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Triple Negative Breast Cancer in a Private Immunohistochemistry Laboratory in Abuja Nigeria

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Abstract: This work is aimed at determining triple negative breast cancer cases received in our centre and to forecast the Nigerian scenario for this cancer. Immunohistochemical staining for estrogen and progesterone receptors and Her-2/neu was performed on 10% formalin-fixed, paraffin-embedded primary carcinoma of the breast from 82 patients, between 2013 and 2014 using monoclonal antibodies for ER and PR (Dako Carpentaria, CA, USA) and ER (ID5; 1:50), with PR (PgR636; 1:400) and HER-2/neu performed using rabbit anti-human c-erbB-2 oncoprotein as primary antibody at 1:100 dilutions. During the 2-year period, 82 histologically confirmed cases of infiltrating ductal breast carcinoma were assessed for estrogen receptor, progesterone receptors and Her-2/neu status. Resultsshowed46.3%:42.6% estrogen receptor(ER+) positivity and progesterone receptor (PR+) positivity respectively. Her-2/neu oncogenes positivity was 25.6% while triple negative breast cancer was 31.7%. ER+PR+/ER-PR+ was 32.5%:50%.The Mean±SD for HER-2/neu variance between women below 50ears and above 50years was 10±4.8 and 11±4.5, t=0.07, DF=6 and was not statistically different at $p < 0.05$ while estrogen and progesterone hormone was 10±4.1 and 11±2.1 respectively. This study indicated that triple negative breast cancer (TNBC) is increasing and thus the chances of breast reoccurrence after mastectomy is predicted in this study. Equally the difference in the ratio of estrogen receptor positivity to progesterone receptor positivity among studies could be largely attributed to masking of antigen due fixation and duration of the archived paraffin embedded tissues in the Nigerian environment.

Key words: Triple Negative · TNBC · Progesterone · Amassoma · Bayelsa · IHC · Abuja · Nigeria

INTRODUCTION

Breast cancer is characterized by genetic heterogeneity which makes its diagnosis and treatment challenging [1-4]. Breast cancer with similar histological appearance can exhibit tremendous variations in clinical presentation, disease aggressiveness and treatment response in different patients and ethnic populations [5, 6]. It is currently regarded as a heterogeneous disease that has been classified into various molecular subtypes according to the gene expression profile of Estrogen receptor (ER), Progesterone receptors (PR) and human epidermal growth factor receptor 2(Her-2/neu) including: basal cell-like or triple negative (ER-, PR-and HER2-), luminal A (ER + and/or PR+, HER2-), luminal B (ER + and/or PR+,HER2+) and normal breast like [7, 8]. Estrogen Receptor is a member of a family of nuclear receptors that functions as transcriptional regulator that mediates the biological responses to the sex hormone- estrogen which is essential for reproduction, cardiovascular, skeletal and nervous systems. Currently there are two different forms of estrogen receptors (A and B) receptors that are co-expressed in many cell types each encoded by separate genes (ESR1 and ESR2) located on different chromosomes [9]. The Estrogen receptor 1 (ESR1) gene is located on q arm of chromosome 6, while ESR2 gene is located on q arm of chromosome 14. ESR1and ESR2 show significant overall sequence homology with the greatest homology (close to100%) in their DNA binding domains and both are composed of seven domains [10, 11]. ESR1 comprises eight exons of more than 140Kb separated by seven introns of 40kb each exon encodes a certain region of the protein [12].

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Researchers have identified 20 ER alpha splice variants in human breast cancer and the most abundant variants are created by splicing deletions in one or more exons. The generation of human Estrogen Receptor alpha (Era) mRNA transcripts is a complex process that involves at least seven different promoters exhibiting cell line-dependent promoter usage. Most ER alpha variants differ in the 5-untranslated regions and result in the expression of the full-length 66-kDa form of ER alpha. Among others, 46-kDa isoforms of ER alpha(ER alpha 46) generated from an internal ATG start codon lack exon 1 and consequently the N-terminal AF-1region.Wang and co-workers (2008) has cloned a 36-kDa isoform of ER alpha -named ER alpha -36 [28]. It is generated from a promoter located in the first introns of the ER alpha gene and it lacks both of the two transcriptional activation domains AF-1 and AF-2. Also in some estrogen-dependent cells and tissues ER might directly activate G-protein-coupled receptors and/or heterodimeric human epidermal growth factor receptors, resulting in downstream activation of MAPK and PI3K pathways and in turn, p38 (Protein 38), a member of the MAPK family, is capable of phosphorylating and activating ER in a ligand-independent manner. This cross-talk activation of ER results in the loss of inhibitory effects of tamoxifen therapy in the management of breast cancer [13, 28].

