Journal of Morphology and Anatomy

Research Article Open Access

Assessment of the Proliferation of Breast Cancer Cells among Women in Osogbo, Osun State, Nigeria

Iyiola S[1]*, Enweani IB[1], Ngokere AAAA[1], Avwioro OG[2], Komolafe AO[3] and Ekundina VO[4]

[1]*Department of Medical Laboratory Science, Nnamdi Azikiwe University, Awka, Nigeria*
[2]*Faculty of Science, Delta State University, Abraka, Nigeria*
[3]*Department of Morbid Anatomy and Forensic Medicine, Obafemi Awolowo University Teaching Hospitals Complex, Ile Ife, Nigeria*
[4]*Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti, Nigeria*

Abstract

Background: It was reported that 4 breast cancer patients die out of every 5 patients in Nigeria. It appears Nigerian women have aggressive breast cancer cells by their molecular biology or late presentation time.

Methods: The bio data of 154 breast cancer patients were obtained including clinic presentation time lag. The breast tissues were processed, stained with H&E and graded, representative sections were taken and MKi67 immuno-histochemical staining was performed and scored as percentage of tumour cells stained brown.

Results: Out of 154 breast cancer samples, 4 (2.6%), 62 (40.3) and 88 (57.1%) respectively were on Grades 1, 2 and 3. The mean value of MKi67 was 21.88 ± 21.19, median 15.00. The mean value for Grade 2 cancer was 13.31 ± 13.54; Grade 3 cancer was 35.83 ± 23.39. There was moderate correlation between MKI67 scoring and H&E grading ($r=0.526$, $p<0.05$). Only 65.2% of breast cancer patients came to clinic after 6 months of lumps awareness in their breasts.

Conclusion: The proliferation index of breast cancer among the Nigerian women is similar to other women from most parts of the world. The delay in presentation leads to high grades and advanced disease at presentation which eventually cause death.

Keywords: Breast cancer; Mki67; Presentation time lag; Tumour

Introduction

The rate at which breast cancer is diagnosed daily in Nigeria is alarming. In 2008, GLOBOCAN estimated 12.7 million cancer cases worldwide with 7.6 million deaths. It is also reported that 56% of these deaths occur in the developing countries in which Nigeria is among. Breast cancer accounted for 23% of these deaths. Breast cancer is the most common cancer in women worldwide. It is also the principal cause of death from cancer among women globally. Despite the high incidence rate, in Western countries, most women diagnosed with breast cancer are still alive 5 years after their diagnosis, which is due to early detection and prompt treatment [1].

In Nigeria, 100,000 new cases of cancer are diagnosed annually out of 681,000 diagnosed in Africa [2,3]. In the North West geopolitical zone of Nigeria, cancer of the breast was second to cancer of the cervix, while at University College Hospital, Ibadan [situated in the South West geopolitical zone of Nigeria]; it was the leading malignancy among women [4]. The North central geopolitical zone has reported 60 new cases of breast cancer each year that constituted 22.41% of new cancer cases registered in 5 years and accounted for 35.41% of all cancers in women [5].

Breast cancer like any other cancer can arise from a benign lesion such as fibrocystic disease or start de novo from normal breast cells [1]. It is a condition in which normal cellular regulation ceases to function, and cells in the breast are allowed to multiply unchecked and to invade adjacent tissues. For this reason, it is called invasive breast cancer. Eventually, these cells gain the ability to leave their original location (i.e. the breast) and spread to other parts of the body [e.g. axillary lymph nodes, the lungs, liver, bones and brain], where they continue to grow and disrupt normal function. This latter development is known as metastasis and is essentially the reason that breast cancer causes death.

In Nigeria, the great number of deaths from breast cancer is depressing and highly worrisome [6]. More people die from cancer every year than human immunodeficiency virus [HIV], tuberculosis and malaria combined [7]. The proliferative index of breast cancer cells vary with geographical regions and ethnicity [8]. High value of MKI67 shows an aggressive tumour therefore poor prognosis. This study is designed to study the reasons associated with high death rate of breast cancer in Osogbo, South-west, Nigeria. This is the first study on MkI67 assessment of breast cancer in Nigeria.

