IJAAR PUBLISHING® DOI: 10.46654

International Journal of Advanced Academic Research | ISSN: **2488-9849**
Vol. 7, Issue 10 (October, 2021) | www.ijaar.org

A Comparative Diagnosis of Breast Tuberculosis using Ziehl Neelson Stain and GeneXpert® Assay in North Central Nigeria.

Oyero S.K.[1], Onwuliri F.C.[2], Itelima J.U.[2], Abdulkadir B.[3], Adeniyi D.S.[4], Egbere J.O.[5], Dakwom N.G.[6], Eshioramhe M.P.[7], Ebeh E.O.[4], Ju G.[8]

[1] Department of Histopathology, Jos University Teaching Hospital, Jos
[2] Department of Plant Science and Biotech, Faculty of Natural Science, University of Jos.
[3] Department of Microbiology, Faculty of Science, Umar Musa Yar'adua University, Katsina
[4] APIN Public Health Initiatives, Jos, Nigeria.
[5] Department of Microbiology, Faculty of Natural Science, University of Jos
[6] North Central Tuberculosis Reference Laboratory, Jos University Teaching Hospital, Jos
[7] Department of Medical Microbiology Genomics Research Laboratory, University of Jos.
[8] The Cancer Registry, Jos University Teaching Hospital, Jos

[*]**Corresponding Author**: dadeniyi@apin.org.ng, Mobile (+2348036261703)

ABSTRACT
Tuberculosis (TB) can affect any part of the body, including the breast tissue (breast tuberculosis). This rare occurrence may be mistaken for breast carcinoma. In unique instances, both pathological states may also exist contemporaneously. This study examined 297 randomly selected formalin-fixed paraffin-embedded (FFPE) breast tissue specimens of women accessing healthcare in three states of North Central Nigeria, namely Plateau, Nasarawa and Benue, to investigate occurrence of breast tuberculosis. A staining technique - Ziehl Neelson (Z-N) and a molecular assay – GeneXpert® MTB/Rif (GX) were employed for this investigation. The Z-N staining method demonstrated the Mycobacterium tuberculosis (MTB) bacilli in 1(0.3%) of the examined breast samples while the GX detected MTB complex in 3(1.01%) of the samples. This finding reveals the comparative usefulness of these techniques in the diagnosis of breast tuberculosis in formalin fixed paraffin embedded breast tissue specimens.

Key Words - Xpert MTB/Rif, Ziehl Neelson stain, Breast carcinoma, Breast tuberculosis, Formalin-fixed, Paraffin-embedded breast tissue.

INTRODUCTION

Mammary (breast) tuberculosis is a rare manifestation of extra-pulmonary localization of tuberculosis which accounts for less than 0.1% of breast conditions in developed countries, but reaches 3–4% in regions where the disease presents with high incidence (India, Africa). It appears mostly in women of reproductive age, multiparous, lactating as well as women of older age (Spyridon *et al.,* 2012). It has been scarcely reported to infect male patients (Quaglio, *et al*, 2018). For histopathological diagnosis, presence of granulomas, caseation, and demonstration of AFB have been commonly used to define a positive test. However, the granulomas can be seen also in nontuberculous mycobacterial disease, fungal infections, brucellosis, or syphilis. Therefore, cautious interpretation is required. More so, the smallness and paucity of tubercle bacilli in tissue section make it very arduous and time-consuming searching for them in stained tissue sections. Clinical examination often fails to differentiate carcinoma of the breast from tuberculosis and a high index of suspicion is necessary. Mammography is not of much help as the findings in carcinoma in advanced stage are similar to that of tubercular lesion (Jayshree *et al.,* 2013). This study seeks to investigate the usefulness of the Z-N staining method as well as the GX MTB/RIF system assay as tool in the detection of extra-pulmonary TB (EPTB) in formalin-fixed, paraffin-embedded breast cancer specimens.