Human epidermal growth factor receptor-2(Her2) also known as c-erb-B2, HER-2/neu, p185, or CD340) is a proto-oncogene located at the long arm of human chromosome 17(17q21–q22).It is part of a family of genes that play roles in regulating cell growth [14, 15]. It is a tyrosine kinase (TK) growth factor receptor expressed by a number of normal tissues and probably has a role in normal cell function, regulating growth and proliferation. The full length (4.6 Kb) of Her-2/neu transcript encodes 185kDa transmembrane glycoprotein receptor with intrinsic tyrosine kinase (TK) activity [16]. It contains an N-terminal extracellular domain (ECD), a single transmembrane helix, a TK domain and an intracellular regulatory domain. The ECD is 600 residues long and contains four (I–IV) domains including a site of potential ligand binding between domains I and III. The ECD can undergo proteolytic cleavage in the juxta-membrane region that releases soluble Her-2/neu ECD (90–110 KD) while the intracellular domain is 500 residues long and consists of a TK domain and an intracellular regulatory domain. The TK domain contains the enzymatic sequences necessary for TK activity with the intracellular domain also contains multiple tyrosine residues which serve as substrates.

The progesterone receptors (PR) is a nuclear receptor subfamily 3, group C, member 3 (NR3C3), a protein found in somatic cells and is activated by steroid hormone progesterone. In humans, PR is encoded by a single PGR gene residing on chromosome11q22 having two main forms A and B that differs in their molecular weight [17]. The level of progesterone receptor in a breast cancer is routinely evaluated since the expression is independent of estrogen receptors levels. It is very common to find a PR positive tumor which is ER negative (only 1% of all breast cancers are PR+ER-). Breast tumor with high levels of ER but low level of PR are common and it is generally believed that the response to endocrine therapy in metastatic breast cancers is better where both are evident [8]. This work aimed at determining triple negative breast cancer received in Gwarimpa medical diagnostic and cervical centre LTD, suite 3 Gwarimpa plaza estate federal capital tertiary Abuja Nigeria and to forecast the Nigerian scenario since our centre is a major immunohistochemical laboratory in the country which receives malignant breast samples from different hospitals in Nigeria for evaluation of hormonal status.

MATERIALS AND METHODS

Breast Cancer Specimen: We studied 82 surgical specimens from female patients with breast cancer, received within the period between January 2013 and December 2014.The samples were pathologically confirmed as invasive ductal carcinoma by the respective client pathologist. Clinical details of the patients were obtained from the archives of the forms sent. Approval was obtained from our clients and all patients gave informed consent. The samples were formalin-fixed and paraffin-embedded, according to the routine procedure.

Procedure: Immunohistochemical (IHC) analysis using monoclonal antibodies for ER and PR (Dako Carpentaria, CA) ER (ID5; 1;50), PR(PgR636; 1;400) and IHC of HER-2/neu protein was performed on 3 to 4ìm thick paraffin embedded tissue sections placed on poly-L-Lysine coated slides. After deparaffinization and blocking of endogenous peroxidase, HER-2/neu immunostaining was performed using rabbit anti-human c-erbB-2 oncoprotein as primary antibody (Dako, Copenhagen, Denmark) at 1:100dilutions. Binding of the primary antibody was checked by Dako Quick-Staining, Labelled Streptavidin–Biotin System (LSAB; Dako, USA), followed by the addition of diaminobenzidine (DAB) as a chromogen. Each slide was scored in a blinded fashion according to the manufacturer's recommended criteria.

