Histological and Immuno-Histochemical Evaluation of Mast Cells in a Retrospective Cross – Sectional Study of Breast Tumour Patients

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ABSTRACT

Background: Breast tumours are the most important lesion of the female breast, the most common invasive cancer in women and second leading cause of cancer death in women after lung cancer. This is becoming most common and more deadly with over 200,000 cases per year in Nigeria and Africa in general.

Methods: A retrospective cross-sectional study was carried out from 2017 to 2023. One hundred and fifty (150) archived breast tumour tissue samples were retrieved from the histopathology Laboratory of Madonna University Teaching, Hospital, Elele. Patients' data were retrieved from the histopathology reports. Tissue blocks were re-embedded in fresh paraffin wax and 4µ thick serial sections were cut and stained accordingly.

Results: Benign tumours showed increased number of mast cells and mast cell membrane permeability making it hyper-chromatic with hypertrophy specifically having the highest immune reactivity count. Malignant tumours had decreased number of mast cells surrounding the periphery of the tumour with lobular carcinoma specifically showing the lowest number of mast cell per high power field. Patients' ages at presentation ranged from 15 to 64 and breast tumour was highest between the ages of 25 ≤34.

Conclusion: The evaluation and mast cell expression pattern in breast tumours maintained different activities of mast cells in benign and malignant tumours and could serve as a positive prognostic indicator for patient's stratification and specific treatment. However, further studies on immune-histochemical evaluation of mast cells in breast tumours should be extended to frozen sections.

Keywords: Breast tumours, mast cells, retrospective, cross-sectional.

INTRODCTION

Breast tumours are the most important lesion of the female breast and the most common invasive cancer in women and second leading cause of cancer death in women after lung cancer [1]. In Nigeria in particular and Africa in general, cancer is becoming most common and more deadly with over 200,000 cases per year. This is more than double the mortality in North America which is about 60,000 cases per year [26]. Breast tumours arise from either connective tissue or epithelia structures [8]. It is the latter that gives rise to the common breast neoplasm accounting for 22.7% of all new cancer cases with 12,000 deaths in Nigeria in 2018 [8]. The increase rate is attributed to age, age at menarche, first live birth, first degree relative with breast cancer, breast biopsies, race, oestrogen exposure, radiation exposure, carcinoma of the contralateral breast or endometrium, geographical influence, diet, obesity, exercise, breast feeding, environmental toxins and tobacco [15]. The uncontrolled division and multiplication of cells results in two basic types of tumour or neoplasm called benign and malignant tumours [10]. Benign tumour remains localized where it originally occurred [11]. Malignant

tumour is a group of cancer cells that may invade surrounding tissues or spread (metastasize) to distant areas of the body [11]. Benign disease is much more common than malignant cancer, particularly in young women and includes acute inflammation of the breast, abscess formation and benign breast lumps which may be fibro-adenosis, chronic mastitis or fibrocystic disease [19]. Malignant tumour includes ductal carcinoma in situ (DCIS), Infiltrating or invasive ductal carcinoma (IDC), medullary carcinoma, lobular carcinoma in situ (LCIS), infiltrating lobular carcinoma (ILC), tubular carcinoma, mucinous carcinoma or colloid, Paget's inflammatory breast cancer, Triple negative breast cancer and metastatic breast cancer [11]. Mast cell which is a resident cell of connective tissue and part of immunological defense system accumulate around the periphery of tumour cells [13]. It is believed that the inter-interactions between mast cells and tumour cells were important for the growth and invasive properties of the tumour [11]. It is also emphasised that there is a favourable prognostic significance of mast cells in tumour pathology [12]. A rare histological study of mast cells in group of histogenetically different tumour reveals that a less aggressive tumour is associated with a higher number of tumour and peri-tumour mast cells irrespective of the histological type [17]. These histological evaluations are not specific but immune-histochemical evaluation is highly sensitive and specific; allowing for the detection of even small numbers of mast cell in tissue samples [15]. In addition, considering other inflammatory cells in tumour pathology, mast cells have significant correlation with breast tumour and the survival status [13]. This current study, therefore, aimed to evaluate the expression pattern of mast cells in breast tumours in a tertiary healthcare facility. The overall prevalence and burden of breast tumours will be made plausible.

