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PATTERN OF IMMUNOHISTOCHEMICAL EXPRESSION OF INHERITED BREAST CANCER GENES AND COLLAGEN CHANGES AMONG AFRICAN WOMEN WITH EARLY BREAST CANCER IN CALABAR, NIGERIA

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ABSTRACT

The disparity in age of diagnosis and genetic testing of breast cancer among African women is a major cause of concern. The common inherited breast cancer genes like breast cancer gene 1 (*BRCA*1), breast cancer gene 2 (*BRCA*2) and Tumour protein 53 (*TP*53 or p53) as well as increase collagen deposition in the stroma predispose women to early breast cancer. The aim of this study was to establish the immunohistochemical expressions patterns of BRCA1, BRCA2, and p53 proteins as well as collagen changes in females with early onset breast cancers in University of Calabar Teaching Hospital. Data on breast tumours occurrences among 96 females were obtained from the Histopathology register. Ten randomly selected paraffin wax-embedded breast tissue blocks from Histopathology laboratory, University of Calabar Teaching Hospital were sectioned at 4 micrometer, stained histologically with haematoxylin and eosin, van Gieson for collagen fibres and immunohistochemically for BRCA1, BRCA2 and p53 protein expressions. Results showed that of the 96 women with breast tumours, 84.4% were ≤50 years while 15.6% were >50 years. Among the 10 tissues, 60% were BRCA1(-) and 40% BRCA1(+), 10% BRCA2(-) with 90% BRCA2(+), and 30% p53(-) with 70% p53(+) for protein expressions, although these were not significant. The BRCA1(+) tissues had significant lower staining intensity than BRCA2(+) (50.5±12.5; p=0.011) and p53(+) (53.8±8.6; p=0.040) counterparts. Majority of the breast tumours had significant increases in collagen fibre sizes consistent with type of tumour and grade of carcinoma but was irrespective of BRCA or p53 statuses. In conclusion, breast tumours are common among women below 50 years in Calabar and the selected early breast cancers were mostly characterized by negative expressions of BRCA1, positive expressions of BRCA2 and p53 proteins as well as increase deposition of collagen fibres. There is urgent need to carryout wider studies on these inherited breast cancer genes and collagen alterations to determine the risk of early breast cancer development.

KEYWORDS: *BRCA* gene, p53, inherited breast tumours, collagen

INTRODUCTION

Breast cancer is the commonest cancer among women worldwide but there is disparity in age of diagnosis in women of African ancestry. In Caucasians, breast cancer is prevalent among women above the age of 50 years (Friebel *et al*., 2019; Ebughe *et al*., 2019). But, in Nigeria the trends over the years revealed that high number of cases of breast cancer occurred among young women mostly below 50 years of age (Ebughe *et al*., 2019; Ikpatt *et al*., 2002; Huo *et al*., 2009; Jedy-Agba *et al*., 2016).

Huo *et al*. (2009) and Rosenthal *et al*. (2017) have stated that one of the implicating risk factors for this

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early onset of breast cancer is inheritance of breast cancer susceptibility genes. The common inherited genes are *BRCA1*, *BRCA2* and *TP53* (Zheng *et al*., 2018). Most studies have reported the mutation of these genes among African women (Friebel *et al*., 2019) and in women in South West region of Nigeria (Zheng *et al*., 2018; Fackenthal *et al*., 2005; Fackenthal *et al*., 2012; Pitt *et al*., 2018). However, apart from paucity of data, there is also disparity of molecular testing on the occurrence of inherited breast cancer genes among women in Calabar, South South, Nigeria.

