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# Prediction of Predisposing Factors to Breast Tumours Using Artificial Neural Network

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## ABSTRACT

Breast tumour occurrences are increasing among women worldwide. The predisposing factors remain unclear because of their heterogeneity, hence the need for computer-aided approach in prediction. This study investigated the occurrence of breast tumours; connective tissue changes; estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), cytokeratin 7 (CK7), and Kiel-67 (Ki-67) expressions; and used artificial neural network (ANN) to predict the important predisposing factors. Data from ninety-six women aged 13-78 years and fifteen breast biopsies from the Histopathology Laboratory, University of Calabar Teaching Hospital were obtained. The formalin-fixed-paraffin-wax-embedded tissues were stained with hematoxylin and eosin (H&E), van Gieson, aqueous beetroot, and Colloidal iron-PAS. Ten malignant tissue blocks were immunohistochemically-stained for ER, PR, HER2, CK7, and Ki-67. Data were analyzed with Chi-square and ANN of Statistical Package of Social Sciences (SPSS) software. In the results, breast cancers were 35(36.5%) of the 96 womenand age was a predisposing factor (p=0.001). Beetroot demonstrated stroma hyalinization. The malignant tumours were mostly HER2+ 8(80%). Chi-square showed HER2+, CK7+, and Ki-67+-tissues had collagen and mucin depositions (p=0.001). Using ANN, mucin deposition, stroma hyalinization, CK7, HER2+, and age were the most important predisposing factors (p=0.006). Breast cancer is characterized mostly by acid mucin deposition and stroma hyalinization.

**Keywords**: Breast cancer, Artificial neural network, Connective tissue, Immunohistochemistry, Beetroot, Calabar

## INTRODUCTION

Breast tumours, especially cancer, have continued to affect women in Calabar, Nigeria (Udonkang *et al.*, 2023). Breast cancer was previously reported to cause significant morbidity and mortality among women and about 37 out of 100,000 women were affected in Cross River State (Ikpatt *et al.*, 2002; Ebughe *et al.* 2016). Most of these breast cancer cases were reported among premenopausal women of less than 50 years (Udonkang *et al.*, 2021a).

Among these tumours, fibroadenoma and invasive ductal carcinoma have remained the most common benign and malignant types of breast tumours (Ebughe *et al.* 2016; Udonkang *et al.*, 2021a; Udonkang *et al.*, 2023). Breast tumour behavior, aggressiveness, and response to treatment have been associated with its biology (Luparello, 2013). The aetiology and biology of breast tumours among women in the study has not been fully understood till date as there is disparity in research towards this direction. Breast tumours have been linked to environmental and genetic factors (Nwafor and Keshinro, 2015). However, Udonkang *et al.* (2021a) showed that collagen changes and inheritance of mutant *p53* and breast cancer (*BRCA*) genes 1 and 2 were genetic risk factors of breast tumours among women in Calabar.

Previous study by Omoruyi *et al.* (2018) on immunohistochemical markers of ER, PR and HER2 had stated that majority of the cases were ER+ in their study area. Triple negative breast cancer was also reported among women of African ancestry and was linked to their aggressiveness among the affected persons (Huo *et al.*, 2009, Rahman *et al.*, 2014; Nwafor and Keshinro, 2015). Apart from these genetic markers, CK7 and Ki-67 are other valuable prognostic markers. CK7 is a prognostic marker of breast tissue differentiation utilized to identify cancers from breast origin (Shao *et al.*, 2012). There has been concern about the loss of expression of this marker in breast tumours thereby increasing the risk of poor prognosis. Ki-67 is a precise cell proliferation marker that predicts the proliferative potential of breast cancer cells and currently utilized as a prognostic marker of the disease (Davey *et al.*, 2021). The presence of Ki-67 in breast cancers was reported in South-Western Nigeria and cases had poor prognosis (Agboola *et al.*, 2013). Reports of studies correlating the presence of these markers in breast tumours in Calabar are scarce. Apart from these immunohistochemical markers, benign and malignant breast tumours have been shown to undergo heterogeneity in extracellular matrix composition and this has been associated with tumour progression, aggressiveness, and prognosis (Luparello, 2013). Breast tissues undergo changes in the epithelium and stroma. The stroma makes up the extracellular matrix. It consists mostly of collagen fibres and ground substances (predominantly mucin). Collagen fibres are double edged swords as excess deposition has been linked to cancer progression and aggressiveness; but can also serve as treatment targets (Liu *et al.*, 2016). Beetroot stain has been used to demonstrate these collagen fibres changes in the stroma of breast tumours (Udonkang *et al.*, 2021). Also, the presence of neutral and acid mucin in benign and malignant tumours has been found to influence their behavior and progression (Mukhopadhyay *et al.*, 2011). There is also correlation between increase collagen and mucin deposition with expression of ER, PR, and HER2 proteins (Trentham-Diez *et al.*, 2014).