MATERIALS AND METHODS

Study Site and Study Population

This is a retrospective, cross-sectional, hospital based study. The study material comprised tissue specimens from women diagnosed with breast cancer (Bca), collected during the period from January 2017 until December 2019. These women accessed healthcare services in tertiary hospitals in three states (Plateau, Nasarawa, and Benue) of North Central Nigeria. By random selection, 279 formalin-fixed, paraffin-embedded breast cancer tissue samples were examined. The samples were analyzed at the Histopathology Laboratory of the Jos University Teaching Hospital (JUTH), the APIN Public Health Initiatives Laboratory in Jos, the Genomics & Postgraduate Research Laboratory, and at the University of Jos, Nigeria.

Tissue Sections Preparation

The cut surface of paraffin tissue blocks were placed facing down on an ice-cold plate for at least 20 minutes. A manual rotary microtome used to obtain tissue sections was thoroughly cleaned with xylene-wet tissue gauze. Cleaning was also performed in between every patient specimen sectioning using xylene- soaked sectioning brush and forceps to remove residue paraffin wax. To minimize the potential for cross DNA contamination, gloves were worn and the microtome and surrounding station were cleaned with bleach-wet gauze between specimens sectioning. In addition, for each block, a new microtome blade and pair of gloves were used. The block was trimmed at 15–30µm. Thinner tissue sections were cut at 5µm and floated onto thermostatically-controlled water bath and picked onto clean microscope glass slides before drying on a hot plate for the Z-N stain. In addition, 10µm thick sections were cut, de-paraffinzed with three changes of pure xylene; and followed with descending grades of alcohol (absolute alcohol, 90%, 70%, 50%) to remove xylene before washing in water for de-alcoholization and hydration respectively.

These sections were then collected into sterile Cryovials containing 1.2ml phosphate buffered solution (PBS).

Procedure for GX MTB/RIF Assay

To the tissue-PBS in Cryovials, 0.05ml proteinase K was added. The Cryovials were then placed on a thermomixer and operated at 1,400rpm at 70^{o}c for tissue lysis and homogenization. The preparation was carefully inspected to ensure complete homogenization. The lysis time varied depending on the consistency of the tissue section. The average lysis time observed in this work was 45 minutes. The Cryovials (containing the homogenate) were then packaged into Eppendorf® boxes, frozen at -40^{o}c and later transported in clean, strong transport boxes packed with dry ice slabs, to another laboratory for the GX system assay.

The GX Assay

Whenever possible, specimens should be transported and stored at 2–8 °C prior to processing (the maximum time for storage and processing is 7 days (WHO, 2014). Using a transfer pipette, approximately 0.7ml of the homogenized tissue specimens was transferred to a sterile, conical screw-capped tube. With the use of a transfer pipette, a double volume of the GX MTB/RIF Sample Reagent (1.4 ml) was then added to 0.7 ml of the homogenized tissue. The tube was vortexed for at least 10 seconds, incubated for 10 minutes and vortexed again for another 10 seconds. The specimen was incubated at room temperature for an additional 5 minutes. Using a fresh transfer pipette, 2 ml of the processed sample was introduced into the GX MTB/RIF cartridge. The cartridge was loaded into the GeneXpert® analyzer following the manufacturer's instructions.

Procedure for Z/N staining

Tissue section was de-paraffinized with xylene, before taken to descending grades of alcohol to remove the xylene and then hydrated in water. The section was covered with carbol-fuchsin. Gentle heat from a Bunsen burner flame was applied until vapor rose from the slide. The preparation was not allowed to boil. The Bunsen burner flame was removed and the heated stain allowed to remain on the slide for ten minutes for the carbol-fuchsin to penetrate and stain the cells adequately. The stain was then gently washed from each slide with a stream of cold water until all the free stains were effectively washed away. Following this, the stained section was differentiated with 1% acid alcohol for three minutes, rinsed again carefully with water and tilted to remove excess water. The section was thereafter counterstained with 3% aqueous methylene blue for one minute, rinsed again carefully with water, drained and dehydrated with alcohol. The stained section was thereafter cleared with xylene, mounted with DPX and examined with the light microscope. A breast tissue section with confirmed acid-fast bacilli was used as a positive control and stained alongside the test samples.