These BRCA1, *BRCA2* and *TP53* are tumour suppressors and high penetrance genes that are inherited in autosomal dominant pattern (Rosenthal *et al*., 2017; Ayub *et al*., 2014; Mehrgou and Akouchekian, 2016). *BRCA1* and *BRCA2* genes are located on long arms of chromosomes 17 and 13 respectively (Petrucelli *et al*., 2010). Their major roles involve ensuring that any damaged deoxyribonucleic acid (DNA) is repaired to maintain cell integrity (Petrucelli *et al*., 2010; Venkitaraman, 2014). The *TP53* is located on the short arm of chromosome 17 and plays major role in inducing apoptosis, ensuring cell cycle arrest, and repair of damaged DNA (Brosh and Rotter, 2009). However, mutations in these genes interfere with normal DNA repair mechanisms causing accumulation of damaged DNA in the cells and consequently lead to increased susceptibility to development of breast cancer (Ayub *et al*., 2014; Mehrgou and Akouchekian, 2016; Brosh and Rotter, 2009; Marchina *et al*., 2010).

Another risk factor for breast cancer development is increase in collagen deposition in the extracellular stroma (Luparello, 2013). Increase in collagen synthesis and deposition is found to cause matrix stiffness, which interferes with diffusion of substances such as oxygen. Hypoxia, in turn up regulates progression to carcinogenesis (Luparello, 2013).

These alterations in collagen fibre production in breast cancers have been associated with the expressions of these genes as stated by Lee *et al*. (2019) that reported increase frequency of hyaline fibrous changes, which were identified as collagen type 1 in *BRCA1* and *BRCA2* mutations. Also, *p53* mutation was found to cause secretion and deposition of extracellular matrix notably collagen in the sclerotic stroma of early onset breast cancers (Packwood *et al*., 2009).

Hence, this study determined the pattern of expression of BRCA1, BRCA2 and p53 proteins as well as collagen changes in women with early onset breast cancers in Calabar.

MATERIALS AND METHODS

Data of study subjects/selection of tissue blocks

Data from 96 subjects were collected between January 2018 and March 2019 to access the occurrence of breast tumours. This study was carried out in accordance with institutional protocol of the University of Calabar Teaching Hospital, Calabar, a hospital-based cancer registry and tertiary health facility where most cases of breast cancers in Cross River State are diagnosed. The data on age of subjects and histological diagnosis of the subjects were obtained from the Histopathology Laboratory Register of the facility. Ten tissue blocks comprising 5 malignant from subjects below the age of 50 years (Test), 3 benign and one normal breast tissue below the age of 50 years, and one malignant tumour above 50 years (positive control) were selected. Ethical approval was obtained from the University of Calabar Teaching Hospital Research and Ethics Committee with approval number UCTH/HREC/33/694.

Histological tissue preparation

The ten buffered neutral formalin and paraffin wax embedded tissue blocks were sectioned at 4micrometer with Leica RM2125 rotary manual microtome (USA). The sections were stained with haematoxylin and eosin and van Gieson dyes, and viewed with OMAX 40X-2500X light microscope (China). The initial diagnosis of benign and malignant breast tumours was done and confirmed by at least two pathologists. Histological grading of breast tumours was based on the Scarff-Bloom-Richardson tumour grading system. Line measurements of the collagen fibres were made with the 400x magnification of van Gieson- stained sections using AmScope digital software. A total of ten measurements were made from two photomicrographs of each tissue for reproducibility.

Immunohistochemical (IHC) staining techniques

The ten tissue blocks were also sectioned for immunohistochemical staining. The BRCA1 (E-AB-40282) 1:100 dilutions and BRCA2 (E-AB-40288) 1:100 dilutions rabbit primary antibodies (Elabscience, China) and p53 (DO7) 1:100 dilutions rabbit primary antibody (Bio SB, USA) were purchased and the procedures were carried out based on manufacturer's instructions. The brief protocol involved antigens retrieval with heated citrate buffer (pH 6.0), peroxidase blocking in 3% hydrogen peroxide, primary antibody (BRCA1, BRCA2, p53) staining, colour development with 3,3′-diaminobenzidine (DAB) chromogen, and counterstaining in haematoxylin. Slides were viewed microscopically and photomicrographs were taken with AmScope MD500 digital camera and software (USA). Brown colour staining of nucleus or cytoplasm was observed as positive immunostaining. Normal expressions of BRAC1, BRCA2 and p53 proteins were recorded as <10 positively-stained cells. Over expression was observed and reported as staining in >10 positively-stained cells. Negative result was reported in slides with complete absence of any immunostaining.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 (Armonk, New York: IBM Corporation) was used to analyze the results. Results were presented as percentages and mean±standard deviation. Chi-square test using 2X2 cross tabulation was used to establish associations between the presence of the proteins with class and grade of the disease as well as the distribution of the negative and positive expression of the proteins after the datasets were weighed. Student t-test was

used to analyze the mean staining intensities among the positive expressed proteins and the mean of the collagen sizes. Pearson correlation was used to establish associations between expressions of BRAC1, BRCA2 and p53 proteins and mean collagen sizes. All results were statistically significant at probability level less than or equal to 0.05.