This heterogeneity of breast tumours has pose difficulty in understanding their biology. Till date, there is disparity in research tailored towards understanding the predisposing factors of breast tumours especially cancers affecting women in the study population. The use of computer-aided approach has become imperative to surmount this challenge. Previous studies have used ANN in breast cancer research. Nwoye *et al.* (2014) utilized ANN-based models for breast cancer classification. Another study used ANN for breast cancer diagnosis and prognosis (Ogah and Ugah, 2022). Nasien *et al.* (2022) also demonstrated that ANN can be applied in breast cancer prediction. Aside breast cancer, ANN has been applied in predicting the aetiologic and predisposing factors of leiomyoma (Udonkang *et al.*, 2022). Thus, this study investigated the occurrence of breast tumours; connective tissue changes; estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), cytokeratin 7 (CK7), and Kiel-67 (Ki-67) expressions; and used artificial neural network (ANN) to predict the most important predisposing factors.

## METHODS

### Data and sample collection

Data was obtained from ninety-six women from the Histopathology Laboratory Register, University of Calabar Teaching Hospital, Calabar from January 2018 to March 2019. Data of subjects included patient's age, nature of tissue, breast part, and histological types and grades of breast tumour. The criterion for selection of the breast biopsies for staining was based on sample size calculation. Fifteen randomly selected paraffin wax-processed breast tissue blocks from women who were diagnosed of breast tumours were selected. The histological diagnosis was based on the Scarff-Bloom-Richardson classification using H&E-stained tissues. Samples from women with incomplete data and from men were excluded. Ethical approvals were obtained from the Research Ethical Committee of the University of Calabar Teaching Hospital, Calabar with numbers UCTH/HREC/33/527, UCTH/HREC/33/632, and UCTH/HREC/33/694.

### Sample size calculations of the study

The sample size for the subjects' data was based on the sample size formula $Z^2P1(1\text{-}P1) + P2(1\text{-}P2) / d^2$ of difference of two proportions of Goyal (2013), where Proportions, P1=80%, P2=90%, Z is confidence interval at 95% =1.96, and d is relative precision =10%. This gave a sample size of 96 subjects. The sample size for the tissues was calculated based on sample size formula of difference of two proportions given as $Z^2P1(1-P1) + P2(1-P2) /d^2$, where proportions, P1=97%, P2=99%, Z is confidence interval at 95% =1.96, and d is relative precision =10%. This gave a sample size of 15 tissue samples.

### Tissue microtomy and staining methods

The tissue blocks were sectioned with a rotary microtome (Leica 2035, USA) at 4 µm, and sections were picked on albumenized slides for staining. Sections from the fifteen blocks were stained for Cole hematoxylin and eosin method (H&E) for general tissue morphology (Bancroft *et al.*, 2019), van Gieson for collagen fibres (Bancroft *et al.*, 2019), aqueous beetroot for general connective tissue architecture (Udonkang *et al.*, 2018), and Colloidal iron-PAS methods for acid and neutral mucins (Bancroft *et al.*, 2019). Also, ten selected tissue sections were stained immunohistochemically with antibodies for ER (BSB-2, BIO SB), PR (BSB-2, BIO SB), HER2 (BSB-3, BIO SB), CK7 (OV-TL, Thermoscientific), and Ki-67 (ER5, BIO SB) according to manufacturers' instructions. Positive immunohistochemical staining was seen as brown nuclear or cytoplasmic staining.

### Image analysis

Light microscope (OMAX 40X-2500X, China) and AmScope MD500 digital camera (USA) were used for photomicrography. Line Measurement and Color Cube tools of AmScope software were used to measure the size of the collagen fibres and color intensities of mucin staining respectively. Line measurement was done by using the Measurement-Line panes of the software. Colour intensity was measured by using the Process-Segmentation & Count-Colour Cube panes. Ten measurements were made per image for reproducibility.

### Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 was used to analyze the results. Pearson correlation was used to analyze the association between age and the tumour types. Chi-square was used to establish associations between the collagen size and mucin intensity with the expression of ER, PR, HER2+, CK7, and Ki-67. All results were statistically significant at $p \leq 0.05$. Multilayer perceptron of ANN was used to establish associations between the expressions of these markers with breast tumour types. The 60:20:20 ANN model was used. Hyperbolic tangent and softmax were the activation functions for the hidden and output layers respectively. The cross entropy error was the statistical error.

## RESULTS AND DISCUSSION

### Data analysis of the subjects

Histopathological diagnosis and age of the subjects are shown in Table 1. The tissue samples were classified into benign tumours with 56(58.3%) cases and malignant tumours with 35(36.5%) cases. Fibroadenoma (benign) accounted for the highest number with 43(44.8%) and invasive ductal carcinoma (malignant) accounted for the second highest occurrence with 28(29.2%) of cases. The age range 13-23 years, had the highest number of cases 32(33.3%), followed by 35-45 years with 26(27.1%) cases, and 23-34 years with 15(15.6%), and least was in 68-78 years with 2(2.1%). Fibroadenoma (benign) had the highest occurrence in age group 13-23 years with 31(32.3%) cases, Invasive ductal carcinoma (malignant) had the second highest occurrences with early onset in 24-34 years 4(4.2%) and peak occurrence at 35-45 years in 9(9.4%) cases. There was statistically significant association between age and the histopathological diagnosis of breast tumours ($r=0.662$, $p=0.001$). The mean age group of subjects with breast tumours was 35.2±15.4 years. The study observed high occurrence of benign and malignant breast tumours. This is similar to report by Ebughe *et al.* (2016). Breast tumours occur because the breast has high predisposition to undergo DNA mutations due to the extensive hormonal changes throughout a woman's lifetime or from environmental factors. Secondly, it has been suggested that exposure to environmental factors such as chemical carcinogens and diets, and endogenous stimulation from free radicals produced by oxidative stress can initiate tumour formation (Santarpia *et al.*, 2013; Zaha, 2014; Lee and Sultanian, 2015; Nwafor and Keshinro, 2015).

An early age of onset of breast tumours especially fibroadenoma and invasive ductal carcinoma were observed in this study. Majority of the affected women were below the pre-menopausal age. This is similar to earlier research in the study area by Udonkang *et al.* (2021a) that showed that women below the age of 50 years were mostly affected by breast tumours. Fibroadenoma has been reported to give a moderate increase risk of developing breast cancer as both have similar clinical manifestations and are induced by hormonal changes in young women. Li *et al.* (2018) had stated that early detection and treatment of fibroadenoma can aid in reducing the increased risk of developing invasive ductal carcinomas in young women.

**Table 1:** Histopathological diagnosis and age of the subjects

| Class | Type | n (%) | Age range (years) | n (%) |
|---|---|---|---|---|
| **Benign** | Fibroadenoma<br>Ductalhyperplasia<br>Fibrocystic change<br>Hyperplastic/fibrocystic change<br>Adenomyoepithelioma<br>Ductal papiloma | 43 (43.8)<br>4 (4.2)<br>3 (3.1)<br><br>2 (2.1)<br>2 (2.1)<br>2 (2.1) | 13-23<br>35-35<br>13-45<br><br>35-56<br>13-34<br>24-45 | 32 (33.3) |
| - | Total | 56 (58.3) | 24-34 | 15 (15.6) |
| **Malignant** | IDC<br>IPC<br>Malignant | 28 (29.2)<br>1 (1.0)<br>6 (6.3) | 35-45<br>46-56<br>46-67 | 26 (27.1) |
| | Total | 35 (36.5) | 46-56 | 11 (11.5) |
| **Normal** | | 1(1.0) | 57-67 | 10 (10.4) |
| **Suspicious** | | 4(4.2) | 68-78 | 2 (2.1) |
| **Overall Total** | | 96(100)<br>r=0.662 | Total<br>p=0.001 | 96(100.0) |