Materials and Methods

Area of study and sample collection

This study was approved by our institutional review board. Formalin Fixed Paraffin Embedded [FFPE] tissue blocks of samples of female patients attending Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria were retrieved from Histo-pathological archive. The patient bio data were retrieved from the histopathology request cards from year 2011 to 2016, while tissue blocks from 60 primary breast cancer patients obtained in the year 2014 and 2015 were used for immunohistochemistry.

***Corresponding author:** Iyiola S, Department of Medical Laboratory Science, Nnamdi Azikiwe University, Awka, Nigeria, Tel: 0708 161 0453; E-mail: iyiolasina@gmail.com

Received June 14, 2018; **Accepted** February 14, 2019; **Published** February 21, 2019

Citation: Iyiola S, Enweani IB, Ngokere AAAA, Avwioro OG, Komolafe AO, et al. (2019) Assessment of the Proliferation of Breast Cancer Cells among Women in Osogbo, Osun State, Nigeria. J Morphol Anat 3: 118.

Patients' data and sample analysis

Bio data of 154 breast cancer patients were obtained along with the reasons and period it took them to report at the clinic from the date of breast lump awareness on them were noted in months. All the tissues were stained with H&E and graded with Elston-Ellie modification of Scaff-Bloom-Richard [9] Methods. 60 cancer blocks, one block from a patient were selected for MKI67 Staining.

Delay at presentation time [T] was classified as follows; Less than one month (<1), one to three months (1>3), 3 to 6 months (3 > 6), 6 to 12 months (6 >12) and above months (12>).

Antigen retrieval methodology and Immuno-histochemical staining

The slides were processed for immunohistochemistry as follows; Tissues were cut at 4 µm and floated in water bath onto charged slides. The sections were allowed to dry on hot plate at 60°C for 2 hours. The slides were arranged in a slide rack, dewaxed and hydrated with distilled water. A litre of retrieval solution was boiled in a pressure pot and the hydrated slides were immersed in it, lid tightened and boiled until full pressure was reached. The samples were left to boil for three minutes, thereafter left to cool for thirty minutes at room temperature. Micropolymer detection kit from Abcam was used with monoclonal MKI67 using Diaminobenzidine Horseradish peroxidase method. Staining technique was performed according to manufacturer's instruction. Counter staining was done using Iyiola-Avwioro haematoxylin for 1 min [10].

MKI67 scoring system

The percentage of tumour cell nuclei stained brown was noted under light microscope. Values obtained for MKI67 were scored according to St. Gallen (2013) recommendation and other publications [11,12]. The classification was as follows:

Scores	Proportion
1	0–9%
2	10–18%
3	19–25
4	26+.

Statistical Analysis

The results were analyzed statistically. The mean was calculated in each case. The results were compared on quartile basis and level of significance found with student t-test.

Results

The modal age group for breast cancer patient is 41-50 years, 28% (Tables 1 and 2).

Table 2 reveals that 80.6% of grade 2 cancer patients came to hospital after 3 months while 77.2% of patients with grade 3 tumours came to hospital after 3 months.

Table 3 shows that all grade1 cancers were on low score; 71.88% of grade 2 cancers were on lower 2 quartile scores while 70.83% of grade 3 cancers were on 2 quartile high score (Figure 1).

Discussion

In this study, the age of the cancer patients ranged from 24 to 83 years. Similarly, Titiloye et al. [13] got an age range of 22 to 82 years in South-west Nigeria. Omoniyi et al. [14] reported 23-92 years in Ife south west Nigeria as well. The age range obtained in this work was similar to 24 – 99 years obtained in Germany [11]; 27-80 years in China [15]. The modal age class for breast cancer in the present study was 41-50 years old. Contrarily, Ebughe et al. [16] obtained 30-39 years in

Age	No of Patients (%)
<20	0
21-30	4(2.6%)
31-40	26(16.9%)
41-50	43(27.9%)
51-60	36(23.4%)
61-70	41(26.6%)
>70	4(2.6%)

Table 1: Age distribution of breast cancer patients.