ETHICAL CONSIDERATION

Ethical clearances (Ref: FMH/FMC/MED.108/VOL.1/X; FMC/KF/HREC/232/18; JTH/DCS/ADM/127/XXVII/789) were sought and obtained from the Medical Ethics and Research Committee of the hospitals concerned, before conducting the study.

STATISTICAL ANALYSIS

For purpose of data analyses, a descriptive statistics of frequency and percentage was employed to analyze the data using SPSS statistical software (SPSS version 16, Chicago Ill, USA).

RESULTS

297 archived blocks of formalin-fixed, paraffin-embedded tissues from patients with diagnosis of Bca were comparatively examined in this study. The sample size by year and the result obtained are shown in the following Table.

Breast Cancer (Bca) Tissues investigated for Tuberculosis of the Breast by the Z/N AFB (Acid Fast Bacilli) Stain and the GX assay

Year	Sample Size	Z/N* Positive	GX* Detected	% Z/N Positive	% GX - Detected	p=0.05 (95% CI)
2017	81	0	0	0.00	0.00	
2018	102	1	2	0.34	0,67	
2019	114	0	1	0.00	0.34	0.10351
TOTAL	**297**	**1**	**3**	**0.34**	**1.01**	

Z-N*= Ziehl Neelson; GX*= GeneXpert®

At p-value of =0.05(95% confidence level), the p-value obtained from the statistical analysis is 0.10351 which is greater than 0.05, implying that there is no significant difference between the Z/N stain and the GeneXpert assay, for the identification of the *Mycobacterium tuberculosis* from the data obtained in this study.

DISCUSSION

Tuberculosis constitutes a challenge to public health globally. Effective diagnosis and control of this malady is presently lacking especially in the developing countries where conventional diagnostic tools and strategies are grossly inadequate to easily capture all latent and active TB cases and obviate the raging spread of the disease. These challenges are particularly acute in Sub-Saharan Africa which endures the highest burden of active TB, where in both the community and hospital settings, many cases of active TB and drug-resistant TB remain undiagnosed (Bates *et al*., 2013; Lawn *et al*., 2015). Although there have not appeared to be any causal link between breast tuberculosis and breast carcinoma, and there is no recorded evidence that tuberculosis is carcinogenic at any site (Bani-Hani, 2005), in this study 297 formalin-fixed paraffin-embedded (FFPE) breast cancer tissue specimens were screened for MTBC using the Z-N AFB stain and the GeneXpert® MTB/RIF molecular test. This research was conceived in view of the fact that breast cancer on one hand and tuberculosis on the other, are burdensome diseases among women, in the study locations. MTBC was detected in 3(1.01%) of the 297 specimens examined. The GX MTB/RIF assay that is currently used for the diagnosis of EPTB through analysis of biopsy specimens, including various body fluids or fresh and frozen tissues, was, in this case, found to be very sensitive. When compared with other conventional TB diagnostic methods, the GX MTB/RIF assay has a generally high specificity in a range of specimen types (Maynard-Smith *et al*., 2014; Polepole *et al*., 2017). This novel, automated, cartridge-based nucleic acid amplification test (NAAT) is considered useful for rapid molecular diagnosis of EPTB (Bowles *et al*., 2011). The smallness and paucity of tubercle bacilli in tissue section make it very arduous and time-consuming searching for them in stained tissue sections. These, coupled with lack of adequate sample and non-uniform distribution of bacteria in tissues complicate the