## Tissue characteristics

The histopathological diagnosis, connective tissue changes and the age distribution of the breast tissues are shown in Table 2. Collagen fibre staining showed 1+ grade for the normal tissue 1(6.7%). There was 1(6.7%) with 2+ grade out of 3(30%) of the benign tumour. The malignant tissues had 5(33.3%) with 2+ grade and 2(13.3%) with 3+ grade. The beetroot stain showed coarse stroma in the normal tissue 1(6.7%), loose and coarse stroma in the benign tissues 3(20%), hyalinized and coarse stroma in 10(66.7%) and hyalinized stroma in 1(6.7%) of the malignant tumours. The mucin staining showed acid mucin in 1(6.7%) of the normal tissue. Neutral and acid mucin was stained for 3(20%) of the benign tissues, and acid mucin for 11 (73.3%) of the malignant tissues. Out of the 15 tissues, 2 (13.3%) were benign tumours diagnosed in <20 years, malignant tumours were 6 (40%) in 31-40 years, 3 (20%) from 41-50 years and 2 (13.3%) from 51-60 years.

## Photomicrographs

The histological staining of the tissues using haematoxylin and eosin method further confirmed the presence of the malignant and benign breast lesions. Plate 1 is the photomicrographs of the stained tissues. Plate 1A stained with H&E is fibroadenoma showing pericanalicular pattern of enlarged duct. Invasive ductal carcinoma stained with van Gieson in Plate 1B showed enlarged bundles of red collagen fibres. Beetroot stained the collagen fibres and desmoplastic ducts of the IDC black-brown colour while the stroma showed hyalinized transparent appearance in Plate 1C. Fibroadenoma in Plate 1D showed neutral and acid mucin staining.

The benign pericanalicular fibroadenoma occur due to proliferative changes in the stroma of the breast tissues (Brandt, 2012; Tse *et al.*, 2013).

The fibroadenoma were shown to undergo changes involving low to moderate increase in collagen size/ deposition and remodeling of collagen fibres into a loose framework around the ducts. This remodeling in benign tumours is a major risk factor for progress to malignant tumour. The malignant invasive ductal carcinoma had hypertrophied collagen fibers/deposition. This is comparable to report that benign and malignant tumours had increase in collagen and hypertrophied fibers (Bredfeldt *et al.*, 2014; Araújo *et al.*, 2017; Li *et al.*, 2018). Increase collagen production occurs due to change in gene expression for collagen production in fibroblasts because of a change in phenotype to cancer associated fibroblasts. This cancer associated fibroblasts then cause increase DNA replication and synthesis of collagen fibres in the rough endoplasmic reticulum leading to increase extracellular matrix collagen (Luparello, 2013). This increase in deposition of collagen is a body defense initially aimed at causing extracellular matrix stiffness to disrupt cancer cell attachment and migration. However, the large collagen fibres turn around to cause hypoxia and promote proliferation and metastasis of the cancer cells via stimulation of angiogenesis (Luparello, 2013).

Beetroot has previously been used to stain breast tumours because it is rich in the pigment betanin (Udonkang *et al.*, 2021b; Udonkang *et al.*, 2018). In the carcinomas, the stroma around the desmoplasia underwent hyalinization. Hyalinization of stroma has been observed as an abnormal deposition of materials from tumour cells on the basement membrane to form a homogeneous, translucent material which causes treatment resistance and promotes progression (Kato *et al.*, 2010; Augustine *et al.*, 2021). This may explain the poor outcome from treatment in the affected women. This emphasizes the importance of adding beetroot stain in routine staining of breast tumours to aid in screening for the risk of stroma hyalinization.

The luminal epithelial cells in the fibroadenoma and invasive ductal carcinomas had increase deposition of acid mucins. This is comparable to work by (Shedge *et al.* 2018) who reported predominance of acid mucins in invasive ductal carcinomas. The genetic changes that lead to the over expression of these mucins in breast carcinogenesis has been linked to changes during glycosylation (Rachagani *et al.*, 2009). Mucin secretion is an early marker of secretory change in epithelial cells because there are secreted first in early carcinogenesis long before there is manifestation in nuclear changes and has been found to influence their behavior and progression (Mukhopadhyay *et al.*, 2011).

