International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 10; Issue 05 (D); May 2021; Page No.24494-24496
DOI: http://dx.doi.org/10.24327/ijcar.2021.24496.4860

Research Article

MYCOBACTERIAL BACILLI IN FORMALIN-FIXED, PARAFFIN-EMBEDDED BREAST TISSUE SPECIMENS OF PATIENTS HISTOLOGICALLY DIAGNOSED WITH BREAST CARCINOMA

***Oyero S.K[1]., Onwuliri F.C[2]., Abdulkadir B[3]., Itelima J.U[2] and Dakwom Nanbam G[4]**

[1]Department of Histopathology, Jos University Teaching Hospital, Jos
[2]Department of Plant Science and Biotechnology, Faculty of Natural Science, University of Jos, Jos
[3]Department of Microbiology, Faculty of Science, Umar Musa Yar'adua University, Katsina
[4]North Central Tuberculosis Reference Laboratory, Jos University Teaching Hospital, Jos

A R T I C L E I N F O

Article History:
Received 15th February, 2021
Received in revised form 7th March, 2021
Accepted 13th April, 2021
Published online 28th May, 2021

Key words:

Acid-fast bacilli, Breast carcinoma, Breast tuberculosis, Formalin-fixed tissue, Histological diagnosis, Ziehl-Neelsen staining

A B S T R A C T

Tuberculosiss (TB) of breast, caused by *Mycobacterium tuberculosis*, is a rare disease and often mimics breast cancer. The smallness and paucity of tubercle bacilli in the tissue section make it very arduous and time-consuming to search for them in stained tissue sections. This present study was performed to investigate the usefulness of acid-fast bacilli (AFB) staining in determining breast tuberculosis in breast biopsy specimens of patients histologically diagnosed with breast carcinoma (Bca). Ziehl-Neelsen staining was done for acid-fast bacilli on 333 formalin-fixed, paraffin embedded sections of breast tissue blocks. Mycobacterial tubercle bacilli were seen in only 2 (0.6%) of the 333 specimens. This study reveals that the Ziehl Neelson stain can be a valuable tool in diagnosing extrapumonary TB in formalin-fixed, paraffin-embedded specimens.

INTRODUCTION

Tuberculosis (TB) constitutes a major challenge to global public health. The ability to tackle this malady has been severely hampered by inadequate diagnostic assays. Diagnosis of extrapulmonary TB (EPTB) remains especially challenging since the number of *Mycobacterium tuberculosis* (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain (Choudhary *et al*., 2017). EPTB refers to TB involving organs other than the lungs, including the pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges, among others (Ji, 2015). EPTB has existed for centuries and is a milder form of disease in terms of infectivity as compared to pulmonary tuberculosis (Jayshree *et al.,* 2013). It is important that both the infectious and non-infectious forms of TB are diagnosed and treated as both can be fatal[5]. Mammary (breast) tuberculosis is a rare manifestation of extra-pulmonary localization of the disease 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.

Corresponding author:* **Oyero S.K
Department of Histopathology, Jos University Teaching Hospital, Jos

It has been scarcely reported to infect male patients, mainly before puberty, as well as women of older age (Spyridon *et al*, 2012). The Ziehl-Neelsen (ZN) microscopy to detect Acid Fast Bacilli (AFB) in clinical specimens is still the cornerstone and the only cost-effective tool for diagnosing TB and to monitor anti-TB chemotherapy in most resource-poor settings (Caviedes *et al*., 2012). For histopathological diagnosis, presence of granulomas, caseation, and demonstration of AFB have been commonly used to define a positive test. However, loss of host immune function can result in histopathologic findings demonstrating greater suppurative response and less well-formed granulomas. Additionally, the granulomas can be seen also in nontuberculous mycobacteria disease, fungal infections, brucellosis, or syphilis, so cautious interpretation is required. ZN stain is commonly used throughout the world and still remains the standard method against which new tests must be measured (Jayshree *et al.,* 2013). This study is designed to investigate the sensitivity of the Ziehl Neelson stain as a tool in the diagnosis of extrapumonary TB in formalin-fixed, paraffin-embedded breast specimens.

MATERIALS AND METHODS

Study Site

This is a retrospective cross-sectional, hospital based study conducted at the Jos University Teaching Hospital (JUTH); a

tertiary health facility located in Jos, Plateau state, North-Central Nigeria. This area has high rates of tuberculosis.

Study Population

The study material comprised tissue specimens from women 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, Benue) of North Central Nigeria and were diagnosed with breast carcinoma. By random selection, 333 formalin-fixed, paraffin-embedded breast cancer tissue samples were examined.

Tissue Sections Preparation

Paraffin blocks were placed cut surface down on an ice-cold plate for at least 20 minutes. A thoroughly cleaned manual rotary microtome was used to obtain sections of tissues..Cleaning was performed before each sectioning session using xylene to remove residue paraffin wax. To avoid the transfer of materials from one specimen to another, a new blade was used for each specimen. The block was first trimmed at 15–30 µm, followed by 5µm thin sections (attached to slides) obtained for Z/N staining. Ziehl-Neelsen staining was performed for acid fast bacilli on each section of tissue. All ZN-stained sections were examined under a conventional light microscope.

Procedure for Z/N staining

Tissue section was taken to water (deparaffinzed and hydrated) and then covered with carbol-fuchsin. Using a bunsen burner, gentle heat was applied until vapour rose. 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 stain has washed away. Followig 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. Section was then cleared with xylene, mounted with DPX and examined with the light microscope.