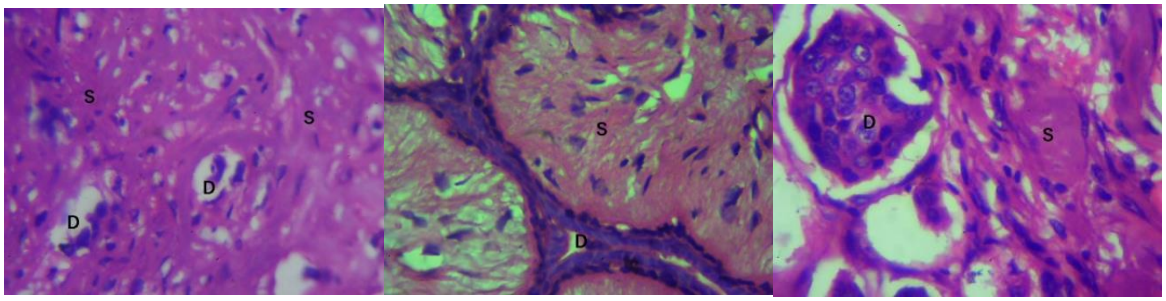
RESULTS

Occurrence of breast tumours at early age

Early breast cancer was regarded as breast cancer occurring below the age of 50 years. Majority of subjects had early breast cancer cases. The distribution of age by histological types of breast tumours is shown in Table 1. Subjects ≤50 years accounted for 81(84.4%) with mean age of 30.7±2.5 years while those >50 years were 15(15.6%) with mean age 64.5±2.23 years. The total benign tumours were 56(58.3%) with all recorded within ≤50 years and malignant tumours were 35(21.9%) with 21(21.9%) within ≤50 years and 14(14.6%) in women >50 years. This distribution of the tumours by age of subjects was statistically significant (χ^2=26.596, p=0.001). Plate 1 shows photomicrographs of sections stained with haematoxylin and eosin method.

Table 1: Distribution of breast tumours by age of subjects

Age (years)	Normal n(%)	Benign n(%)	Malignant n(%)	Unclassified n(%)	Total n(%)	Mean Age±SD (years)	Statistics
≤50	1(1.0)	56(58.3)	21(21.9)	3(3.1)	81(84.4)	30.68±2.50	χ^2=26.596
>50	0(0.0)	0(0.0)	14(14.6)	1(1.0)	15(15.6)	64.54±2.23	p=0.001
Total	1(1.0)	56(72.9)	35(21.9)	4(4.2)	96(100)	35.16±15. 36	



A B C

Plate 1: Haematoxylin and eosin-stained breast tissues. A is normal tissue, B is benign fibroadenoma with intracanalicular pattern of duct, and C is malignant invasive ductal carcinoma with desmoplasia in duct. D is duct and S is stroma (H&E 400x magnification).

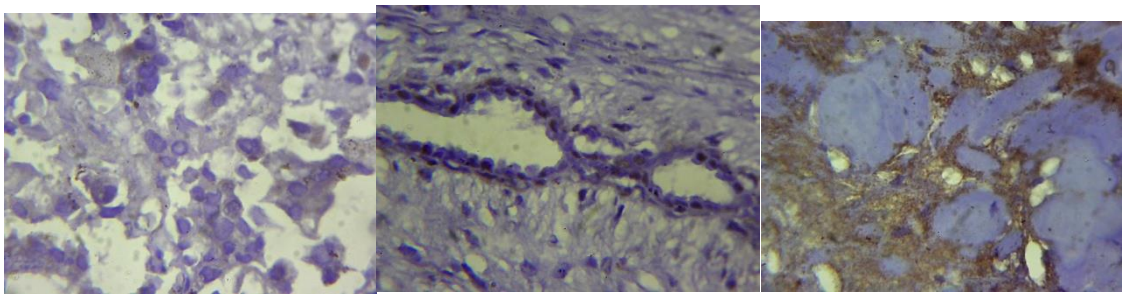
Loss of expression/low staining intensity of BRCA1 and over expression of BRCA2 and p53 proteins found in most early breast cancers