METHODOLOGY

Study Area

This study was carried out at Madonna University Teaching Hospital (MUTH), Elele, Rivers State. Madonna University Teaching Hospital is a tertiary teaching hospital with (3) out stations located at Okija, Akpugo and Enugu. The hospital's catchment area extends beyond Rivers State to include parts of the neighboring states; Bayelsa and Imo State and is located in Elele, a town 42km from the state capital city. The city is located at the eastern part of the Niger River and about 80km South East of Owerri in Imo State, Nigeria. Elele is a medium-sized town in Rivers State [16]. The city spans 4.277 square miles (2,423 km^2) in Rivers State. The population census of Elele is 21,391 (male 11.048 and female 10,343) as of 2015. The predominant economic activity of her people is farming [14].

Study Design

A retrospective cross-sectional study was undertaken using archived breast tissue samples collected from a tertiary healthcare institution in south south Nigeria from 2017 to 2023.

Ethical consideration

Ethical approval to conduct this study was granted by the Ethics Committee of Madonna University Teaching Hospital (MUTH), Elele (Appendix V).

Breast tumour tissue block collection and processing

One hundred and fifty (150) archived formalin-fixed paraffin-wax-embedded breast tumour tissue samples were retrieved, sorted and selected from the Histopathology Laboratory of Madonna University Teaching Hospital (MUTH), Elele. Patients'socio-demographic and clinical data were retrieved from the daily surgical register folders and histopathology reports of the patients. Retrieved tissue blocks were re-embedded in fresh molten paraffin wax and 4µ thick serial sections were cut with a rotary microtome (Leica). Cut sections were floated out on a 48 degree Celsius water bath, mounted on clean grease-free identified frosted light microscope slides, drained and dried on a Leica hot plate at a temperature 5 -10 degree above the melting point of paraffin wax to affix the tissue on the light microscope slides before staining. Cut sections were stained by the haematoxylin and eosin (H&E) method for blind assessment to reconfirm diagnosis of breast tumour subtype

and grade, toluidine blue method to stain mast cells and immune-histochemistry with monoclonal anti AA1, AA3 and AA5 to determine mast cell count on each sample using eye estimation count technique [23].

Immune-histochemical Staining (IHC)

Immune-histochemical staining of test and control slides was carried out using avidin-biotin immune-histochemistry method [9]. The principle is based on simple indirect antigen and antibody reaction. One block of formalin-fixed, paraffin-embedded breast tumour tissue was selected per case and 4 micrometer thickness was cut, de-paraffinized and rehydrated by passing through 2 changes of xylene, then 4 changes of descending grades of alcohol (100%, 90%, 80%, 70%) and finally to water. Antigen retrieval was performed by immersing and heating sections on a citrate buffer solution of pH 6.0 using the microwave at power 100 for 15minutes. The sections were equilibrated gradually with cool water to displace the hot citrate buffer for at least 5min for the section to cool. Endogenous peroxidase blocking was done on the sections by simply covering section with 3% hydrogen peroxide (H_2O_2) for 15min. Sections were washed 2 times in phosphate buffered saline (PBS) for 3 minutes each. The slides were incubated with protein blocker (avidin) for 20mins to prevent non-specific binding and then washed in phosphate buffered saline (PBS). Endogenous biotin in tissue was blocked using biotin for 15min and washed with PBS. Serial sections then were incubated overnight with primary antibodies; mouse monoclonal antibodies ((anti-tryptase antibody) against AA1, AA3 and AA5proteins in humid chamber for 1 hour at room temperature. Excess antibodies were washed off with PBS and a secondary antibody (LINK) was applied on sections for 15mins. Sections were washed and the (LABEL) horseradish peroxidase (HRP) was applied on the sections for 15min. A working 3.3'-diaminobenzidine (DAB) solution was made up by mixing 1 drop (20microns) of the DAB chromogen to 1ml of the DAB substrate. This working solution was applied on sections after washing off the HRP with PBS for at least 5minutes. The brown reaction began to appear at this moment especially for a positive target. Excess DAB solution and precipitate were washed off with water. Sections were counterstained with Mayer's haematoxylin solution for 5 minutes and blued briefly. Sections were dehydrated in alcohol, cleared in xylene and mounted in Dibutyl Phthalate Polystyrene Xylene (DPX). Stained sections were reviewed for nuclear reactivity and Photomicrographs were taken using Olympus PM-10AK and an Olympus BX - 50 microscope. Mast cells were counted under X100 magnification, using the microscope equipped with a 0.1 X 0.1mm ocular grid.

Data analysis

Data were entered and analysed using Statistical Package for Social Science (SPSS) version 22. Descriptive statistics was used to summarize the data obtained from the archives and also employed in evaluating mast cells in malignant and benign breast tumour patients. Numerical data was presented as mean (standard deviation) or median (Inter-quartile range). Categorical data were summarized and presented as percentages.