Table 2: The histopathological diagnosis, connective tissue changes and the age distribution of the breast tissues

| **Parameters** | Normal n(%) | Benign n(%) | Malignant n(%) | Total n(%) |
|---|---|---|---|---|
| **Histopathological diagnosis** | 1(6.7) | 3(20) | 11(73.3) | 15(100) |
| **Collagen size** (pixel) | | | | |
| <50 (+) | 1 (6.7) | 2 (20) | 4 (26.7) | 8 (53.3) |
| 50-100 (++) | 0 (0) | 1 (6.7) | 5 (33.3) | 5 (33.3) |
| >100(+++) | 0 (0) | 0 (0) | 2 (13.3) | 2 (13.3) |
| **Total** | 1 (6.7) | 3 (20) | 11 (73.3) | 15 (100) |
| **Beetroot stroma** | | | | |
| Coarse | 1 (6.7) | 0 (0) | 0 (0) | 1 (6.7) |
| Loose/coarse | 0 (0) | 3 (20) | 0 (0) | 3(20) |
| Hyalinized/coarse | 0 (0) | 0 (0) | 10 (66.7) | 10 (66.7) |
| Hyalinized | 0 (0) | 0 (0) | 1 (6.7) | 1 (6.7) |
| **Total** | 1 (6.7) | 3 (20) | 11 (73.3) | 15 (100) |
| **Mucin** | | | | |
| Acid | 1 (6.7) | 3 (20) | 11 (73.3) | 15(100) |
| Neutral | 0 (0) | 3 (20) | 0 (0) | 3 (20) |
| **Total** | 1 (6.7) | 3 (20) | 11 (73.3) | 15 (100) |
| **Age range (years)** | | | | |
| <20 | 0 (0.0) | 2 (13.3) | 0 (0.0) | 2 (13.3) |
| 21-30 | 0 (0.0) | 0 (0.0) | 8 (53.3) | 8 (53.3) |
| 31-40 | 1 (10) | 0 (0.0) | 2 (20) | 3 (20) |
| 41-50 | 0 (0.0) | 0 (0.0) | 1 (6.7) | 1 (6.7) |
| 51-60 | 0 (0.0) | 0 (0.0) | 1 (6.7) | 1(6.7) |
| **Total** | 1 (10) | 2 (13.3) | 12 (80) | 15 (100) |

Plate 1: Photomicrographs of stained tissues

## Immunohistochemical staining for ER, PR, HER2, CK7, and Ki-67

The ER, PR, HER2, CK7, and Ki-67 positive expressions are shown in Plate 1E to 1I respectively. The expression of immunohistochemical markers were 1(10%) each for ER+ and PR+ statuses with 3+ grade among the IDC 3. The HER2+ overexpressed was in 8(80%) with 4(40%) having 3+ grade and 7(70%) occurring in the IDC 3 cases. Triple negative (ER-, PR-, HER2-) occurred in 2(20%) of the cases. The CK7+ expression was in 6(60%) with 2(20%) in benign and 4(40%) occurring in the malignant cases while 5(50%) had 3+ grade. In the Ki-67 expression, 4(40%) had positive expression with 1(10%) occurring in fibroadenoma and 3(30%) occurring in the IDC cases while 2(20%) had 3+ grade (Table 3). There was low expression of ER+/PR+ tumours while majority were HER2+ overexpressed. Concerning these expressions, Nwafor and Keshinro (2015) had reported that ER+ and PR+ statuses were associated with good prognosis but HER2+ status was associated with poor prognosis and survival. This could explain why most of the breast cancer cases were aggressive with poorer survival. The high HER2+ overexpressed cases gave a different result from previous studies that reported the predominance of triple negative breast cancer among women of African ancestry (Huo *et al.*, 2009; Rahman *et al.*, 2014; Nwafor and Keshinro (2015). The reason for the difference could be because the tissue biopsies were fixed in neutral buffered formalin within 72 hours and stained for immunohistochemistry within a short duration of storage, thereby maintaining the antigenicity for HER2. Positive expression of CK7 was observed as was similarly stated by Maniar (2020) who reported presence of CK7 in breast cancers. CK7 is a type II keratin intermediate filament that is a marker of breast tissue differentiation. However, loss of CK7 expression in breast cancers may be associated with poor prognosis (Moatamed *et al.*, 2015). Also, Ki-67 was expressed in fibroadenoma and invasive ductal carcinoma with high proliferation index. Ki-67 is an important proliferation marker and positive cases have an increased risk of proliferation, re-occurrence, and poor prognosis. Poor prognosis of Ki-67 positive breast cancers was reported in the South-West part of Nigeria by Agboola *et al.* (2013). The expression of Ki-67 was also reported in breast tumours (Moatamed *et al.*, 2015; Davey *et al.*, 2021) and in leiomyoma in Calabar (Udonkang *et al.*, 2022). This study showed that there was increase in collagen size and mucin deposition among the immunohistochemical positive tissues. This is similar to reports by Trentham-Dietz *et al.* (2014) and Walsh *et al.* (2015) that observed that large collagen fibres were linked to ER+/PR+ and HER2+ statuses. It has also been reported that HER2+ tumours cause fibroblast proliferation and fibroblast growth factor-$\beta$ upregulation which leads to increase collagen density (Turgut *et al.*, 2015). Acid mucins have been reported to promote HER2 gene expression while decreasing ER1 gene expression (Auvinen, 2000; Hanoux *et al.*, 2018). As such, in addition to targeted therapies for ER+, PR+, and HER+ expressions, therapies targeted at collagen and mucin syntheses in breast cancers may help in improving prognosis in Calabar.