diagnosis of EPTB tuberculosis by AFB stain even where the lesions appear active histologically (Jayshree *et al.,* 2013). In practice, in resource-limited settings, the diagnosis of TB relies on Z-N stained specimen examined with the light microscope. Z-N stain and light microscopy are however a relatively insensitive methodology for the diagnosis of TB (Singal *et al*., 2013). This probably is why only 0.3% AFB positivity was found in this study, using the Z-N stain and microscopy method. The result of Z-N stain and microscopy is however vital for clinical and epidemiological evaluation since it gives a quantitative estimation of the number of bacilli present and thus an insight into the degree of infectivity as well as the severity of the disease. Most studies to date have used fresh or fresh-frozen tissue or biopsy specimens for GX analysis, but the routine method for handling biopsy or postmortem tissue in a diagnostic histopathology laboratory is to fix the specimen with formalin and embed it in paraffin wax. Whether fresh from the tissue processor or from archived biobanks, FFPE tissues are important diagnostic research materials as they are noninfectious, have a better-preserved cellular architecture, and hence are suitable for morphological evaluation and can be kept for a long time, making them useful for retrospective studies. In this research, the molecular detection of *Mycobacterium tuberculosis* was done on formalin-fixed, paraffin-embedded tissue specimens.

CONCLUSION

Though very rare, the coexistence of breast carcinoma and breast tuberculosis is grievous. Where it occurs, it could also create a dilemma in the diagnosis and treatment since the former often mimics the latter. This research finding suggests that though the Z-N method is useful for the diagnosis of TM, the use of molecular methods such as the GeneXpert® MTB/RIF assay on FFPE tissue may be a more reliable, effective tool for detecting EPTB.

REFERENCES

Bani-Hani, K., Yaghan, R., Matalka, I. & Mazahreh, T. (2015). Tuberculousmastitis: A Disease not to be forgotten. *International Journal of Tuberculosis and Lung Diseases*, 9(8): 920–925.

Bates, M., O'Grady, J., Maeurer, M. (2013). Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: a prospective descriptive study. *Lancet Infectious Diseases*, 13, 36– 42.

Bowles, E.C., Freyée, B., van Ingen, J., Mulder, B., Boeree, M.J., van Soolingen, D. (2011). Xpert MTB/RIF®, a novel automated polymerase chain reaction-based tool for the diagnosis of tuberculosis. *International Journal of Tuberculosis and Lung Diseases*, 15(7):988-9.

Jayshree D.P., Sanjay D.R., Parimal H. P., Parul D. S., Lata R. P., Jignesh A. P. (2013). Screening of extrapulmonary tuberculosis samples by zeihl neelsen staining in patients presenting at tertiary care hospital Ahmedabad. *National Journal of Community Medicine*, 4:11

Lawn, S.D. & Zumla, A.I. (2011). "Tuberculosis". *Lancet*, 378 (9785): 57–72.

Maynard-Smith, L., Larke, N., Peters, J.A., Lawn, S.D. (2014). Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. *BioMed Central Infectious Diseases*, 14:709.

Polepole, K., Kasonde, T., Shibemba A. (2017). Performance of the Xpert MTB/RIF assay in the diagnosis of tuberculosis in formalin-fixed, paraffin-embedded tissues. *International Journal of Mycobacteriology*, 6(1):87-93

Quaglio, G., Pizzol, D., Bortolani, A., Manenti, F., Isaakidis, P., Putoto, G., & Olliaro, P. L. (2018). Breast tuberculosis in men: A systematic review. PloS one, 13(4), e0194766.

Singal R., Dalal A.K., Dalal U., Attri A.K. (2013). Primary Tuberculosis of the Breast Presented as Multiple Discharge Sinuses. *Indian Journal of Surgery*, 75:66–7.

Smith, I. (2003). Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. *Clinical Microbiology Review*, 16(3):463-96.

Spyridon M., Dionysia L., Thomas G., Constantine D., Irini P. & Aris A. (2012). Breast tuberculosis: Diagnosis, management and treatment. *International Journal of Surgical Case Reports*, 3(11): 548–550.

WHO (2014). Xpert MTB/RIF implementation manual; Technical and operational 'how to': practical considerations. Available at: *http://www.who.int/tb/laboratory/policy_statements/en/*