Global Journal of Scientific Researches

Available online at gjsr.blue-ap.org
©2014 GJSR Journal. Vol. 2(6), pp. 154-164, 31 March, 2014
E-ISSN: 2311-732X

Histological and Immunohistochemical Study of Androgen Receptor Expression in Normal Breast Tissue and Invasive Duct Carcinoma

Mohammed F* and Bahaa A Khaled

1-Darweesh Pathology Department, Faculty of Medicine, Cairo University.
2-Anatomy Department, Faculty of Medicine, Cairo University

Corresponding Author: Mohammed F

Received: 25 May, 2014 *Accepted: 10 June, 2014* *Published: 30 June, 2014*

ABSTRACT

Background: steroids and their nuclear receptors including androgen receptor (AR) play significant role in the development and maintenance of normal function of human breast. AR is also expressed in 70-90% of invasive breast cancers and could be utilized as a prognostic indicator and a target for hormonal therapy. **Aim of the work:** the aim of the present work was to evaluate the expression of AR in the normal tissue of the four quadrants of the female breast and invasive duct carcinoma and to compare the androgen receptor expression in the primary tumor and the adjacent non-neoplastic (normal) tissue of the affected breast quadrant. **Material and methods:** a total of 50 pathological specimens of female breast were studied in this work. For all specimens, clinical parameters such as: age of the patient, breast quadrant affected by the tumor, size of the tumor and axillary lymph nodes status were obtained from the patients' records. Tissue samples from each breast quadrant as well as separate samples from the tumor were obtained from each mastectomy specimen, and prepared for histological examination (haematoxylin and eosin and Masson's trichrome stains) as well as immunohistochemical staining for AR. **Results:** the upper outer quadrant showed the highest mean area percentage of both collagen fibers and AR expression while the tumor presented higher area percentage than any of the four quadrants regarding both collagen fibers and AR expression. Statistically significant negative correlations were found between tumor AR expression and clinicopathological parameters. **Conclusion:** AR is expressed in both normal breast tissue and invasive duct carcinoma. AR expression in invasive tumors could be associated with better prognosis and survival and could be used as a therapeutic target in hormonal treatment of breast carcinoma.

Keywords: *breast, carcinoma, androgen receptor, Immunohistochemical.*

INTRODUCTION

Several studies concerned to apply the affected quadrant of the breast as a prognostic factor in breast cancer and found that most of tumors develop in the outer quadrants (66%) compared to the inner quadrants (22%) or central region (12%); tumors occur most frequently in the upper outer quadrant (48-54%) (Bevilacqua, 2002; Janni, 2003).

An earlier study by Haagensen (1986) attributed the high incidence of occurrence of breast cancer in the upper outer quadrant to the presence of more breast tissue in such quadrant. However, Ellsworth . (2004) reported greater genomic instability in the outer quadrants of the breast, compared to the inner quadrants, which could provide a better explanation than the simple volume-related theory.

The role of estrogen receptor (ER) and progesterone receptor (PR) in breast carcinomas is well established, but less data is available on the functional and clinical significance of androgen receptor (AR) in such cases. Steroids and their nuclear receptors play crucial roles in the development and maintenance of normal function of the human breast. In addition to estrogen receptor-α, estrogen receptor-β and progesterone receptors, androgen receptors are present in both normal and neoplastic breast tissue. Their stimulation leads to mammary epithelial proliferation and apoptosis; both are important in tissue homeostasis (Zhou, 2000; Dimitrakakis, 2002).

Androgen receptor is expressed in approximately 70-90% of invasive breast cancers, a frequency comparable to or even higher than those reported for estrogen receptor (70-80%) or progesterone receptor (50–70%) (Hall, 1996; Lillie, 2003; Riva, 2005).