Table 2 shows the age distribution of tumours and immunohistochemical staining statuses of the proteins. The ten tissues selected for immunohistochemical staining were 5(50%) malignant (test), 3(30%) benign, and 1(10%) normal tissues within ≤50 years while 1(10%) malignant positive control was from >50 years. Among the 10 tissues, 6(60%) showed negative expression and 4(40%) had positive expression for BRCA1 proteins. The distribution of negative expression for BRCA1 proteins was 4(40%) for test (malignant tumours) group and 1(10%) each for normal tissue and positive control. The positive expression for BRCA1 proteins was found in only 1(10%) of test group and 3(30%) of benign tumours. Only 1(10%) of benign tumour had negative expression of BRCA2 proteins while 9(90%) including all 5(50%) malignant tumours (test) had positive expression of BRCA2 proteins. There were 3(30%) cases with negative expression for p53 and 7(70%) cases with positive expression for p53. Among these, the test group had 2(20%) with negative expression and 3(30%) had positive expression for p53. The expression patterns were not statistically significant for BRCA1 (χ^2=3.016, p=0.389), BRCA2 (χ^2=2.593, p=0.459) and p53 (χ^2=2.222, p=0.528) respectively.

Plate 2, 3, and 4 show the photomicrographs of slides stained for BRCA1, BRCA2 and p53 respectively. The staining intensities were measured using the colour cube tool under segmentation and count section of Amscope software. Six measurements per slide for each protein were recorded. The mean staining intensity shown in Figure 1 of BRCA1 (33.5±10.6) was significantly lower when compared with mean staining intensities of BRCA2 (50.5±12.5; t=3.935, p=0.011) and p53 (53.8±8.6; t=2.764, p=0.040) respectively.

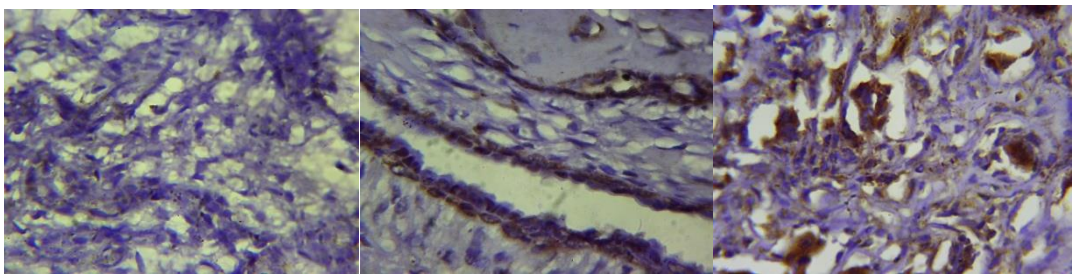
Table 2: Age distribution of tumours and immunohistochemical staining status

Parameters	Normal (Negative control) n(%)	Benign (Negative control) n(%)	Malignant (Test) n(%)	Malignant (Positive control) n(%)	Total n(%)	Statistics
Age (years)						
≤50	1(10.0)	3 (30.0)	5(50.0)	0(0.0)	9(90.0)	
>50	0(0.0)	0(0.0)	0(0.0)	1(10)	1(10.0)	
Total					10(100)	
IHC status						
BRCA1(-)	1(10)	0(10)	4(40)	1(10)	6(60)	χ^2=3.016
BRCA1(+)	0(0)	3(30)	1(10)	0(0)	4(40)	p=0.389
BRCA2(-)	0(0)	1(10)	0(0)	0(0)	1(10)	χ^2=2.593
BRCA2(+)	1(10)	2(20)	5(50)	1(10)	9(90)	p=0.459
p53(-)	0(0)	1(10)	2(20)	0(0)	3(30)	χ^2=2.222
p53(+)	1(10)	2(20)	3(30)	1(10)	7(70)	p=0.528