Table 3: Positive immunohistochemical expression of immunohistochemical markers and connective tissue changes among the tumour types

| **Diagnosis n=10** | **ER n(%)** | **PR n(%)** | **HER2 n(%)** | **CK7 n(%)** | **Ki67 n(%)** |
|---|---|---|---|---|---|
| **Normal** | 0 (0) | 0 (0) | 0(0) | 0 0) | 0 (0) |
| **Benign** | 0 (0) | 0 (0) | 0 (0) | 2(20) | 1(10) |
| Fibroadenoma | 0 (0) | 0 (0) | 0 (0) | 1 (10) | 1(10) |
| +2 | 0 (0) | 0 (0) | 0 (0) | 1(10) | 1(10) |
| Duc. papilloma | 0 (0) | 0 (0) | 0 (0) | 1 (10) | 0 (0) |
| +3 | 0 (0) | 0 (0) | 0 (0) | +3 (1) | 0(0) |
| **Malignant** | 1(10) | 1(10) | 8(80) | 4(40) | 3(30) |
| IDC 2 | 0(0) | 0 (0) | 1(10) | 1(10) | 1(10) |
| +1 | 0(0) | 0(0) | 0(0) | 0(0) | 1(10) |
| IDC 3 | 1(10) | 1(10) | 7(70) | 3(30) | 2(20) |
| +1 | 0(0) | 0(0) | 1(10) | 0(0) | 0 (0) |
| +2 | 0(0) | 0(0) | 3(30) | 0(0) | 0 (0) |
| +3 | 1(10) | 1(10) | 4(40) | 4(40) | 0 (0) |
| **Total (positive)** | 1(10) | 1(10) | 8(80) | 6(60) | 4(40) |
| **Total (negative)** | 9(90) | 9(90) | 2(20) | 4(40) | 6(60) |
| **Average collagen size (pixel)** | 34 | 34 | 76 | 52.6 | 55.0 |
| **Average mucin intensity (pixel)** | 46.4 | 46.4 | 59 | 61 | 71.5 |
| **Statistics** | $\chi^2$=12.800 | p=0.001 | | | |

Keys: 1+(low, <5 cells), 2+(moderate, 5-10 cells), and 3+(high, >10 cells)

Figure legends

Plate 1A: Photomicrograph of benign fibroadenoma. There is pericanalicular pattern of enlarged duct (D). (Haematoxylin and eosin x400).

Plate 1B: Invasive ductal carcinoma stained with van Gieson. Verhoeff's-van Gieson x400

Plate 1C: Invasive ductal carcinoma stained with beetroot. Beetroot x400

Plate 1D: Fibroadenoma stained with Colloidal iron-PAS method. Colloidal iron-PAS x400

Plate 1E: Immunohistochemical staining of estrogen receptor. (IHC x400).

Plate 1F: Immunohistochemical staining of progesterone receptor. (IHC x400).

Plate 1G: Immunohistochemical staining of HER2. (IHC x400).

Plate 1H: Immunohistochemical staining of CK7. (IHC x100).

Plate 1I: Immunohistochemical staining of Ki-67. (IHC x100).