Rakha . (2007) concluded that in cases of invasive breast carcinomas, tumor size, lymph node status and AR positivity are the most useful prognostic markers, rather than tumor grade, expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), epidermal growth factor receptor (EGFR) or p53 gene. However, Narita . (2008) found a positive correlation between AR expression and the grade of differentiation of tumors; most of AR-positive carcinomas were well or moderately differentiated. On the other hand, a negative association was found between AR expression and the number of the affected lymph nodes by tumor spread.

Gonzalez . (2008) emphasized that AR is commonly expressed in breast cancer. Although they concluded that the value of AR expression could be considered as a prognostic indicator in breast cancer, the functional role of AR in these neoplasms is still unclear and further data is needed to determine its biological significance and if it could be used as a marker for efficiency of endocrine therapy.

Since AR is expressed in a significant percentage in both normal and tumor tissue, the present study aimed at evaluation of AR expression in both the four breast quadrants and invasive duct carcinoma. As well as, comparing between AR in the normal tissue and the tumor, and finding the correlation between AR expression and the clinicopathological parameters.

Material and methods:

A total of 50 mastectomy specimens of female breast were studied in this work. The specimens were obtained from patients admitted to Kasr Al-Ainy Hospital, diagnosed as "breast invasive duct carcinoma not otherwise specified (NOS)" and subjected to modified radical mastectomy. The Specimens were collected from the Department of Pathology, Faculty of Medicine, Cairo University, to which the specimens were transferred following mastectomy from December 2011 to March 2013.

Inclusion criteria:

- Female gender.
- Preoperative diagnosis of "invasive duct carcinoma not otherwise specified (NOS)" established with excisional biopsy, true cut needle biopsy or fine needle aspiration cytology, based on the WHO classification of tumors (Tavassoli and Devilee, 2003).
- Normal non-neoplastic tissue sections of the affected breast were chosen according to the following criteria: composed of histologically normal ducts and stromal fibroblasts and free from atypical ductal hyperplasia or residual tumour (duct carcinoma in situ) (Ellsworth ., 2004).

Exclusion criteria:

- Previous exposure to radiotherapy.
- Previous hormonal therapy.

For all specimens, the following parameters were obtained from the patients' clinical and medical reports:

- Age of the patient.
- Breast quadrant affected by the tumor.
- Size of the tumor.
- Axillary lymph nodes status.

Histopathological evaluation

Tissue samples from each breast quadrant as well as separate samples from the tumor were obtained from each mastectomy specimen, fixed in 10% buffered formalin, dehydrated through ascending concentrations of ethanol and embedded in paraffin at 56°C for 30 minutes. Histological sections 4 µm thick were obtained using a microtome from each paraffin block and subjected to:

- Routine haematoxylin and eosin (Hx & E) staining to verify the preoperative diagnosis of the tumor and to examine the disease-free quadrants and the tissues adjacent to the tumor in the affected quadrant to ensure that they contain normal breast tissue (free of malignant cells).
- Masson's trichrome stain for detection and quantitative evaluation of the collagen fibers.
- Immunohistochemical staining: for detection and quantitative evaluation of the androgen receptor in normal tissue of the breast quadrants and the tumor.

Androgen receptor immunostaining

Immunohistochemical staining was carried out using avidine-biotin immunoperoxidase detection system {Ecno Tek HRP Anti-Polyvalent (DAB)} and androgen receptor mouse monoclonal antibody (clone AR 441) diluted at 1:25. The antibodies (cat. #MS-443-PO) were obtained from Neomarker (Lab Vision Corporation, Fremont, CA, USA) while the detection system was obtained from Sky Tek Laboratories (Utah, USA). Androgen receptor immunostaining interpretation.

The pattern of immunostaining for AR was nuclear. For semiquantitative evaluation of AR, ten tissue sections were examined from each mastectomy specimen; two from each breast quadrant and a further two from the tumor. Sections were considered positive when at least 10% of the cells showed nuclear staining (Monifar ., 2003;Park ., 2010). Mastectomy specimens were considered positive for AR when tissue sections of at least three quadrants exhibited 10% or more positive nuclear staining.