Table 1: Scoring Pattern

SCORE	Protein expression assessment	Staining pattern
0	Negative	No reaction or membrane activity in ? 10% of tumour cells.
1	Negative	Faint/barely perceptible membranous reactivity in >10% of tumor cells; cells are reactive only in part of their membrane
2	Equivocal(negative)	Weak to moderate complete, basolateral membranous reactivity in >10% of tumor cells
3	Positive	Strong complete, basolateral or lateral membranous reactivity in > 10% of tumour cells

Pathological Investigations: The mean age of patients ranged between 34-97years (53.50 ± 13.7). The histopathological diagnosis of breast carcinoma was established by standard light-microscope using sections stained with Hematoxylin (Genzyme, England) and Eosin (Chematec, UK) (H&E), by the pathologist before they were sent to our centre.

Staining Interpretation: The criteria of positive reaction for ER and PR are dark brown intra-nuclear precipitate. The staining was assessed by scoring the proportion and intensity performed at X 40 objective lens. The immunostaining was read in a semi quantitative manner and graded as follows: 0, 1+, 2+ and 3+. Intensity scores of 0 or 1+ were designated as negative expression and 3+ were designated as positive expression for HER-2/neu. Scores of 2+ were taken as equivocal cases. For ER and PR scores, specimens with <1% nuclei staining were considered negative (Score 0) and those with weak (1% to 10%, score 1), moderate (10% to 33%, score 2) and strong (>33%, score 3) (Table 1). Staining was classified as hormone-receptor positive, denoted ER+ and PR+ respectively. A positive control sample was included in all batches. Breast cancer subtypes were also classified as triple-negative (TRN: Her2/neu-, ER- and PR-).Her-2/neu oncogenes were scored as Haider *et al*. [18].

Statistical Analysis was done using One-way analysis of variance and the Tukey's post hoc test was used to assess the significance of differences between groups. Values were expressed mean ± SD. A P value < 0.05 was considered to be significant. Analysis of variance was performed using Graph-Pad Prism 5 (GraphPad Software, San Diego, CA).

RESULTS

The study period span between January 2013 to December 2014 and 82 breast cancer samples were received, all were infiltrating ductal carcinoma. Table 2 shows the percentage distribution of Estrogen (53.6, 46.3%) and progesterone (57.3 and 42.6%) immunonegative and positive cases respectively. Also Her2+ was presented as 74.4% negative and 25.6% positive with triple negative breast cancer (TNBS) at 31.7 %.(Table 2)

Table 3 shows phenotypic expression of hormonal receptor and Her2 oncogenes by age of patients, ≥50 years show ER+/PR+ expression at 32.5% compared with ≤50 years at 31.0%.

Table 4 presents the mean and standard deviation of estrogen-progesterone positivity and epidermal growth factor 2 phenotypic expressions with age.

While photomicrograph shows the immunohistochemical status (positivity and negativity) for progesterone receptor status, Estrogen receptor status and epidermal growth factor 2 in infiltrating ductal carcinoma of the breast.

Table 2: Esrtogen, Progesterone Receptors and Epidermal Growth Factor 2 among Subjects

Hormonal status		No	Percentages
ER Negative		44	53.6
ER Positive		38	46.3
	Positive stain score +1 =10		26.3
	+2 = 12		31.5
	+3= 16		42.1
PR Negative		47	57.3
PR Positive		35	42.6
	Positive stain score +1=9		25.7
	+2=11		31.4
	+3 =15		42.8
HER	2 Positive score+3=	21	25.6
HER 2	Negative	61	74.4
	Negative stain score 0 = 47		77.0
	+1 = 9		14.8
	+2 =5		8.1
Triple Negative		26	31.7

Table 3: Phenotypic outcome of progesterone and estrogen receptors and her2 status among subjects