**ANN Multilayer perceptron network prediction**

The results of the multilayer perceptron network are presented in Table 4. The 60-20-20 model had 9 factors in input layer (24 units), 1 hidden layer (4 units) and 1 output layer (2 units). The sample training had 8(57.1%), testing 5(25.7%) and holdout 1(7.1%). The model summary showed cross entropy error for training as 0.006 and testing as 0.031 which signified good prediction of the model with percent incorrect prediction as 0% each for the training and testing layers. The ROC curve analysis gave perfect accuracy of prediction as 1.000 for the benign and malignant tumours diagnosis respectively. Normalized importance of the independent variables is shown in Figure. 2. The markers/ risk factor of the breast tumour in order of importance were mucin (100%), beetroot stroma coarseness/hyalinization (91.1%), CK7 (80.5%), HER2+ (77.1%), and age (65.1%). The association between these independent variables and the diagnosis of the benign and malignant tumours was statistically significant at probability values of the cross entropy error for training as 0.006 and testing as 0.031. Figure 3 is the multilayer perceptron network diagram showing the interconnections between risk factors/genetic markers with benign and malignant tumours diagnosis.

The MLP neural network showed that the most important predisposing factors of the benign and malignant tumours involved the expression of acid mucin, stroma hyalinization, CK7+, HER2+, and age at diagnosis. Acid mucin produced by the cancer cells has been found to influence the behavior and progression of the disease (Mukhopadhyay *et al*., 2011). Stroma hyalinization causes treatment resistance and promotes tumour progression (Augustine *et al*., 2021). Loss of CK7 expression in breast cancers is associated with poor prognosis (Moatamed *et al*., 2015) and HER2+ overexpressed status is associated with poor prognosis and survival (Nwafor and Keshinro, 2015). As such, treatment tailored to these tissue changes can aid in reducing tumour aggressiveness and improve prognosis. On the other hand, age has remained a major risk factor for breast cancer as most occur during the premenopausal period. This is similar to the association of early age at diagnosis with breast tumours in previous study by Udonkang *et al*., 2023. The way forward is for women to have routine breast cancer screening during their reproductive age for early diagnosis and treatment.

**Table 4:** Summary of MLP results for breast tumours diagnoses with genetic markers

| MLP RESULTS | | | | |
|---|---|---|---|---|
| **Parameter** | | N (%) | Cross entropy error | |
| **Sample** | Training | 8 (57.1) | 0.006 | |
| | Testing | 5 (35.7) | 0.031 | |
| | Holdout | 1 (7.1) | 0.0 | |
| | Valid | 14 (100) | | |
| **Training time** | 0:00:00.02 | | | |
| **Classification** | | Benign | Malignant | % Correct prediction |
| **Training** | Overall % | 12.5 | 87.5 | 100 |
| **Testing** | Overall % | 20.0 | 80.0 | 100 |
| **Holdout** | Overall % | 100 | 0.0 | 100 |
| **ROC Area** | Normal | 1.000 | | |
| | Benign | 1.000 | | |
| | Malignant | 1.000 | | |