Presentation time	Grade			Total
	1	2	3	
Count T<1	0	2	2	4
%within grade	0%	3.2%	2.3%	2.5%
1<3 months	2	10	18	30
%within grade	50.0%	16.1%	20.5%	19.4%
3<6 months	2	8	12	22
%within grade	50.0%	12.9%	13.6%	14.3%
6<12 months	0	16	15	31
%within grade	0.0%	25.8%	17.1%	20.1%
>12 months	0	26	41	67
%within grade	0.0%	41.9%	46.6%	43.5%
Count	4	62	88	154
Total	100%	100%	100%	100%

Table 2: Distribution of breast cancer grade as related to time period in months of patients' presentation at the breast clinic.

Scores	Grade 1	Grade 2	Grade 3
	Value[%]	Value[%]	Value[%]
1.	2[50]	13[40.63]	4[16.67]
2.	2[50]	10[31.25]	3[12.5]
3.	0[0]	5[15.62]	2[8.33]
4.	0[0]	4[12.50]	15[62.50]
Total	4[100]	32[100]	24[100]

Table 3: MKI67 against H&E grading.

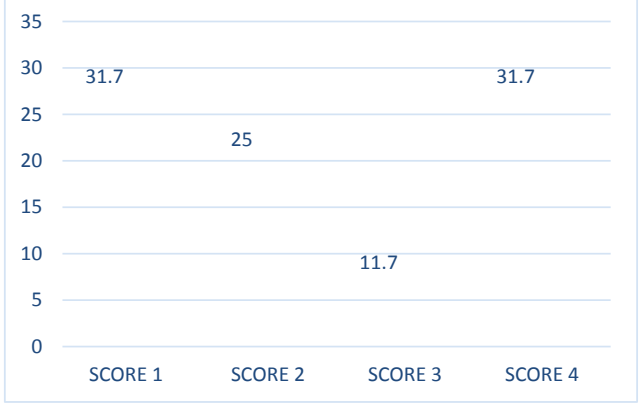

Figure 1: Staining pattern of Mki67 among malignant lesions.

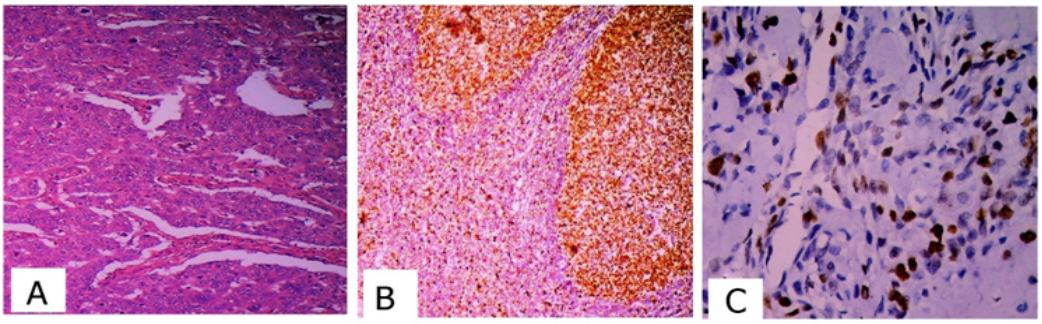

Figure 2: a) Grade 3 breast cancer; **b):** MKI67 immuno-histochemical staining of Tonsil positive control; **c):** positive staining of breast cancer cells by MKI67. The positive cells are stained golden brown to dark brown.