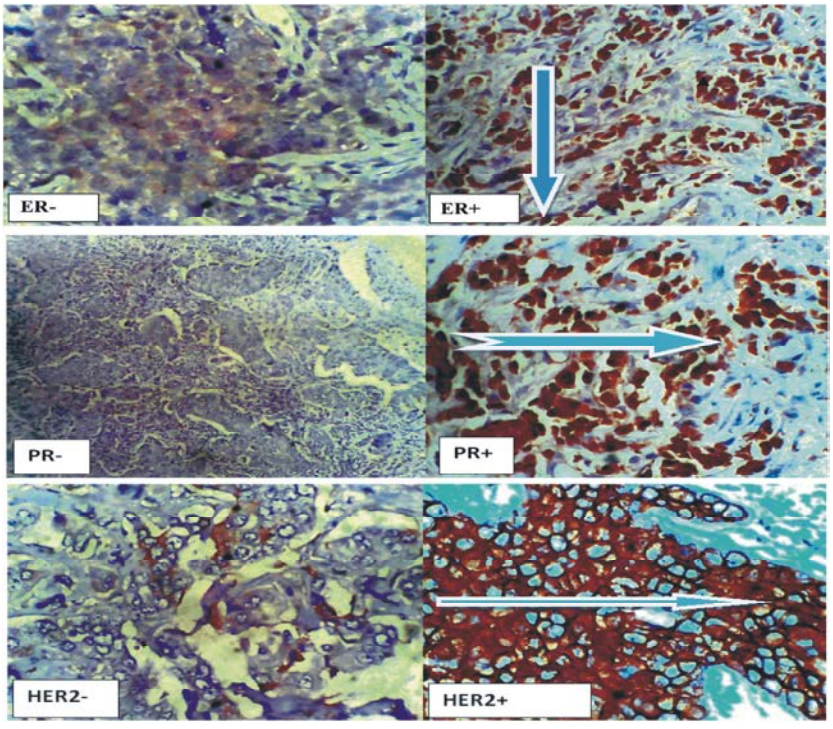
Age Phenotype	Number	Percentage by Age	Total Percentage
≥ 50yrs ER^+ PR^+	13	32.5	15.8
ER^+ / PR^-	6	15.0	7.2
ER^- / PR^+	01	2.5	1.2
ER^- / PR^+	20	50.0	24.4
Total		40	
Her2 Status Score 0 Negative	24	60.0	29.3
Score 1 Negative	5	12.5	6.1
Score 2 equivocal	3	7.5	3.7
Score 3 Positive	8	20.0	19.0
Total		40	
≥ 50yrs ER^+ PR^+	13	31.0	15.8
ER^+ / PR^-	6	14.3	7.3
ER^- / PR^+	8	19.0	9.7
ER^- / PR^-	15	35.7	18.2
Total		42	
Her2 Status Score 0 Negative	22	52.3	26.8
Score 1 Negative	05	11.9	6.1
Score 2 equivocal	02	4.7	2.4
Score 3 Positive	13	30.9	15.8
Total		42	

Table 4: Mean ±Sd of Hormones Status and Her2+ among Participant by Age

Age(yrs)	Er^+pr^+ Phenotype	Her2+	Comment
≥ 50	10±4.1	10±4.8	NS
≥50	11±2.1	11±4.5	NS

X^{2}= 0.07, DF=6, P > 0.05

Photomicrograhy of Er and Pr Hormones and Her2/neu



ER= Estrogen receptor positive and negativity for antibody against the corresponding antigen, PR= Progesterone receptor positive and negativity for antibody against the corresponding antigen and HER2= Epidermal growth factor 2 while arrows are pointing reactive site.

DISCUSSION

Breast tumors area heterogeneous disease with diverse biological, pathological and clinical characteristics and response to treatment has been attributed partially to various risk factors including reproductive, genetic and environmental. Breast cancer prognosis and management is dependent on histopathology type of tumor, grade, size, involvement of lymph nodes, immunohistochemistry profile of hormone receptors and in recent years status of HER2. [3,19].The current basis of ER, PR and HER2 evaluation in mammary gland neoplasm is immunohistochemical staining which has become wide spread in health institutions[10,20-22] however the opposite is the case in many tertiary health institution in Nigeria.