RESULTS

In Microscopic Count Pattern of mast cells and tumour type, hypertrophy had the highest number of mast cell count while lobular carcinoma had the lowest number of mast cell count as shown in table 1. Invariably, mast cell was on the increase in benign tumours while on the decrease in malignant tumours (Table 1). In the same vein, the normal breast (control) was mast cell – free and the totality of mast cell per high power field by 4 is 1,285 with a total mean value of 321.25 (25%) as also shown in table 1.

Table 1: Microscopic Count Pattern of Mast Cells and Tumour Type

Breast Diseases	Mast Cells (phf)*4	Mean Value (%)
Hypertrophy	210	52.50(4.08)
Granulomatous mastitis	189	47.25(3.67)
Fibrocystic changes	170	42.50(3.60)

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Carcinoma Insitu	105	26.25(2.06)
Ductal carcinoma insitu	101	25.22(1.96)
Ductal papilloma	101	25.25(1.96)
Cystadenomas	93	23.25(1.80)
Ductal carcinoma	90	22.50(1.75)
Lobular carcinoma	75	18.75(1.46)
Phyllodes	150	37.50(2.9)
Normal	-	-
Total	1,285	321.25(25)

The reaction pattern of mast cells with toulidine blue among breast tumour cases showed high percentage positivity in benign tumour (80) compared to malignant tumour (51). Only five cases out of the eight-five cases of benign tumour showed negativity while fourteen out of the sixty-five cases in malignant tumour reacted negatively as shown in table 2. This high percentage positivity in benign tumour is attributed to the high number of mast cell in benign tumour than in malignant tumour. There is metachromatic staining of mast cell with toluidine blue with mast cell membrane permeability in benign tumour making it hyperchromatic as shown in plate 1 and plate 2. A total number of positivity and negativity in both benign and malignant tumours were one hundred and thirty-one and nineteen respectively amounting to one hundred and fifty which is the total number of cases studied as also shown in table 2.

Table 2: Staining Reactions of Mast Cells with Toulidine Blue among Case Study % Staining Reaction

Disease Condition	No Examined	No/Positive %	No/Negative %
Benign tumour	85	80(53.3%)	5(3.4)
Malignant tumours	65	51(34.0)	14(9.3)
Total	150	131(87.3)	19(12.7)

In mast cell expression pattern of breast tumour based on age, the highest occurrence of breast tumour was found in the ages of 25 ≤ 34 and the lowest occurrence of breast tumour was found in ages of 55 -≤ 64 as a result of high and low oestrogen level attributed to reproductive age respectively (Table 3).

Table 3: Expression Pattern of Breast Tumour based on Age of Patients

Age(years)	No. of Subjects Examined	% No of Breast Tumour
15 - ≤ 24	21	16(4.0)
25 - ≤34	50	40(33.3)
35 - ≤44	30	30(20.0)
45 - ≤ 54	40	37(24.7)
55 - ≤ 64	9	8(5.3)

Total	150	131(87.3)

In immuno-reactivity pattern of mast cell in breast tumours, benign tumours in general showed an increased number of mast cell with hypertrophy specifically showing the highest immune-reactivity. Malignant tumours were on the decreased number of mast cell with lobular carcinoma specifically showing the lowest number of mast cell per high power field. There was no immune-reactivity in the normal breast or control (Table 4).

Table 4: Immuno-reactivity Pattern of Mast Cells in Breast Tumours

Breast Diseases	Mast Cells (phf)*4	Mean value (%)
Hypertrophy	105	26.25 (2.04)
Granulomatous mastitis	95	23.63 (1.83)
Fibrocystic changes	85	21.25 (1.80)
Carcinoma insitu	52,5	13.13 (1.03)
Ductal carcinoma insitu	50.5	12.61 (0.98)
Ductal papilloma	50.5	12.61 (0,98)
Cystadenomas	46.5	11.63 (0.90)
Ductal carcinoma	45	11.25 (0.88)
Lobular carcinoma	37.5	9.38 (0.73)
Phyllodes	75	18.75 (1.45)
Normal	-	-
Total	642.5	160.63 (12.6%)