Calabar Nigeria. This may be due to difference in race and social life. Mean age was 52.87 years, median 52.3 years for the present study. Similarly, researchers got 50.7 years in Ife [14]; 48.62±12.41 years in South West Nigeria [17]; 45.06 years in Calabar [16]. A mean age of 63 years among German [11] and 58.7 years in Finland were recorded [18].

Breast cancers are often asymptomatic during the early stages [19] as obtained in this study in which 64% of the patients presented at the Clinic without any other complaints apart from the lump. Our result confirmed the report by Bello [20] that there were no sustained national campaign programs for breast screening in Nigeria. The high level of breast cancer awareness and breast screening did not translate to early presentation of breast cancer at the clinic in Lagos south west Nigeria [21]. Most breast cancer patients in this study visited the hospital when the lump was well developed. The presentation time for patients to attend the Clinic after lump discovery was 1 week to 3 years. Most breast cancer patients arrived the hospital after 1 year of lump awareness in their breasts, and this accounted for 69 (44.8%) out of 154 patients investigated. According to Sharma et al. [22] a delay of more than 3 months is associated with poor survival; as only 34(23.4%) of the patients presented within this period. Delay in presentation may lead to high stage, 70% of breast cancer patients presented at stages 3 and 4 in the south west of Nigeria [13]. Report by Agbo et al. [23] in Nigeria was shocking as no patient was seen with stage 1 disease in the 5 year study period. It is noteworthy that 99.4% presented with late disease (55.4%, stage 3 and 44.0% stage 4) and 0.6% [5] presented with stage 2b disease. In Ibadan Nigeria, Ntekim et al. [24] reported 2 % (5) stage 1, 13 % [24-29] stage 2 and 85% late stage 3 and 4. Similar occurrence was reported in Kumasi, Ghana [25] in which 82% of patients presented with stages 3 and 4 disease.

Our examination of breast slides revealed that out of 154 breast cancer samples, 4 (2.6%), 62 (40.3%) and 88 (57.1%) were on Grades 1, 2 and 3. In a similar manner, in Southwest Nigeria, 90% of breast cancer were on Grades 2 and 3 [13]; 99% high grade in Calabar [16]; 99% of breast cancers were on high grade in Lagos [23]; Nwafor et al. [24-26] found 50% and 37.6% to be Grades 2 and grade 3 respectively in Nigeria as a whole.

Immuno-histochemical quantification of MKI67 has revolutionized the study of mitotic index. K167 is expressed in all phases of the cell cycle i.e., G [I] S G [2] M, phase but absent in a resting G [0] phase of cells. It's presence at all phases of cell cycle has made MK167 to be an excellent proliferative cell marker. Higher proliferation rates are seen in cases of cancer. K167 is situated in the nucleolus, especially in the dense fibrillar component [27]. During mitosis K167 surrounds mitotic chromosomes preventing and maintaining the sister chromatids from being scattered in to the cytoplasm following nuclear envelop disassembly [28]. MK167 value of less than 10% is considered a good prognosis of survival for more than 10 years, while 10% to 20% is said to be fair, and more than 25% of stained tumour cells (Class 4) is an indication of poor prognosis [11]. This study revealed that 19(31.7%) out of 60 samples had a Score 1 that is, less than 10% of tumour cells were stained brown. Similarly, 19 [31.76%] of them had Score 4. The result also showed that 56.7% belong to the lower half while 43.3% belong to the upper half. The mean value was 21.88%, median value was 15.0% and range from 0-70%. The mean and median values were similar to the one obtained among Germans [11] in which the Mki67 mean was 20.3±18.1 and median 15, range from 0-99%; mean 26.2%, median 20.5% in Japan [29]; mean of 20% in Japan [30]; mean 18% among the Norwegian [31]; mean 20% among the Sudanese [32] but different from mean 26.9% obtained in Pakistan [33] and mean 26.7±22.9% among German [34]. On quartile basis, our result was between the 36% upper two quartile reported among the Italian women [35] and 47.6% reported by Sullivan and colleagues [8]. Also, very similar to 62.2% reported among Germans [11].