ER, PR and HER2 are essential in the estimation process of breast cancer prognosis and play central role in its management and treatment of choice worldwide [6].Determination of estrogen and progesterone receptors status is helpful in selecting the patients most likely to receive benefit from endocrine therapy and provide prognostic information on recurrence and survival since their expression is related to the degree of the tumors differentiation, the highest response rates to endocrine therapy are observed in tumors, which are positive for estrogen and progesterone receptors [10]. Estrogen enacts a crucial function in cell proliferation and breast cancer progression and is described as the major mutagenic steroid in neoplastic transformation for the cells of luminal epithelium and may play important role in prognosis [12, 17].

In this work 82 cases of infiltrating ductal carcinoma (all females) were received within a period of two years. ER positive receptors were 46.3% (38/82) and about 31.2%(score 2) of the positive cases were equivocal (12/38) while 42.1%(score +3) were strongly positive as compared to PR positive receptor of 42.6% (35/82) with a 31.4% equivocal and 42.8% strongly positive for progesterone positive. Findings from this work showed Estrogen receptors positive tumors were 46.3% of the cases and progesterone receptor positive tumors were 42.6% of the cases. This result of present work agrees with the works of Iraqi cancer registry in 2007 [7] were 65.1% Estrogen receptor positive and 45.1% progesterone receptor positive, also Khabaz (2014) had equally recorded 77.8% estrogen positive cases and 62.1% progesterone positive of all ductal carcinoma studied in pathology department of king Abdullazzi university kingdom Saudi Arabia[23]. However the high ER+ compared with PR+ as obtained in these studies is in agreement the work of Huo *et al*. (2009) among indigenous African women with a record of 24% ER+ and 20% PR+ [29] bearing direct relationship between geographical and pattern of hormonal expressions breast tumor.

The phenotypic representation of the estrogen and progesterone were as follows. ER+/PR+=31.6%, ER+/PR- were 14.5%, ER-/PR+ were 10.9% while ER-/PR- breast cancers were 42.2% (Table 3). This phenotypic representation is in disagreement with that in Iraqi patients where estrogen and progesterone negativity (ER-/PR-) was the highest followed by ER+/PR+. It is important to note the only reason that can be responsible for this large difference is due to tissue fixation resulting in masking of valuable tissue antigen. Secondly the timing from fixation of the sample to the time the samples were evaluated since samples were direct archived samples from representative countries histopathology department. Najafi (2013) reported the connection between the amplification of HER2 gene and bad prognosis and established a relationship between disease-free survival and Her-2/neu amplification making evaluation of Her2/neu so important for treatment of choice especially for patients with metastatic tumors, who respond better for additional medication such as Perception [6, 27]. The proto-oncogene Her-2/neu is amplified and as a result is over expressed in 25 to 30% of human breast cancer and is usually associated with tumor aggressiveness and poor prognosis. In this study 25.6% of the breast cancer studied was Her-2/nue positive score 3(HER2 +3)comparing to Ugiagbe *et al*.[25] who recorded for immunopositivity of breast carcinoma for Her-2/neu at 10.8% in Benin, Edo state Nigeria, While 8.2% recorded in Ile-Ife, 17.1% and 25% documented in Ibadan and Jos, respectively all in Nigeria[24-26].Furthermore this study revealed 30.9%(13/42) of the HER2 of cases occur in patients above the ages of $\geq$50 years and 20.0%(8/40) was below $\leq$50year and from this finding it is true that aggressiveness and prognosis has a relationship with age of detection.

Triple negative breast cancer (TNBC) is a subtype of breast cancer defined by the absence of the hormone receptors for estrogen and progesterone, as well as a lack of expression of a cancer-promoting protein known as Her2/neu. TNBC occurs more frequently in women of color and is twice as common in black women as in white women in the United States. In the United States, about

15% of all breast cancers in white women are TNBC compared with 30% in black women. This means that on an annual basis, approximately 30,000 white women and 8200 black women will be diagnosed with TNBC. As with other breast cancer subtypes, mammography can be an effective means of detecting early stage TNBC. Early stage TNBC can be treated effectively with surgery, often followed by radiation and chemotherapy.