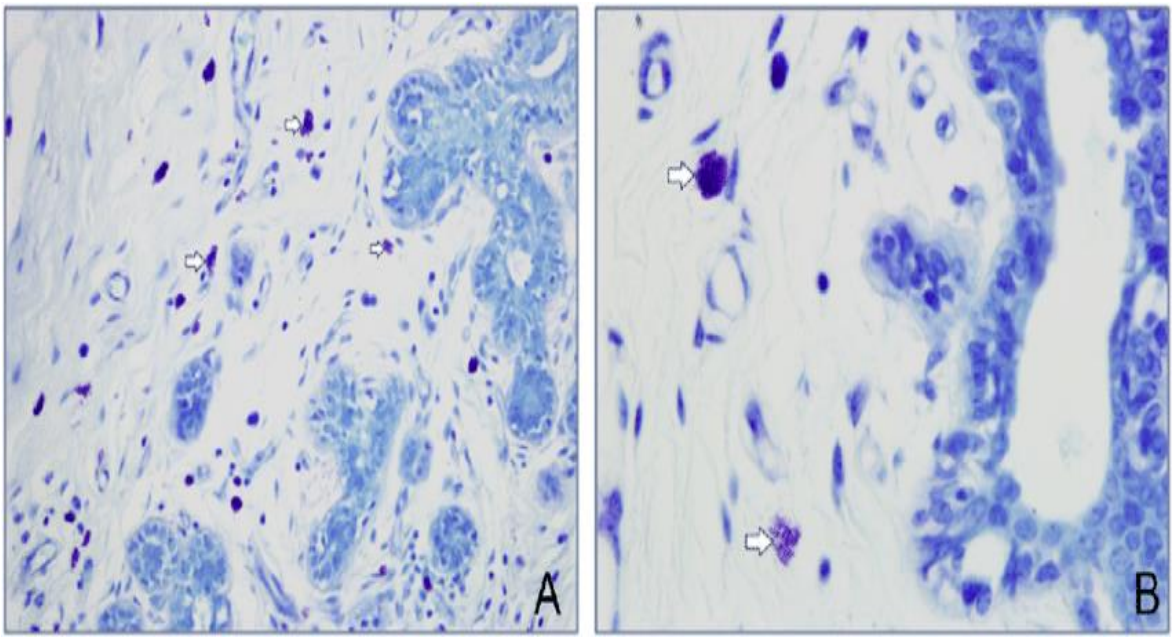

Plate I: toluidine blue staining of mast cell in benign breast tumour (X400 magnification) Toluidine blue stained mast cells whose cytoplasm contain granules rich in heparin and histamine red-purple (metachromatic staining) and the background blue (orthochromatic staining).

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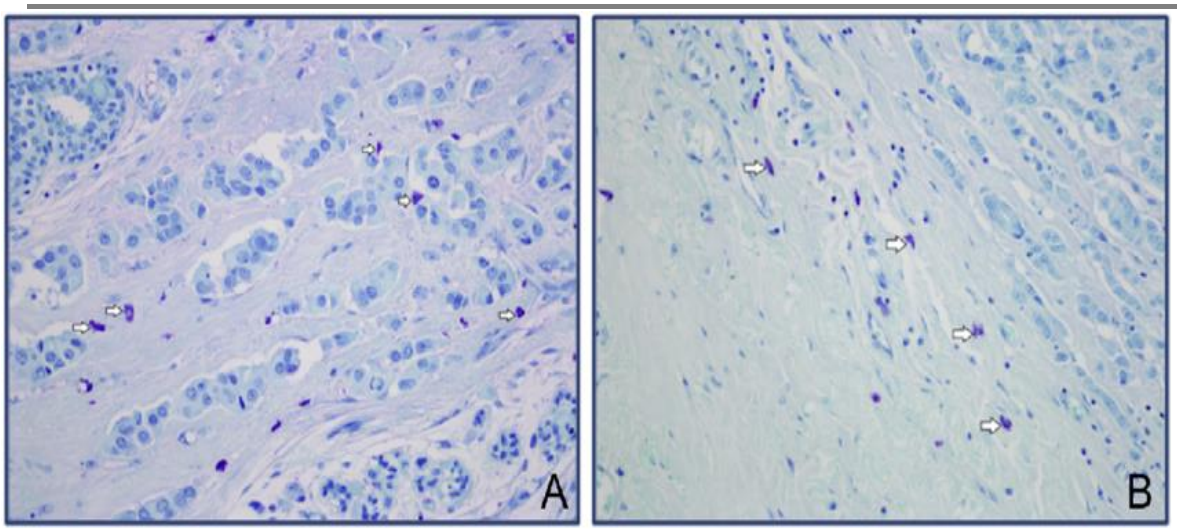

Plate II: Toluidine blue staining showing Intramural mast cells (X400 magnification). Mast cells stained metachromatically with toluidine blue with greater intensity in cells containing smaller granules.