The relationship between H&E grading system and the scoring using MKI67 showed moderate correlation in value ($r=0.526$, $p<0.05$). The result showed that grading and scoring were positively related. The mean value for Grades 1, 2 and 3 were 6.75, 13.31 and 35.83 respectively. Breast cancer in Nigeria had a low mitotic index on Grade 2 when compared with 16% obtained in Germany [11] and 26.97% ± 22.23 obtained in Pakistan [33]. The mean value of Grade 3 was similar to 37% among Germans [11] and 36.10% ± 28.13 among the Pakistan [33].

In conclusion, breast cancers among the Osogbo women were of high grade. Histological grading and MKi67 scoring were positively related (Figure 2a-2c).

References

1. Jemal A, Bray F, MM Center MM (2011) Global cancer statistics. CA Cancer J Clin 2: 69-90.
2. Ferlay J, Shin HR, Bray F (2008) Estimates of worldwide burden of cancer in 2008: GLOBOCAN. Int J Cancer 2010 127: 2893-2917.
3. Sylla BS, Wild CP (2011) A million Africans a year dying from cancer by 2030: What can cancer research and control offer to the continent? Int J Cancer 2: 245-250.
4. Afolayan EAO (2008) Cancer in North Western region of Nigeria: an update analysis of Zaria cancer registry data. Nig J of Med Sci 1: 37-43.
5. Abiodun A, Olatunde O, Michael M, Rakiya S (2012) Breast cancer trends in a Nigerian population: an analysis of cancer registry data. Life Science Pathology 3: 29-34.

6. Jedy-Agba E, Curado MP, Ogunbiyi O, Oga E, Fabowale T, et al. (2012) Cancer incidence in Nigeria: a report from population based cancer registsries. Cancer Epidemiology 36: 271-278.

7. Iyoke CA, Ugwu GO (2013) Burden of gynecological cancers in developing countries. World J Obstet Gynecol 1: 1-7.

8. Sullivan HC, Opreas- Ilies G, Adam A, Page AJ (2013) Triple- negative breast carcinoma in African and Caucasian women; clinicopathology, immunomarkers, and outcome. Appl Immunohistochem Mol Morphol 1:17-23.

9. Elston CW, Ellis IO (1992) Pathological prognostic factors in breast cancer and the value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19:403-410.

10. Iyiola S, Avwioro OG (2011) A new Haematoxylin for The Staining of Nuclear and Extracellular Materials. JPCS 1:21-23.

11. Inwald EC, Klinkhammer- schalke F, Zeman F (2013) K1- 67 as a prognostic parameter in breast cancer patient; results of as a large population. Breast Cancer Res Treat 2: 539-552.

12. Abubakar A, Nick O, Frances D (2016) Prognostic value of automated KI67 scoring in breast cancer: a centralized evaluation of 8088 patients from 10 study groups. Breast Cancer Res 18: p 104.

13. Titiloye NA, Omoniyi-Esan GO, Adisa AO (2013) Breast cancer in a Nigeria cohort; histopathology, immune-histochemical profile and survival. Postgraduate Med J of Ghana 2: 83-86.\

14. Omoniyi-Esan GO, Olaofe OO, Omonisi AE (2015) Hormonal and Her2 Receptor Immunohistochemistry of Breast Cancers in Ile-Ife, Nigeria. Austin J Women's Health 1: 2014.

15. Feng-yan Li, San-gang Wu, Juan Zhou JyS (2014) Prognostic value of K1-67 in breast cancer with positive auxiliary lymph node; a retrospective cohort study. PLoS One 2: e 87264.

16. Ebughe GA, Ugare GU, Nnoli MA (2013) Histological type and tumor grade in Nigeria breast cancer; relationship to menarche, family history of breast cancer, parity, age at first birth, and age at menopause. J Dent and Med Sci 5: 8-63.