Histomorphometric study

Tissue sections were examined by the image analysis computer system using the software Leica Qwin 500. Six randomly chosen fields were captured in each section with a magnification x400 to determine the area percentage of collagen fibers and the area percentage of the positive cells for AR (the pattern of staining was nuclear). Blue binary color was used to mask the blue color of collagen fibers in Masson's trichome sections and the brown color of AR nuclear staining in immunohistochemical sections then the area percentage of the blue binary color in relation to the whole area was calculated.

Statistical Analysis

Statistical Package of Social Science Software program, (SPSS) version 21 was used for statistical analysis. Data were summarized using range, mean and standard deviation for parametric quantitative variables and frequency and percentage for qualitative ones.

Comparison between groups was performed using one way ANOVA (Analysis of Variance) with Tukey's post hoc test. Comparison of different expressions at different quadrants was conducted through paired (dependent) t-test. Pearson or Spearman correlation coefficients were calculated to get the association for quantitative and ordinal variables respectively.
p-values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.

RESULTS AND DISCUSSION

Results

Age distribution

The age of the patients ranged between 47-70 years; the mean ± standard deviation (SD) was 58.6 ± 6.4 years. Thirty cases (60%) were ≤ 60 years and 20 cases (40%) > 60.

Tumor size

Tumor size was determined as the largest diameter measured in centimeters according to the TNM staging system (AJCC, 2009). The tumor size ranged between 1.6-7 cm with a mean ± SD 3.73 ± 1.4 cm. According to tumor size and extension (T) included in TNM staging system, cases were categorized into three groups. The majority of tumors, 34 out of 50 (68%) were categorized as T2 (2-5 cm). T3 tumors (more than 5 cm) constituted 11 cases (22%) and T1 tumors (less than 2 cm) comprised five cases (10%).

Lymph nodes status

Spread of the tumor to the axillary lymph nodes (LN) was classified according to TNM staging system into N 0 (0), N 1 (1-3), N 2 (4-9) and N 3 (≥ 10) (N is the number of lymph nodes which are affected by tumor spread). Twenty three cases (46%) showed negative axillary lymph nodes spread (N 0) while 27 cases (54%) showed positive axillary nodes spread (N 1, 2, 3).

The quadrant affected by the tumor

The upper outer (UO) was the one affected by the tumor in 19 cases (38%), the lower outer (LO) in 12 cases (24%), the lower inner (LI) in 10 cases (20%) and the upper inner (UI) in 9 cases (18%).

Haematoxylin and eosin stained sections

Examination of normal non-neoplastic tissue sections revealed that the normal histological architecture of the breast was formed of parenchyma (lobules and ducts) and fibrofatty stroma (Figs. 1, 2). The lobules were lined with cuboidal epithelium with basophilic vacuolated cytoplasm (lipid globules) and central rounded nuclei (Fig.3). The stroma was formed of connective

tissue fibers and fat cells (Figs.1-3). Blood vessels were illustrated within the stroma (Fig.1). The ducts appeared also surrounded by fibrofatty stroma (Fig.4) and were lined with cuboidal epithelial cells with acidophilic cytoplasm and central rounded nuclei (Fig. 5).

Examination of neoplastic tissue sections of invasive duct carcinoma revealed clusters of malignant ductal cells in variable shaped duct-like structures invading the surrounding dense fibrous stroma (Figs. 6). These malignant cells demonsted the criteria of malignancy: anaplasia, pleomorphism, hyperchromatism and increased nuclear cytoplasmic ratio (Fig. 7). In other sections there was increased ductal proliferation forming malignant ductal cells invading the neighboring fatty stroma (Fig. 8). These malignant cells exhibited the forementioned criteria of malignancy (Fig. 9). Cystic dilatation of some breast ducts which were surrounded with malignant ductal cells and inflammatory cells were also encountered (Figs. 10, 11).