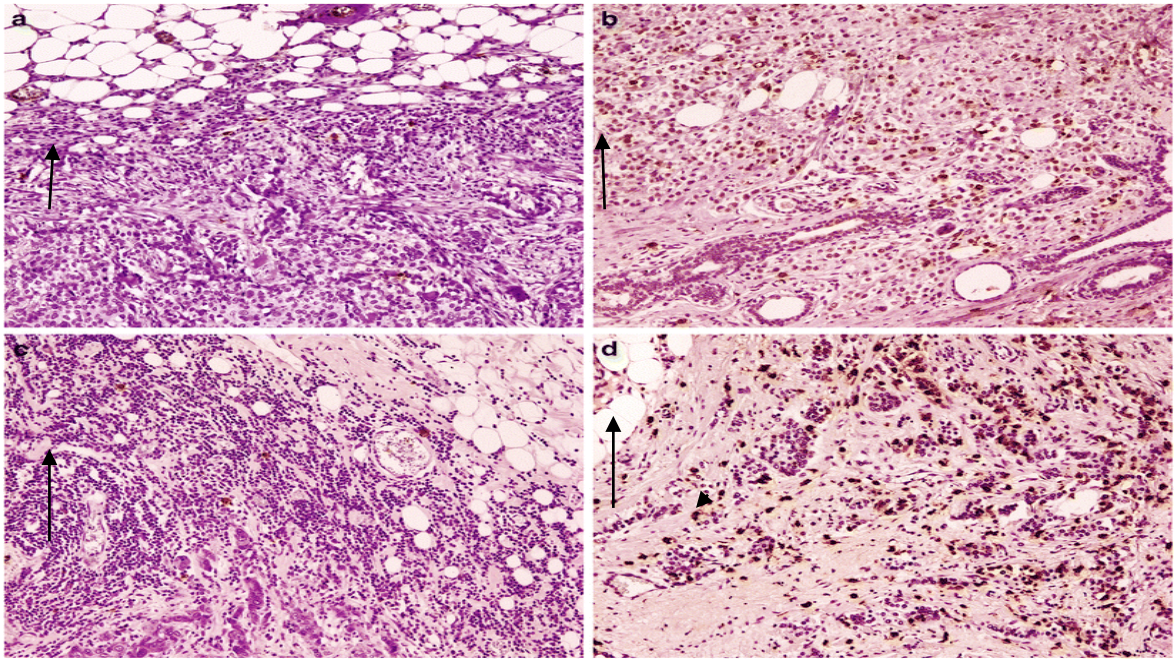

Plate III: Mast cells in invasive breast cancer, low (a) and high (b) mast cells infiltration. Low(c) and high (d) tryptase –positive mast cells infiltration immune-histochemistry X100.