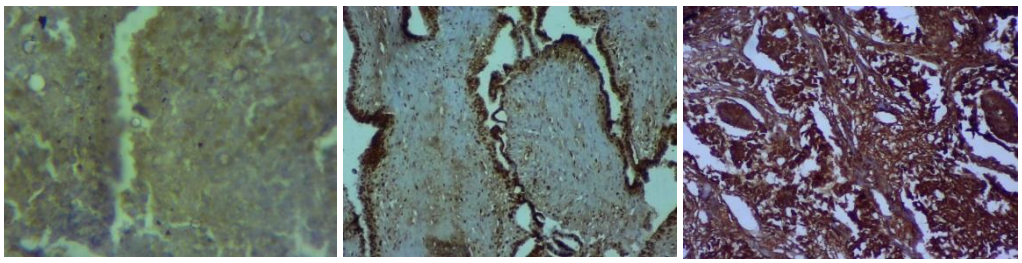
A B C

Plate 2: BRCA1immunohistochemical-stained breast tissues. A is tissue with negative expression, B is benign fibroadenoma with positive expression shown as faint nuclei or cytoplasmic staining. C is malignant invasive ductal carcinoma with cytoplasmic staining indicating positive expression. (IHC, 400x magnification).



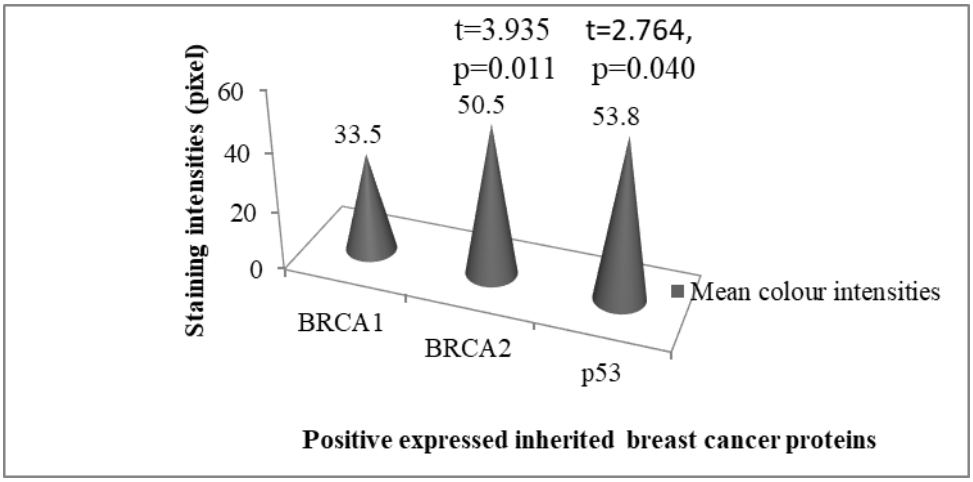
A B C

Plate 3: BRCA2 immunohistochemical-stained breast tissues. A is tissue with negative expression, B is benign fibroadenoma with positive expression shown as cytoplasmic staining. C is malignant intraductal carcinoma with cytoplasmic staining indicating positive expression of the protein. (IHC, 400x magnification).



A B C

Plate 4: p53 immunohistochemical-stained breast tissues. A is tissue with negative expression, B is benign fibroadenoma with positive expression shown as cytoplasmic staining. C is malignant invasive ductal carcinoma with cytoplasmic staining indicating positive expression of p53. (IHC, 400x magnification)



t=T-test and p=probability level

Figure 1: Comparison of staining intensities among the positive expressed inherited breast cancer proteins