Masson's trichrome stained sections

Examination of normal non-neoplastic tissue sections revealed collagen fibers of the breast stroma surrounding the breast parenchyma (Figs. 12, 13). Stromal blood vessels (Fig.12) and intralobular capillaries (Fig.13) could also be illustrated.

Examination of neoplastic tissue sections of invasive duct carcinoma showed malignant duct cells which appeared as clusters surrounded by dense stromal reaction formed of collagen fibers (Figs. 14, 15). The malignant ductal cells exhibited criteria of malignancy in the form of pleomorphism, hyperchromatism and increased nuclear cytoplasmic ratio (Fig. 15).

Immunohistochemical stained sections

Examination of normal non-neoplastic tissue sections demonstrated the AR nuclear staining (Figs. 16-19). The intensity of nuclear staining was mild (Fig. 17) to moderate (Fig. 19).

Examination of neoplastic tissue sections revealed AR nuclear staining of the tumor cells (Figs. 20-23). The nuclear staining showed mild to moderate (Fig. 21) and marked intensity (Fig. 23).

Statistical Analysis

Mean area percentage of collagen fibers and its correlations

The area percentage of collagen fibers in each quadrant as well as in the tumor was expressed in values as range, mean ± SD (Table 1). Comparison between the mean area percentage of collagen fibers of the different quadrants using paired (dependent) t-test is illustrated in table (2). Comparison between mean area percentage of collagen fibers of each quadrant and that of the tumor using paired (dependent) t-test is illustrated in table (3). Correlations between mean area percentage of collagen fibers in each quadrant and the tumor on one hand and the corresponding AR expression on the other hand are illustrated in table (4).

Table 1. Area percentage of collagen fibers in the four quadrants and the tumors

Region	Area percentage of collagen fibers	
	Range	Mean ± SD
UO	20.8-32.0	27.2 ± 2.7
LO	17.8-32.8	24.2 ± 2.7
LI	17.0 – 27.4	22.5 ±2.4
UI	16.2 – 25.9	21.5 ± 2.4
Tumor	39.7 – 48.2	44.8 ± 2.2

SD: standard deviation.

Table 2. Comparison between the area percentage of collagen fibers in each pair of the four quadrants (paired dependent t-test)

Quadrant	Area percentage of collagen fibers (Mean ± SD)	t-value	p-value
UO	27.2 ± 2.7	101.9	<0.001**
TUMOR	44.75 ± 2.2		
LO	24.2 ± 2.7	85.3	<0.001**
TUMOR	44.75 ± 2.2		
UI	21.5 ± 2.4	150.7	<0.001**
TUMOR	44.75 ± 2.2		
LI	22.5 ±2.4	140.6	<0.001**
TUMOR	44.75 ± 2.2		

(*): significant. (**): highly significan

Table 3. Comparison between the area percentage of collagen fibers in the four quadrants and the tumor (paired dependent t-test)

Quadrant	Area percentage of collagen fibers		
	Mean ± SD	t-value	p-value
UO	27.2 ± 2.7	14.1	<0.001 **
LO	24.2 ± 2.7		

UO	27.2 ± 2.7	54.5	<0.001 **
LI	22.5 ±2.4		
UO	27.2 ± 2.7	66.1	<0.001 **
UI	21.5 ± 2.4		
LO	24.2 ± 2.7	8.9	<0.001 **
LI	22.5 ±2.4		
LO	24.2 ± 2.7	13.4	<0.001 **
UI	21.5 ± 2.4		
LI	22.5 ±2.4	2.1	<0.05 *
UI	21.5 ± 2.4		

(**): highly significant

Table 4. Correlations between mean area percentage of collagen fibers and the corresponding AR expression (Pearson correlation coefficient)