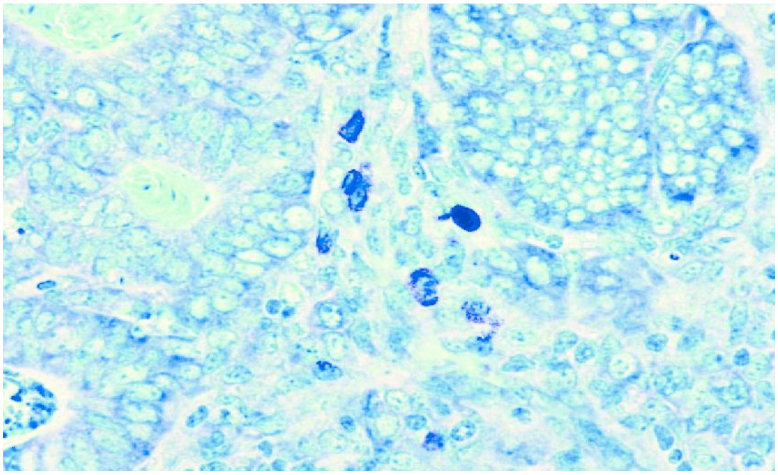

Plate IV: Toluidine blue staining showing mast cell in malignant tumour X40

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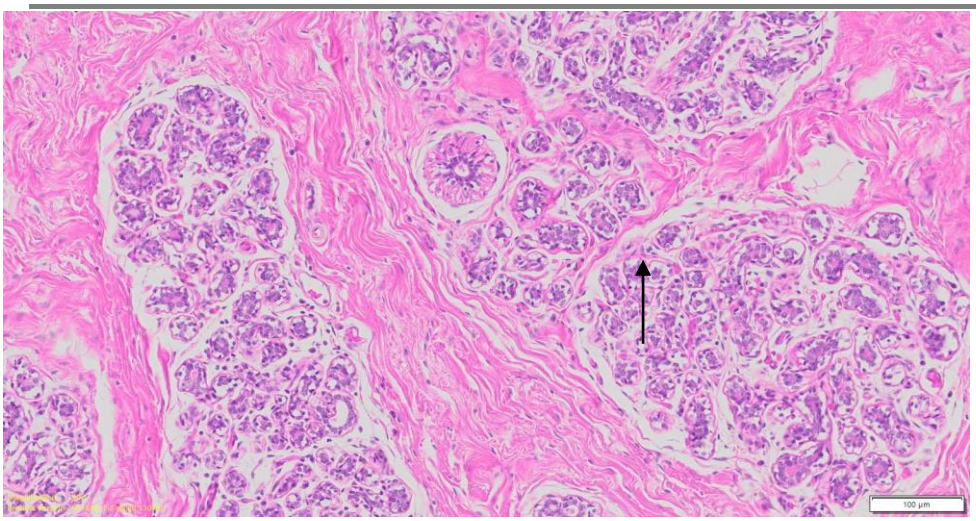

Plate V: Haematoxylin and eosin staining of normal breast tissue X40. There is no abnormal cell features.

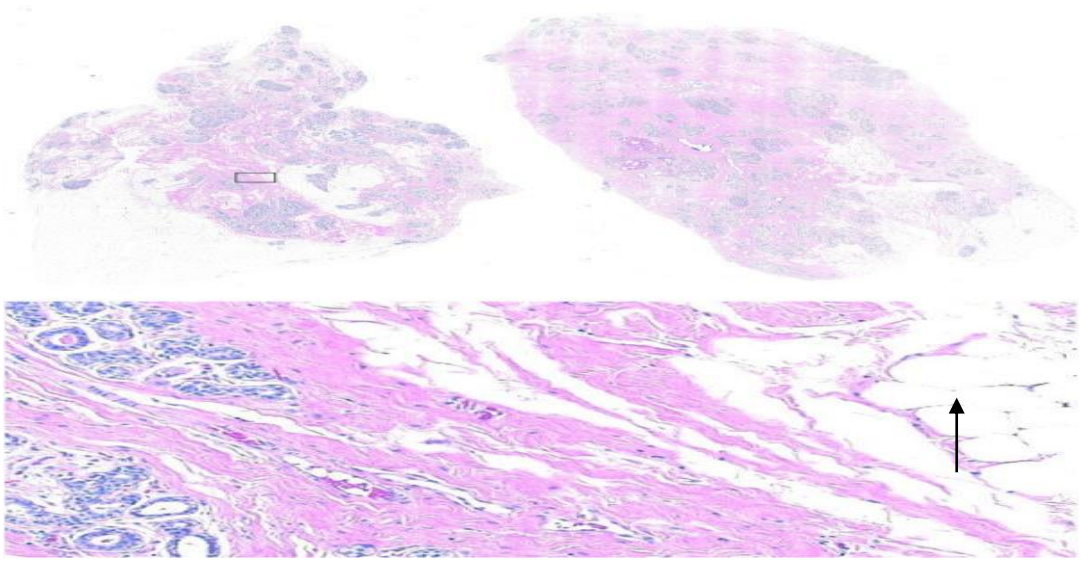

Plate VI: Haematoxylin and eosin staining of benign breast tumour x 40. Histological sections show encapsulated aggregates of benign adipocytes with no form of atypia. There is no mitotic figures present, nucleo-cytoplasmic ratio is not increased (no hyperchromasia), no nuclear membrane lobulation and acantocytic cells.