Increase in collagen fibre size in BRCA1 negative tumours

The mean collagen fibre measurements of the breast tumours were compared with that of the normal tissue. The result is shown in Table 3. Among the test, there were significant increases in mean collagen fibre sizes of invasive ductal carcinoma grade 2 (37.5±17.3, t=2.993, p=0.014) with (BRCA1+/BRCA2+/p53+) status as well as in invasive ductal carcinoma grade 3 (52.7±36.4, t=2.323, p=0.045) and invasive ductal carcinoma grade 3 (94.8±59.4, t=3.978, p=0.003) all having (BRCA1-/BRCA2+/p53+) statuses respectively. There was no significant increase in mean collagen fibre size of the benign ductal papilloma (25.6±9.88, t=0.600, p=0.564), which was positive for all three proteins (BRCA1+/BRCA2+/p53+). The increase in mean collagen fibre size of fibroadenoma (39.9±15.76, t=2.993, p=0.015) with (BRCA1+/BRCA2+/p53+) status was significant. For the positive control, there was significant increase in mean collagen fibre size of the invasive ductal carcinoma grade 3 (55.2±38.7, t=2.587, p=0.029) with (BRCA1-/BRCA2+/p53+) status. The van Gieson-stained photomicrographs are shown in Plate 5.

Table 3: Collagen fibre size in breast tumours and immunohistochemical status

Tumour type	Age (years)	Mean collagen fibre size±SD	BRCA1 status	BRCA2 status	p53 status	t-test	p-value
Normal	≤50	23.2±7.99	-	+	+		
Test							
IDC grade 2	≤50	37.5±17. 3	+	+	+	3.063	0.014*
IDC grade 3	≤50	52.7±36.4	-	+	+	2. 323	0.045*
IDC grade 3	≤50	94.8±59.4	-	+	+	3.978	0.003*
Benign							
Fibroadenoma	≤50	39.9±15.76	+	+	+	2.993	0.015*
Ductal papilloma	≤50	25.6±9.88	+	+	+	0.600	0.564
Positive control							
IDC grade 3	>50	55.2±38.7	-	+	+	2.587	0.029*

* indicates significance at 95% confidence interval

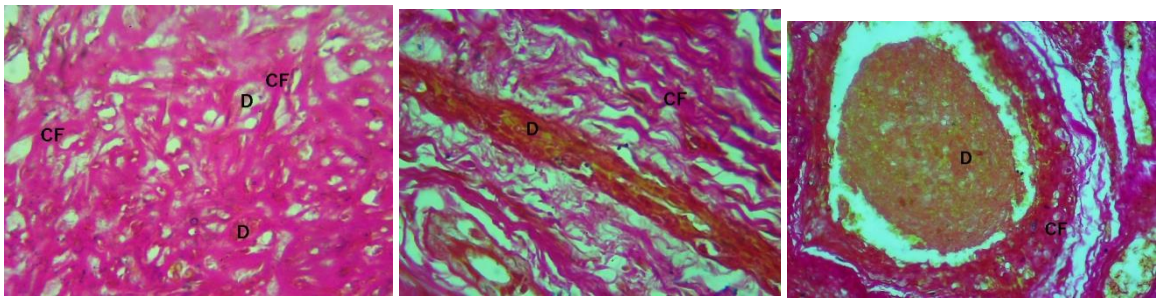


PLATE 5: Collagen fibres in breast tissues. A is normal tissue, B is benign fibroadenoma, and C is malignant invasive ductal carcinoma. D is duct and CF is collagen fibre (van Gieson 400x magnification).

Correlation between BRCA1, BRCA2 and p53 protein expressions with collagen deposition

The Pearson correlation is shown in Table 4. The negative weak correlations between the expressions of BRCA1 protein and collagen sizes were all not significant in the ductal papilloma (r=-0.216, p=0.340), fibroadenoma (r=0.120, p=0.410), and invasive ductal carcinoma grade 3 (r=-0.283, p=0.294). The associations of BRCA2 protein expressions with collagen sizes showed non-significant weak negative correlation in ductal papilloma (r=-0.144, p=0.393) and weak positive correlation in fibroadenoma (r=0.188, p=0.361), but significant strong negative correlation in invasive ductal carcinoma (r=-0. 941, p=0.003). In the p53 protein expressions association with collagen sizes, there were non-significant negative weak correlation in ductal papilloma (r=-0.388, p=0.224) and positive weak correlation in fibroadenoma (r=0.103, p=0.423), but significant negative strong correlation in invasive ductal carcinoma (r=-0.732, p=0.049).