		UOAR	LOAR	LIAR	UIAR	Tumor AAdjacent
UO collagen	r	-0.076	-0.087	-0.113	-0.136	-0.063	0.032
	p	0.598 NS	0.55 NS	0.435 NS	0.345 NS	0.665 NS	0.826 NS
LO collagen	r	0.003	-0.001	-0.028	-0.095	0.014	0.081
	p	0.984 NS	0.995 NS	0.849 NS	0.516 NS	0.925 NS	0.58 NS
LI collagen	r	-0.057	-0.065	-0.088	-0.122	-0.044	0.054
	p	0.695 NS	0.655 NS	0.544 NS	0.4 NS	0.76 NS	0.712 NS
UI collagen	r	-0.079	-0.085	-0.109	-0.136	-0.069	0.033
	p	0.586 NS	0.557 NS	0.453 NS	0.347 NS	0.634 NS	0.823 NS
Tumor collagen	r	-0.125	-0.127	-0.152	-0.115	-0.111	0.011
	p	0.393 NS	0.384 NS	0.298 NS	0.431 NS	0.448 NS	0.938 NS
Adjacent normal tissue collagen	r	-0.097	-0.095	-0.096	-0.101	-0.08	0.354
	p	0.504 NS	0.512 NS	0.507 NS	0.484 NS	0.58 NS	0.012 *

r: correlation coefficient, p: p-value, (*): significant, NS: non significant.

Androgen receptor expression

Out of the 50 mastectomy specimens, 42 specimens were AR positive (84%) and 8 were AR negative (16%). The area percentage of AR expression in each quadrant as well as in the tumor is expressed in table (5).

Table 5. The area percentage of AR expression in each of the four quadrants and the tumors

Region	Area percentage of AR expression	
	Range	Mean ± SD
UO	8.0 – 26.6	19.9 ± 5.2
LO	7.9 – 26.0	19.7 ± 5.4
LI	7.0 – 23.5	17.3 ± 4.4
UI	4.3 – 12.0	7.8 ± 2.2
Tumor	8.6 – 35.1	28.1 ± 8.4

SD: standard deviation

Correlations between AR expression in the four quadrants and in the tumor

Comparison between AR expression of the four quadrants is illustrated in table (6) while comparison between AR expression in each quadrant and that in the tumor is shown in table (7).

Regarding the results of the AR expression in the normal breast tissue adjacent to the tumors affecting the four quadrants, the AR expression of these four groups of cases is shown in table (8).

Table 6. Comparison between AR expression in the four quadrants (paired dependent t-test)

Quadrant	AR expression (Mean ± SD)	t-value	p-value
UO	19.9 ± 5.2	2.0	0.053 NS
LO	19.7 ± 5.4		

UO	19.9 ± 5.2	15.8	<0.001**
LI	17.3 ± 4.4		
UO	19.9 ± 5.2	22.4	<0.001**
UI	7.8 ± 2.2		
LO	19.7 ± 5.4	14.3	<0.001**
LI	17.3 ± 4.4		
LO	19.7 ± 5.4	21.5	<0.001**
UI	7.8 ± 2.2		
LI	17.3 ± 4.4	21.9	<0.001**
UI	7.8 ± 2.2		

(**): highly significant, NS: non significant.

Table 7. Comparison between AR expression in each quadrant and the tumor (paired dependent t-test)

Region	AR expression (Mean ± SD)	t-value	p-value
UO	19.9 ± 5.2	17.2	<0.001**
TUMOR	28.1 ± 8.4		
LO	19.7 ± 5.4	17.5	<0.001**
TUMOR	28.1 ± 8.4		
UI	7.8 ± 2.2	20.3	<0.001**
TUMOR	28.1 ± 8.4		
LI	17.3 ± 4.4	18.2	<0.001**
TUMOR	28.1 ± 8.4		

(**): highly significant

Table 8. Comparison between the different affected quadrants as regard AR expression of adjacent normal tissues (one way ANOVA)

	UO	LO	LI	UI	p-value
AR expression of adjacent normal tissue (Mean ± SD)	19.8 ± 5.5	18.9 ± 6.6	16.6 ± 4.7	8.8 ± 1.8	<0.001**

(**): highly significant

Correlations between AR expression of the tumors in each quadrant and the adjacent normal tissues in the corresponding quadrant are shown in table (9). Correlations between AR expression of both the tumors and the adjacent normal tissues on one hand and the investigated clinicopathological parameters (age, size of the tumor, T staging of the tumor, number of the affected LN by tumor spread and the N staging of the LN) on the other hand are presented in table (10).