CONCLUSIONS

Triple Negative Breast Cancer recursion is more commonly than other types of breast cancer and accounts for a disproportionate percentage of breast cancer deaths. Recurrences almost always occur within the first 5 years after diagnosis. Adjuvant chemotherapy, which is treatment given after surgery for early breast cancer, can be highly effective for preventing disease recurrence. In this study triple negative breast cancer was presented as 31.7% (26/82) compared to other studies. It is therefore imperative for oncology centers with notable cancer registry to be established in the various tertiary health institutions in the country to fight the menace of breast cancer.

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REFERENCES

1. Ikpatt, O.F. and R. Ndoma-Egba, 2003. Estrogen and progesterone receptors in Nigerian breast cancer: relationship to tumour histopathology and survival of patients. Cent. Afr. J. Med., 11: 122-126.
2. Horita, K., A. Yamaguchi, K. Hirose, M. Ishida, S. Norika, Y. Imamura and M. Fakuda, 2001. Prognostic factors affecting disease-free survival rate following surgical resection of primary breast cancer. Eur. J. Histochem., 1: 73-84.
3. Huo, D., F. Ikpatt, A. Khramtsov, D. Jean-Marie, R. Nanda, J. Dignam, I.D. Gukes, B.A. Jenings, B.M. Mandong, G.O. Igun, A.C. Girling, A.N Manasseh, F. Iyare and A. Falusi, 2005. Clinicopathological features and molecular markers of breast cancer in Jos Nigeria. West Afr. J. Med., 24: 209-213.
4. Khokher, S., M.U. Qureshi, S. Mahmood and A.H. Nagi, 2013. Association of Immunohistochemical defined molecular subtypes with clinical response to presurgical chemotherapy in patients with advanced breast cancer. Asian Pac. J. Cancer Prev., 14: 3223-3228.
5. Kadivar, M., N. Mafi, A. Joulaee, A. Shamshiri and N. Hosseini, 2012. Breast cancer molecular subtypes and associations with clinicopathological characteristics in Iranian women, 2002- 2011. Asian Pac. J. Cancer Prev., 13: 1881-1886.
6. Runnak, M.A., M.A. Hazha, H.A. Hemin, A.A. Wasan, R.M. Rekwat and H.D. Micheal, 2012. A population-based study of Kurdish breast cancer in northern Iraq: hormone receptor and HER2 status. A comparison with Arabic women and United States SEER data. BMC Women Health, 12: 16-25.
7. Iraqi Cancer Board. Results of the Iraqi Cancer Registry, 2007. Baghdad, Iraqi Cancer Registry Center, Ministry of Health
8. Moradi-Marjaneh, M.F., S.A. Homaei-Shandiz, A. Shamsianand, M.R. I. Eftekhar-ZadehMashhadi and M. Hedayati, 2008. Correlation of HER2/neu over-expression, p53 protein accumulation and steroid receptor status with tumor characteristics: an Iranian study of breast cancer patients. Iranian J. Publ. Health, 37(3): 19-28.
9. Al-Eid, H.S. and A.D. Garcia, 2012. Cancer incidence report Saudi Arabia 2009. Saudi Cancer Registry, 2012 Ministry of Health, Kingdom of Saudi Arabia.
10. Barnes, D.M. and A.M. Hanby, 2001. Estrogen and progesterone receptors in breast cancer: past, present and future. Histopathology, 3: 271-274.
11. Anderson, E., R.B. Clarke and A. Howell, 1998. Estrogen responsiveness and control of normal human breast proliferation. J. Mammary Gland Biol. Neoplasia, 1: 23-35.
12. Izadi, P., N. Mehrdad, F. Foruzandeh and N.M. Reza, 2012. Association of poor prognosis subtypes of breast cancer with estrogen receptor alpha methylation in Iranian women. Asian Pac. J. Cancer Prev., 13: 4113-4117.
13. Bartlett, J.M., D. Rea and D.L. Rimm, 2011. Quantification of hormone receptors to guide adjuvant therapy choice in early breast cancer: better methods required for improved utility. J. Clin. Oncol., 27: 3715-3716.
14. Kaptain, S., L.K. Tan and B. Chen, 2001. Her-2/neu and breast cancer. Diagn. Mol.Pathol., 3: 139-152