Ethical Consideration

Ethical clearance was sought and obtained from the Medical Ethics and Research Committee of yhe hospitals concerned, before conducting the study.

Statistical Analysis

A descriptive statistics of frequency and percentage was used to analyze the data using SPSS statistical software (SPSS version 16, Chicago Ill, USA).

RESULTS

333 blocks of formalin-fixed, paraffin-embedded tissues from patients with diagnosis of Bca were examined. The sample size by year and the results obtained are revealed in Table 1. Mycobacterium tubercle bacilli were seen in only 2 (0.6%) of the 333 specimens examined. Table 2 outlines the breast cancer type in which these organisms were seen, following staining by the Ziehl Neelson AFB method.

Table 1 Breast Cancer (Bca) Tissues investigated by the Ziehl Neelson AFB (Acid Fast Bacilli) Stain

Year	Sample Size	Ziehl Neelson Positive	Percentage ZN Positive
2017	81	0	0.00
2018	109	1	0.30
2019	143	1	0.30
TOTAL	333	2	0.60

Table 2 Breast tuberculosis in relation to Breast cancer (Bca) Types, determined by Ziehl Neelson Method

Bca Type	2017	2018	2019	TOTAL	AFB (%)
Invasive Ductal Ca	48	61	81	190	2 (0.60)
Invasive Mucinous Ca	12	18	27	57	0 (0.00)
Invasive Lobular Ca	7	10	14	31	0 (0.00)
Invasive Papillary Ca	3	6	8	17	0 (0.00)
Metaplastic carcinoma	3	3	4	10	0 (0.00)
Others	8	9	11	28	0 (0.00)
TOTAL	81	107	145	333	2 (0'60)

DISCUSSION

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 extrapulmonary tuberculosis by acid fast stain even where the lesions appear active histologically (Jayshree *et al.,* 2013). This present study was performed to investigate the usefulness of acid-fast bacilli (AFB) staining in breast biopsy specimens of patients histologically diagnosed with breast carcinoma (Bca). Ziehl-Neelsen staining was done for acid fast bacilli on 333 paraffin embedded sections of tissue blocks. Though it is known that the demonstration of acid fast bacilli (AFB) from breast lesions is usually difficult, it is a documented fact that the gold standard diagnosis of TM is by bacteriological culture of breast tissue or by Ziehl Neelsen (ZN) stain. However, in this study, acid fast bacilli (AFB) were identified only in 0.6% (n=2) of the subjects.

In practice, in resource-limited settings, the diagnosis of TB relies on Ziehl-Neelsen stained specimen examined with the light microscope. Ziehl Neelson stain and light microscopy is however a relatively insensitive methodology for the diagnosis of TB (Singal *et al*., 2013). This probably is why only 0.6% AFB positivity was found in this study, using the Ziehl Neelson stain and microscopy method. As observed by Kent and Kubika (1995), however, an alternative for the light microscope is the fluorescence microscopy, reported to be 10x more sensitive, since the fluorescent bacilli of *M. tuberculosis* can be seen at lower magnification and the specimen can be examined in only 25% of the time taken to read with the light microscope. It has however been difficult to implement the fluorescence microscopy in the diagnosis of TB due to the higher cost associated with purchase aside other technical and infrastructural demands.

The result of Z/N stain and microscopy is 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. The Z/N stain microscopic method is however limited by its sability to detect only the actively growing population of the bacilli (about 10^4 bacilli/ml of specimen) (American Thoracic Society, 2010), while paucy- bacillary cases often

turn out negative. [13] This study shows that the Ziehl Neelson stain can be a tool in the diagnosis of extrapumonary TB in formalin-fixed, paraffin-embedded breast specimens.

CONCLUSION

It can be concluded that result obtained from Z/N stain and microscopy is vital for clinical and epidemiological evaluation since it gives a quantitative estimation of the number of bacilli present in a specimen and thus an insight into the degree of infectivity as well as the severity of disease, hence a useful method for detecting extrapulmonary tuberculosis. Though associated with some limitation, its usefulness in especially resource-limited settings cannot be overemphasized.

References

American Thoracic Society (2010). Diagnostic standards and classification of Tuberculosis. *American Review of Respirtory Disease,* 142: 725-735

Caviedes L, Lee TS, Gilman RH, Sheen P, Spellman E, Lee EH, Berg DE, Montenegro-James S. (2000). Rapid, efficient detection and drug susceptibility testing of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. *Journal of Clinical Microbiology*, 38(3):1203-8.

Choudhary, V., Pankaj, S., Harsvardhan., R. *et al.* Tuberculosis of Breast Mimicking Breast Carcinoma: A Case Report and Review of Literature. *Indian Journal of Gynecological Oncology,* 15, 5.

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

Ji Y. L. (2015). Diagnosis and Treatment of Extrapulmonary Tuberculosis. *Tuberculosis of Respiratory Diseases (Seoul)*, 78(2): 47–55.

Kent P.T. & Kubica G.P. (19855). Public Health Mycobacteriology. A guide to the level III laboratory. *US Department of Health and Human Services*, 1: 30333.

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.

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.

How to cite this article:

Oyero S.K *et al* (2021) 'Mycobacterial Bacilli In Formalin-Fixed, Paraffin-Embedded Breast Tissue Specimens of Patients Histologically Diagnosed with Breast Carcinoma', *International Journal of Current Advanced Research,* 10(05), pp. 24494-24496. DOI: http://dx.doi.org/10.24327/ijcar.2021.24496.4860

****