Table 9. Correlation between AR expression of tumors in each quadrant and the adjacent normal tissues in the corresponding quadrants (Pearson correlation coefficient)

		UO tumors	LO tumors	LI tumors	UI tumors
Adjacent normal UO	r	0.98			
	p	<0.05 *			
Adjacent normal LO	r		0.97		
	p		<0.001**		
Adjacent normal LI	r			0.99	
	p			<0.001**	
Adjacent normal UI	r				0.77
	p				<0.05 *

r: correlation coefficient, p: p-value, (*) : significant, (**): highly significant

Table 10. Correlations between AR expression and the investigated clinicopathological parameters (Pearson and Spearman correlation coefficient)

		TUMOUR AR expression	AR expression of adjacent normal tissue
AR expression of adjacent Normal tissues "	r	0.624	

	p	<0.001**	
AGE"	r	-0.011	0.012
	p	0.941NS	0.936 NS
SIZE"	r	-0.744	-0.438
	p	<0.001**	0.001**
T. Staging"'	r	-529	-0.308
	p	<0.001**	0.03 *
No LNs"	r	-0.871	-0.552
	p	<0.001**	<0.001**
N. Staging"'	r	-0.839	-0.55
	p	<0.001**	<0.001**

r: correlation coefficient, p: p-value, (*):significant, (**): highly significant. ("): the use of Pearson coefficient, ("'): the use of Spearman coefficient, NS: non significant

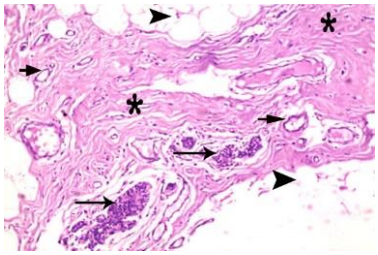

Figure 1. A photomicrograph of the normal non-neoplastic breast tissue demonstrating the breast lobule (long arrows). Dense connective tissue fibers (asterisks), fat cells (arrowheads) as well as blood vessels (short arrows) are illustrated within the fibrofatty stroma. (Hx & E x100)

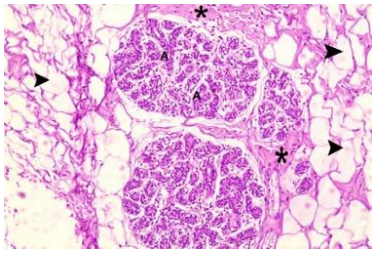

Figure 2. A photomicrograph of the normal non-neoplastic breast tissue demonstrating the lobule of the breast formed of alveoli (A). The lobules are surrounded by extensive fatty stroma (arrowheads) and connective tissue fibers (asterisks). (Hx & E x100)

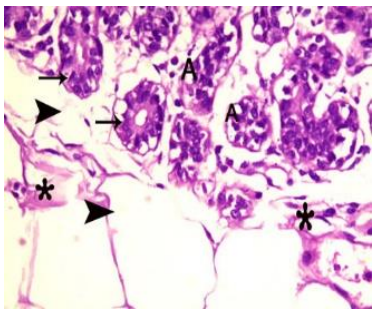

Figure 3. A photomicrograph showing the structure of the breast lobule formed of alveoli (A) lined with cuboidal epithelium (arrows) with basophilic vacuolated cytoplasm and central rounded nuclei. The lobules are surrounded by connective tissue fibers (asterisks). (Hx & E x400)