15. Vaidyanathan, K., P. Kumar, C.O. Reddy, V. Deshmane, K. Somasundaram and G. Mukherjee, 2010. ErbB-2 expression and its association with other biological parameters of breast cancer among Indian women. Indian J. Cancer, 47: 8-15.
16. Ali, H., A. Al-Khafaji, M.A. Fadhil and A.H. Mohammed, 2014. Immunohistochemical Study of Estrogen, Progesterone Receptor and Her-2neu Oncogene with Her-2neu Biomarker Estimation by ELISA Technique in Primary Breast Cancer before Chemical Therapy. Iraqi Journal of Science, 55(1): 132-144.
17. Lazennec, G., D. Bresson, A. Lucas, C. Chauveauand and F. Vignon, 2001. ER beta inhibits proliferation and invasion of breast cancer cells. Endocrinology, 9: 4120-4130.
18. Haider, W., G.M. Arain, K.M. Sohu, S.A. Naqiand and B.B. Younus, 2001. Estrogen receptors as tumour markers in malignant breast disease: A retrospective study of 100 cases. Biomedical, 17: 1-5.
19. Sandhog, D.S., S. Sandhu, R.K. Karwasra and S. Marwah, 2010. Profile of breast cancer patients at a tertiary care hospital in north India. Indian J. Cancer, 47: 16-22.
20. Hammond, M.E., D.F. Hayes, M. Dowsett, D.C. Allred, K.L. Hagerty, S. Badve, L. Patrick, F. Glenn, L. Susan, L. Richard, B.M. Pamela, W. Thomas, L. James, V. Daniel, V. Paul, C.G. Freed and C.W. Antonio, 2010. American society of clinical oncology/college of American pathologists guideline recommendations for Immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J. ClinOncol., 16: 2784-95.
21. Allred, D.C., J.M. Harvey, M. Berardo and G.M. Clark, 1998. Prognostic and predictive factors in breast cancer by Immunohistochemical analysis. Mod. Pathol., 2: 155-68.
22. Zhang, B., T. Grushko, C. Zhang, O. Oluwasola, D. Malaka, S. Malami, A. Odetunde, O. Adeoye, F. Iyare, A. Falusi, C.M. Perou, E.Y. Cho, Y.L. Choi, J.A. Han, K.M. Kim and Y.L. Oh, 2008. Expression and amplification of HER2, EGFR and cyclin D1 in breast cancer: Immunohistochemistry and chromogenic in situ hybridization. Pathol. Inte., 58: 17-25.
23. Mohammad, N., 2014, Immunohistochemistry Subtypes (ER/PR/HER) of Breast Cancer: Where Do We Stand in the West of Saudi Arabia? Asian Pac. J. Cancer Prev., 15(19): 8395-8400.
24. Adeoye, A.O, 2007. Histological and Immunohistochemical patterns of breast cancer at the University College Hospital, Ibadan. A dissertation submitted to the West African College of Physicians, Oct 18.
25. Ugiagbe, E.E., A.N. Olu-Eddo and D.E. Obaseki, 2011. Immunohistochemical detection of Her-2/neu over expression in breast carcinoma in Nigerians: A 5-year retrospective study. Niger J. ClinPract., 14: 332-337.
26. Titiloye, W., 2008. Histological pattern of epithelial breast cancer in Ile-Ife (OAUTH). A dissertation submitted to the National Postgraduate Medical College of Nigeria, May. 2008.
27. Najafi B., S. Anvari and Z.A. Roshan, 2013. Disease free survival between 2001 and 2010 in Iran. Asian Pac. J. Cancer Prev., 10: 5811-5816.
28. Wang, Z., X. Zhang, P. Shen, B.W. Loggie, Y. Chang and T.F. Deuel, 2008. Identification, cloning, expression of Human Estrogen Receptor, 8(5): 392-401.
29. Huo, D.F., A. Ikpatt, A. Khramtsov, J.M. Dangou, R. Nanda, J. Dignam, B. Zhang, C. Tzhang, O. Oluwasola, D. Malaka, S. Malami, A. Odetunde, A.O. Adeoye, F. Iyare, A. Falusi, C.M. Perou and O.I. Olopade, 2009. Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer," Journal of Clinical Oncology, 27(27): 4515–4521.