Table 4: Correlation between BRCA1, BRCA2 and p53 protein expressions with collagen deposition

Protein type	Protein intensity±SD	Collagen size±SD	Pearson correlation value	p-value
Ductal papilloma				
BRCA1	141.83±36.30	25.61±9.88	r=-0.216	p=0.340
BRCA2	50.00±18.05		r=-0.144	p=0.393
p53	47.50±4.88		r=-0.388	p=0.224
Fibroadenoma				
BRCA1	29.17±11.86	39. 90±15.76	r=-0.120	p=0.410
BRCA2	53.33±10.11		r=0.188	p=0.361
p53	62.00±11. 95		r=0.103	p=0.423
Invasive ductal caarcinoma				
BRCA1	50.67±17.05	37.47±17. 30	r=-0.283	p=0.294
BRCA2	60.00±18.58		r=-0. 941	p=0.003*
p53	61.50±17.65		r=-0.732	p=0.049*

* indicates significance at 95% confidence interval

DISCUSSION

This study was to evaluate the expressions of inherited BRCA1, BRCA2 and p53 proteins and collagen changes in selected early breast cancer cases. The detection of benign and malignant breast tumours was common. Women of younger ages (≤50years) were diagnosed of breast cancer in the study consistent with reports in previous works where the mean ages of 42.7 years (Ikpatt *et al*., 2002) and 44.8 years (Huo *et al*., 2009) were reported in Calabar. Others were <50years in Ibadan (Huo *et al*., 2008), 48.2 years in Sokoto (Agbo *et al*., 2014), 41.7 years in Imo state (Anele *et al*., 2014), and 48.98 years in Ilorin (Rahman *et al*., 2014). The link between age and breast tumours shows that majority of the tumours begin as benign diseases and some later progress to malignant diseases with increasing age. These breast tumours are thought to occur as a result of changes in DNA. The high susceptibility of the breast to frequent DNA mutations has been linked to constant hormonal (oestrogen) fluctuations during physiological changes in puberty, menstrual cycle, pregnancy, lactation, and menopause (Lee and Sultanian, 2015). However, there seems to be other underlying factors that may be linked to inherited breast cancer genes such as mutations in BRCA1, BRCA2 and p53 as well as changes in the collagen fibres in the stroma.

Results from this study revealed that majority of the early breast cancer cases had loss of BRCA1 expression but were positive in expressions of BRCA2 and p53 proteins. The few BRCA1(+) cases were found to have reduced staining intensity of the protein. This loss of/or low expression levels of BRCA1 protein may be attributed to its gene mutation. Previous studies have reported mutations in these genes among Nigerian women (Fackenthal *et al*., 2005; Fackenthal *et al*., 2012; Pitt *et al*., 2018) in South West, Nigeria. In Ibadan, South West, Nigeria, Fackenthal *et al*. (2012) reported more frequency of mutations in *BRCA1* than in *BRCA2*. Zheng et al (2018) also reported the frequency of mutations in *BRCA1* (7.0%), *BRCA2* (4.1%) and *TP53* (0.4%) among women in Ibadan. While Fackenthal *et al*. (2005) and Friebel *et al*. (2019)

reported more frequency of *BRCA2* than *BRCA1* genes among the women.

It was found that although there was loss of BRCA1 expression, the same tissues had over expression of BRCA2 protein. This pattern of positive expression of BRCA2 in early breast cancer cases have been reported by Hedau *et al*. (2015) who also suggested that over expressed BRCA2 may aid in tumour aggressiveness. *BRCA1* and *BRCA2* are tumour suppressors that function in DNA repair. It is thought that in the absence of BRCA1, there might be a compensatory increase in *BRCA2* in order to repair the damaged DNA of the cells (Hedau *et al*., 2015).

