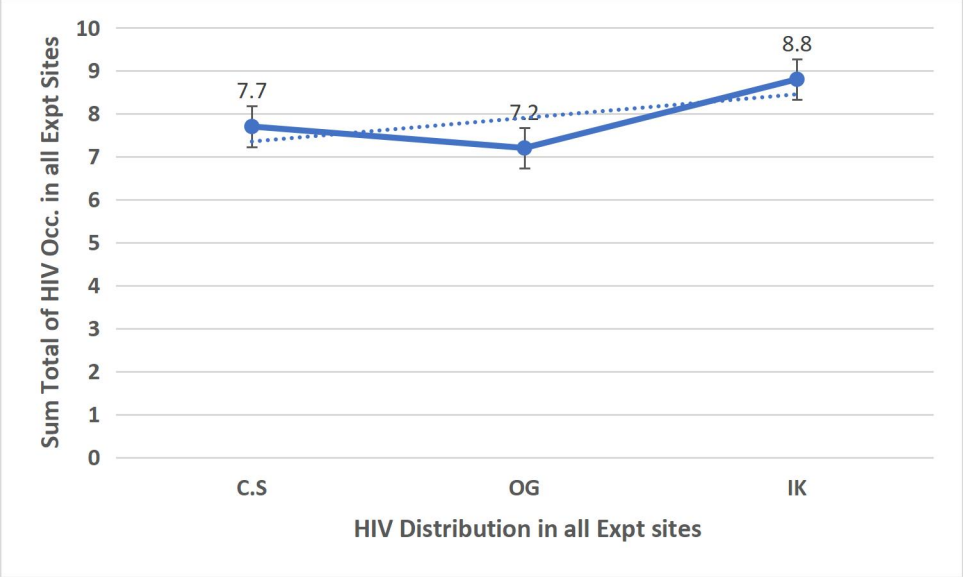


Figure 3b: Showing more TBHIV prevalence in IK among the experimented sites.

4.0 Discussion

Tuberculosis continues to remain one of the most important communicable diseases in the world. In any infection including tuberculosis, early diagnosis, prevention and treatment relies on the capacity of screening methods employ. The index study reports 51.5, 33.2 and 5.1 % as TB prevalence rate

for male, female and TBHIV co-infection in Calabar South with no significant different at *P*= 0.05 in gender and age range in relation to TB detection techniques. 51% TB+ve male, 32.5 % female 7.2 TBHIV co-infection with significance different at *P*=0.005 in gender in relation to tests method from Ogoja. 39.4 % TB+ve male, 44.6 % female, 8.8 % TBHIV co-infection from Ikom. The prevalence reports from the three locations of this study are lower than the rate 66.5 and 44.8% TB +ve for male and female reported by Boum *et al.,* 2014 [5] in Southwestern Uganda. Endemicity of an infection in a given locality determine the prevalence rate of such infection.

The two (AFB and GXPT) TB screening methods employed in this research reports as follow; AFB detected 170 (43.4) TB+ve; 25.8, 17.6 % for male and female from C.S, 157 (40.5); 25, 15.5 for male and female from OG, 89 (46.1); 22.8, 23.3 male and female IK.

The GXPT results documents 162 (43.4); 25.8, 15.6 % male and female C.S, 167 (43.0);26, 17 % male and female OG and 73 (37.8); 16.6, 21.2 % male and female IK respectively.

Different scholars including Caulfield and Wengenack (2016) [6]; Rasool, Khan, Raza and Riaz (2019) [7] document on rapid and specificity of GXPT adding that it is more advantageous for early detection over AFB and MTB culture in Punjab. Another researcher Ejeh, *et al.,* 2019a [8] and Ejeh, *et al.,* 2021b [9] reports similar submission to diagnostic techniques starting that AFB detected 18.4, GXPT 23.8 and 12.3 MTB culture in Tertiary hospital Benue State and also reported in 2012 research carried out at Federal Medical Center Benue State that AFB detected 7.3%, GXPT 16.9 % and 2.2 MTB culture respectively.

The argument of Law *et al.,* 2018 [10]is in line with the present report that despite the short comings of AFB techniques in TB screening it still remains the most widely used tool in TB diagnosis in high-burden developing countries. This method with new innovation (automated whole smear microscopy screening) improves detection level to 85.4 % with little or no Manuel work load.

TB evaluation based on age grade shows consistent increase (55.9, 60.0 and 53.4 %) among participant of age < than 38 years C.S, OG and IK compare to 36.2, 40.0, 30.6% for greater > 39 age range. This commensurate with the work of Lay cock *et al.,*2021 and Snow, *et al.,* 2018 [11 and 4] reporting high prevalence of TB in adolescent and young adults.

Historical data shows that the risk of tuberculosis increases dramatically during adolescence and young people. Wider range of social and interactive contacts outside of house hold, migrants, refugees, homelessness, incarceration, diabetes, the use of tobacco are some among the risk factors more common among young people that directly or indirectly support rapid transmission of this

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