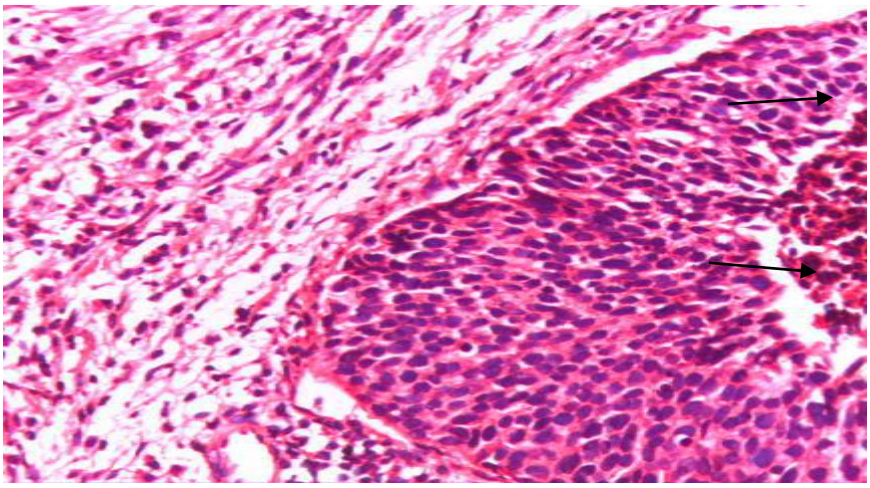

Plate VII: Haematoxylin and eosin staining of malignant breast tumour x 40.; there is presence of mitotic figures and multiple nuceoli, nuclear membrane lobulation, acantocytic cells and increased nucleo-cytoplasmic ratio (hyperchromasia). The resection margins are positive.

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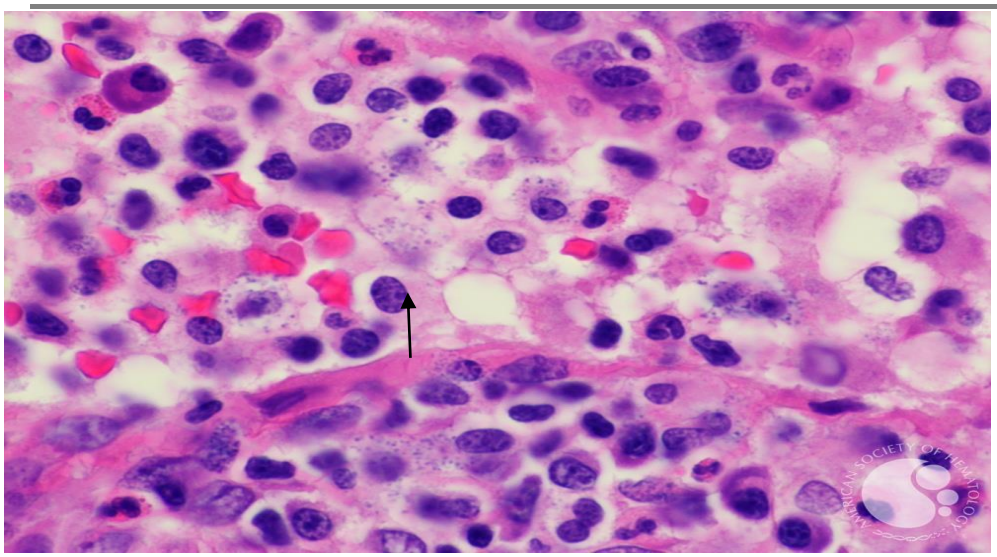

Plate VIII: Haematoxylin and eosin staining of breast tumour tissue showing mast cell infiltration in benign tumour X40