The early breast cancer cases also had over expression of p53 proteins. The p53 protein in normal cells has short life cycle because of frequent degradation. But, when missense *TP53* gene mutations occur, a stabilized p53 protein is produced and this affects posttranscriptional modification causing excess accumulation of *TP53* gene in the nucleus. The accumulated p53 protein results in over expression in immunohistochemical staining (Yang *et al*., 2013).

There are several suggestions on possibly link between the over expressed p53 proteins with loss of BRCA1 and over expression of BRCA2 in breast cancers. First, *TP53* gene maintains the genome integrity by integrating the cellular response to DNA damage. Arizti *et al*. (2000) stated that during response to DNA damage, the wild-type *TP53* down regulates BRCA1 protein expression by negatively affecting its transcription. The postulated mechanism might occur when *TP53* causes cell cycle arrest at the Gap1 and or Gap2/Mitotic phases (Arizti *et al*., 2000).

In addition, the relationship between p53 and BRCA2 protein over expressions shows that their genes act in a synergistic manner as tumour suppressors during homologous DNA repair as they are located on the same chromosome 17 (Arizti *et al*., 2000; Rajagopalan *et al*., 2010). In the early steps of homologous repair, *TP53* binds to *BRCA2* and this may be one of the main reasons they are over expressed (Rajagopalan *et al*., 2010). These mechanisms of loss and over expression of proteins might rightly explain the characteristics of the early breast cancer cases observed in this study.

Another finding was that the early breast cancer had significant increased production and deposition of collagen fibres in the stroma. The collagen sizes were increased as the grade of invasive ductal carcinoma increased. The increases in collagen sizes was observed in the benign states especially in fibroadenomas. Association of the expressions of BRCA1, BRCA2 and p53 proteins show varying results. But, a strong association between the BRCA2 and p53 protein expressions and increase collagen size was significant. This observation is similar to reports of increase collagen deposition in BRCA and p53 mutations (Lee *et al*., 2019; Packwood *et al*., 2009).

High collagen fibre deposition has been reported as a major predisposing factor to the development of breast cancer (Luparello, 2013). It is believed that the collagen fibre gene synthesis alteration leads to their over production in reaction to the changes in the tumour cells and as a defence mechanism to halt their metastasis. However, the excess collagen fibres begin to subject the extracellular matrix to hypoxia which worsens the progression of breast cancer. Studies have shown that increase collagen deposition leads to high mammographic density and increases the risk of development of breast cancer (Luparello, 2013).

The highlights of this study are that these collagen changes in early breast cancer together with loss of inherited breast cancer genes may be major driving forces in initiation, progression and outcome of the disease.

CONCLUSION

The findings showed higher occurrence of breast cancer among young women below 50 years. Further investigations using paraffin wax-embedded blocks from 10 subjects revealed that, most of the early breast cancer cases did not express BRCA1 proteins and the few with positive expression had lower staining intensities. But, BRCA2 and p53 proteins were found to be over expressed. Also, the breast tumours were shown to undergo increases in deposition and size of collagen fibres. This research has established a link between early breast cancer with alterations in tumour suppressor genes and collagen deposition. Overall, these results may aid in better prognosis, treatment and management of early breast cancer in Calabar. Further molecular studies to investigate mutations in *BRCA1, BRCA2* and *TP53* genes as well as collagen genes changes will aid in understanding the nature of gene mutations among these early breast cancer cases. Thus, the findings in this study will serve as basis for future and broader collaborative research in understanding the genetic alterations of early breast cancer among women in Calabar.

Author Contributions

MU, TU and IE did study design and conceptualization. MU, EO, AA, and SJ funded the work. MU, EO, AA, SJ, and DO acquired data and drafted the manuscript. MU, TU, and IE analyzed and interpreted data. All authors revised the manuscript. MU is the guarantor of this work.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Abbreviations

BRCA1, breast cancer 1; BRCA2, breast cancer 2, TP53 or p53, Tumour protein 53; DNA, deoxyribonucleic acid; H&E, haematoxylin and eosin; DAB, 3,3′-diaminobenzidine; SPSS, Statistical Package for Social Sciences; IHC, Immunohistochemistry.

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