17. Titiloye NA, Foster A, Omoniyi GO, AO Komolafe, AO Daramola, et al. (2016) Histological features and tissue microarray taxonomy of Nigeria breast cancer reveal predominance of the high grade triple negative phenotype. Pathobiol 1: 24-32.

18. Ikpatt F, Kuopio T, Erekul A, Collan Y (2002) Apoptosis in breast cancer: Nigerian vs. Finnish material. Analyt Quant Cytol Histol 24: 73-80.

19. Ayoade AB, Tade OA, Salam BA (2012) Clinical Features and Pattern of Presentation of Breast Diseases in Surgical Outpatient Clinic of a Suburban Tertiary Hospital in South-West Nigeria. Nigerian J Surg 1: 13-16.

20. Bello M (2012) Awareness is the first step in battle against breast cancer. Bull World Health Organization 90:164-165.

21. Olajide TO, Ugburo AO, Habeeba MO (2014) Awareness and practice of breast screening and its impact on early detection and presentation among breast cancer patients attending a clinic in Lagos, Nigeria. Nigeria J Clin Pract 17: 802-807.

22. Sharma K, Costas A, Shulman LN, Meara JG (2012) A Systematic Review of Barriers to Breast Cancer Care in Developing Countries Resulting in Delayed Patient Presentation. J Oncol 21873.

23. Agbo PS, Khalid A, Oboirien M (2014) clinical presentation, prevalence and management of breast cancer in Sokoto, Nigeria. Nigeria. J of Women's Health Care 3: p 149.

24. Ntekim A, Nufu FT, Campbell OB (2009) Breast cancer in young women in Ibadan, Nigeria. Afr Health Sci 2009 4: 242-246.

25. Ohene-Yeboah M, Adjei E (2012) Breast cancer in Kumasi, Ghana. Ghana Medical Journal 1: 8-13.

26. Nwafor CC, Keshinro SO (2015) The pathology of Breast biopsies in a sample of Nigeria patients; review and analysis. Ann Afr Surg 12: 89-94.

27. Maccallum DE, Hall PA (2000) The location of PK167 in the outer dense fibrillar compartment of the nucleolus points to a role in ribosome biogenesis during the cell division cycle. J Pathol 190: 537-544.

28. Cuylen S, Blaukopf C, Politi AZ (2016) KI-67 acts as a biological surfactant to disperse mitotic chromosomes. Nature 7611: 308-12.

29. Tashima R, Nishimura R, Osako T (2015) Evaluation of an Optimal Cut-Off Point for the Ki-67 Index as a Prognostic Factor in Primary Breast Cancer: A Retrospective Study. PLOS One **7**: e0119565.

30. Nishimura R, Osako T, Nishiyama Y (2014) Prognostic significance of Ki-67 index value at the primary breast tumor in recurrent breast cancer. Mol Clin Oncol 2: 1062–1068.

31. Knutsvik G, Stefansson IM, Aziz S (2014) Evaluation of Ki67 Expression across Distinct Categories of Breast Cancer Specimens: A Population-Based Study of Matched Surgical Specimens, Core Needle Biopsies and Tissue Microarrays. PLOS One 11: e112121.

32. Khalid D, Renato M, Ihsan O, Massimo C (2012) Ki-67 labelling index in primary invasive breast cancer from Sudanese patient: A pilot study. ISRN Pathology.

33. Haroon S, Hashmi AA, Khurshid A (2013) Ki67 index in breast cancer: correlation with other prognostic markers and potential in Pakistani patients. Asian Pac J Cancer Prev **7**: 4353-4358.

34. Fasching PA, Heusinger K, Haeberle L (2011) Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neo adjuvant treatment. BMC Cancer 11: 486.

35. Querzoli P, Albonico G, Ferretti S (1996) MIB-1 proliferative activity in invasive breast cancer measured by image analysis. J Clin Pathol 49: 926-930.