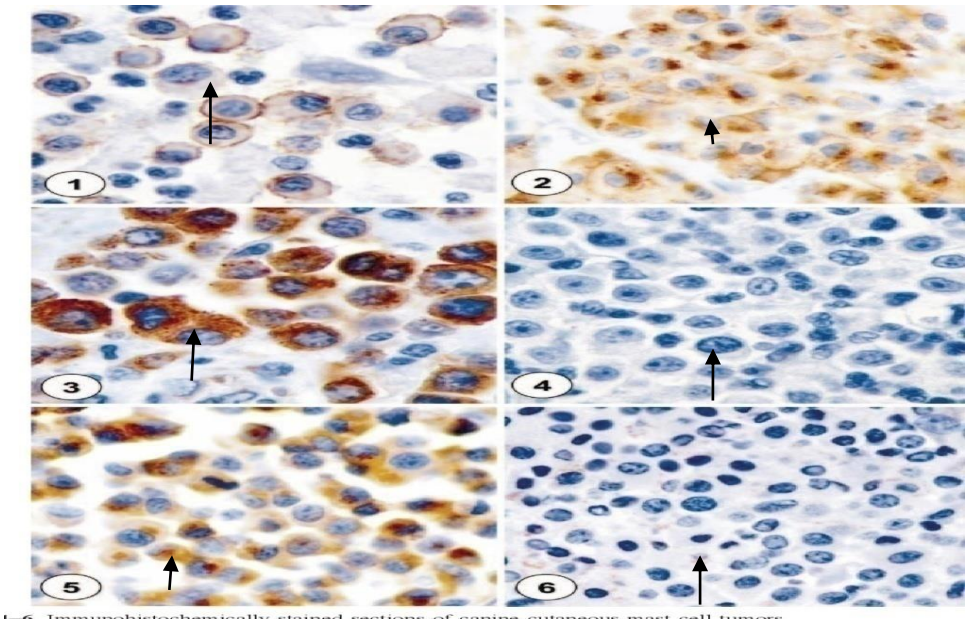

Plate IX: immune-histochemical staining of mast cell in malignant (1, 2, 3 and 5) and benign (4 and 6) breast tumours X100.

DISCUSSION

Mast cells are unique tissue-resident immune cells of the myeloid lineage that have long been implicated in the pathogenesis of allergic and autoimmune disorders [13]. They are derived from CD34+ bone marrow myeloid precursors that circulate in the blood and migrate to peripheral tissues where they develop and differentiate into nature mast cells under the pressure of tissue specific chemokines and cytokines (such as stem cell factor and IL-4), extracellular mature proteins and adhesion molecules [13]. Mast cells are strategically located throughout the body near blood vessels, lymphatics and mucosal surfaces such as the skin and gastrointestinal tract where they interface with the external environment [13]. Their location allows them to mediate systemic responses to local stimuli and orchestrate important aspects of both innate and adaptive immunity as well as other physiologic processes. More recently, mast cells have been recognized as key orchestrators of anti-tumour immunity, modulators of the cancer stroma and have also been implicated in cancer cell intrinsic properties [13]. As such, mast cells are an under recognized but very promising target for breast cancer immunohistochemistry and immunotherapy [13]. A critical role for mast cells in modulating tumour progression is their role as a sentinel immature cell that releases chemokines, cytokines and other factors that

recruit other immune cells to the tumour microenvironment and alter their function [13]. Histological and immune-histochemical evaluation of mast cells in breast tumour was extensively studied using haematoxylin and eosin, toulidine blue dye and Tryptase immunolabelling technique. One hundred and fifty patients attending Madonna University Teaching Hospital Cancer Clinic served as subjects while normal breast samples served as control. A prevalence of 131(87.3%) breast tumour was detected using toulidine blue reaction which clearly demonstrated mast cells in tissue sections. Mast cells were seen proliferating more in benign tumour than malignant tumour - 80(56.6%) and 51 (34.0%) respectively. Hypertrophy had a total mast cell count of 210 with mean of 52.50(4.08%) while lobular carcinoma had a total of 75 with a mean of 18.75(1.46%). This could be attributed to membrane permeability in inflammatory conditions caused by neutrophils and macrophages [18]. The occurrence of breast tumour was highest between the age of 25 - $\leq$ 40 years (33.3%) and this is attributed to high oestrogen synthesis of above 1500mg in ages believed to be actively reproductive [24]. The result is in agreement with the theoretical and studies which emphasizes a favorable prognostic significance of mast cells in tumour pathology [13]. It also agrees with the works that reported an increasing number of mast cells in benign tumours, evaluating its prognostic significance in breast tumours [13]. The correlation between mast cells proliferation and severity of breast tumour was statically significant at $P < 0.05$. Rare histological studies of mast cells in group of histogenetically different tumour agrees that a less aggressive tumour is associated with a higher number of tumour and peri-tumour mast cells irrespective of the histological type [11]; thus with the findings of this study. From the result, the colour pattern of mast cell was similar despite the tumour type.

CONCLUSION

A study on histological and immuno-histochemical evaluation of mast cells in breast tumours among patients attending Madonna University Teaching Hospital Cancer Clinic has shown that the average number of mast cell density in benign tumours were higher than in malignant tumours. Invariably, an increasing number of mast cells are connected with an invasive growth of low aggressive tumour, while a decreasing number is associated with malignancy. This is attributed to mast cell membrane permeability in benign and inflammatory condition of the breast. This would suggest different activities of breast tumours and peri-tumour mast cells in breast carcinoma and could serve as a positive prognostic indicator for patient's stratification and specific treatment. The function of mast cells being a key component of the innate immune system could also be augmented by immune-therapy in breast cancers where they have an anti-tumourigenic role and diligent clinical follow-up of these patients and survival rates of cancer patients are required to determine the exact prognostic significance of these findings.

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