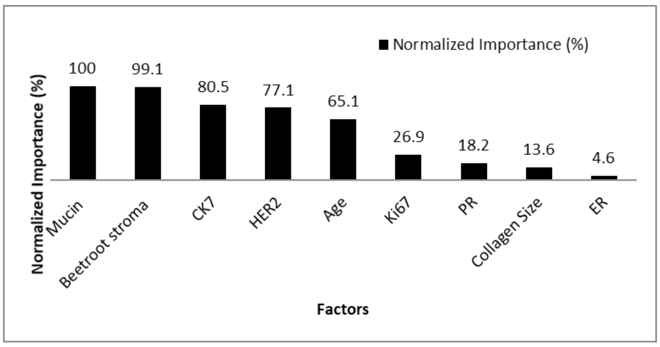


**Fig. 1.:** Normalized importance of age, genetic markers and connective tissue changes among the tumour types

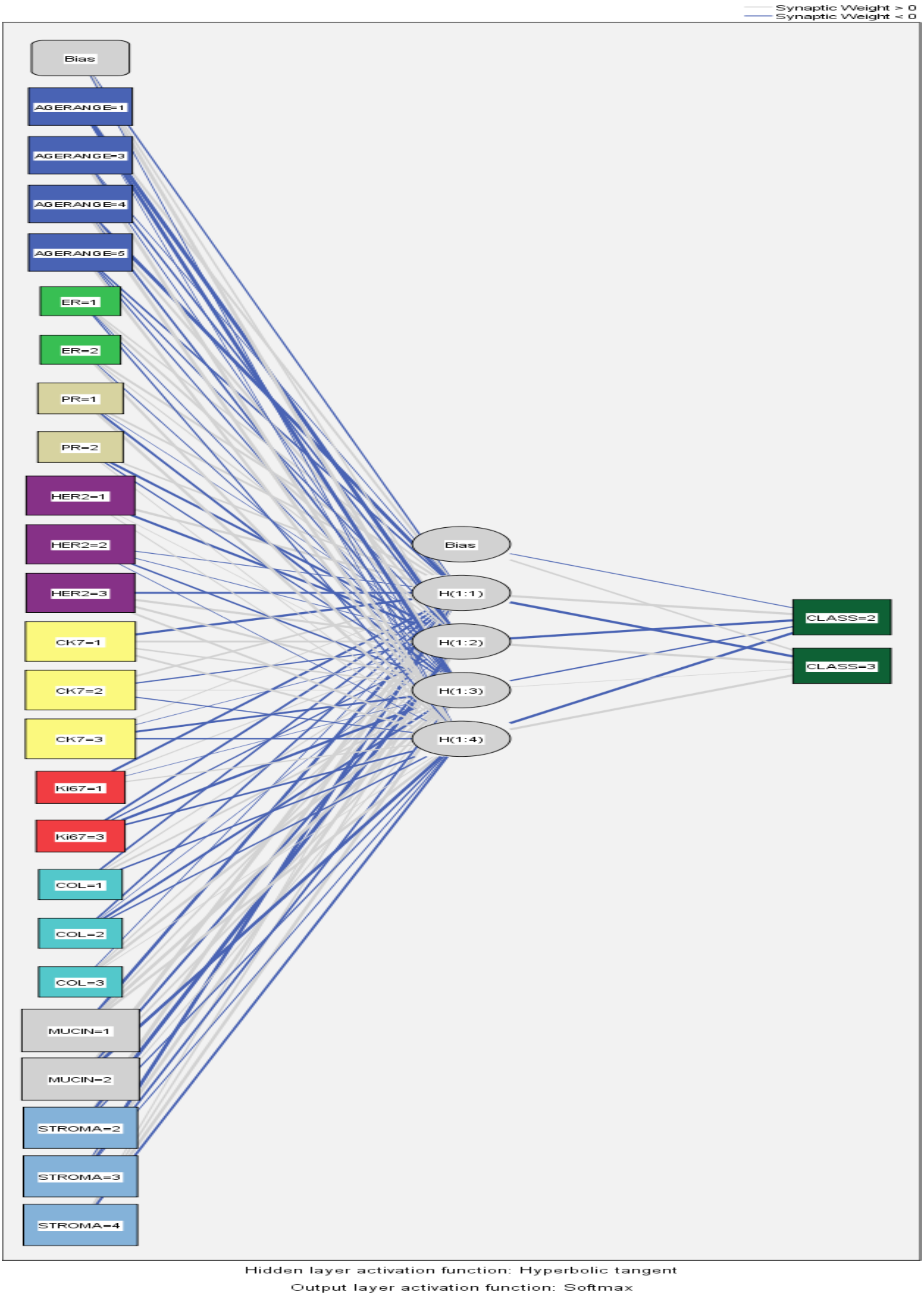


**Fig. 2:** The MLP neural network of age, immunohistochemical markers and connective tissue changes among the tumour types

Keys: Class 2=benign, Class 3=malignant; age (years) 1=<20. 2=21-30, 3=31-40, 4=41-50, 5=>50; markers 1=low, 2=moderate, and 3=high

## CONCLUSION

Benign and malignant breast tumours are common among women in Calabar and the tissues undergo epithelial and stromal changes characterized by ductal enlargement, collagen remodeling, reorientation, overproduction, and desmoplasia. Beetroot stain showed stroma hyalinization changes in the breast cancers. Over-expression of HER2+ was found in the invasive ductal carcinomas which had increase collagen and acid mucin depositions. The ANN gave acid mucin deposition, stroma hyalinization, CK7, HER2+, and age as the most important predisposing factors to breast tumours. These tissue changes have given insight into tumour behavior in the studied cases as they may play important roles in promoting progression and resistance of breast cancer cells to treatment. Breast cancer is characterized mostly by acid mucin deposition and stroma hyalinization.

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