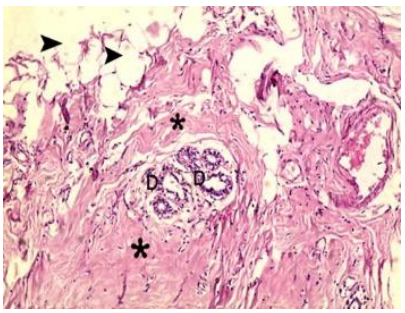

Figure 4. A photomicrograph illustrating the ducts of the breast (D). The ducts are surrounded by fibrofatty stroma {formed of fat cells (arrowheads) and dense connective tissue fibers (asterisks)}. (Hx & E x100)

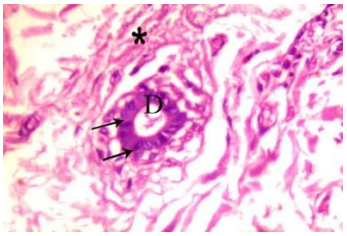

Figure 5. A photomicrograph showing a duct of the breast (D) lined with cuboidal cells (arrows) with acidophilic cytoplasm and central rounded nuclei. The ducts are

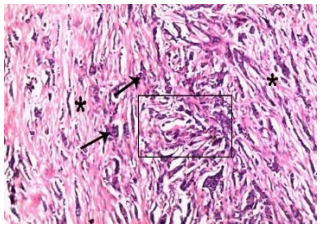

Figure 6. A photomicrograph of a case of invasive duct carcinoma showing clusters of malignant ductal cells arranged in variable shaped duct like structures (arrows)

surrounded by connective tissue fibers (asterisks). (Hx & E x400)

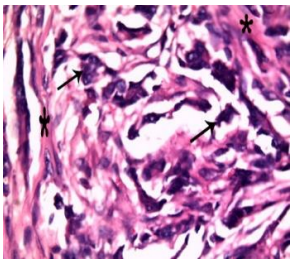

Figure 7. A higher magnification of the inset in figure 6 demonstrating malignant ductal cells (arrows) arranged in variable shaped duct like structures and showing the criteria of malignancy: anaplasia, pleomorphism, hyperchromatism and increased nuclear cytoplasmic ratio. The malignant cells are invading the surrounding dense fibrous troma (asterisks). (Hx & E x400)

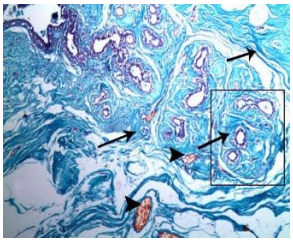

Figure 9. A photomicrograph of normal non-neoplastic tissue showing collagen fibers (arrows) in the stroma which is surrounding the parenchyma. Stromal blood vessels (arrowheads) can also be seen. A higher magnification of the inset is exhibited in figure 10. (Masson's trichrome x100)

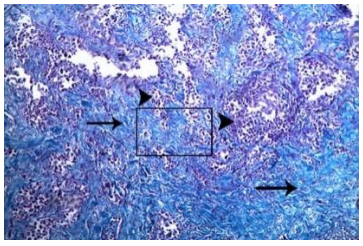

Figure 11. A photomicrograph of invasive duct carcinoma showing clusters of malignant ductal cells (arrowheads) surrounded by dense stromal reaction formed of collagen fibers (arrows). (Masson's trichrome x100)

invading the surrounding stroma. The stroma exhibiting a stromal reaction formed of dense fibrous stroma (asterisks). A higher magnification of the inset is shown in figure 7. (Hx & E x100)

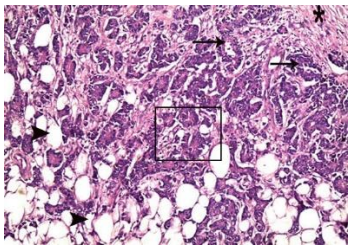

Figure 8. A photomicrograph showing malignant ductal cells (arrows) arranged in variable shaped duct like structures invading the neighboring fatty tissue (arrowheads). At the right upper angle of the photo, the surrounding dense fibrous stroma (asterisks) is seen. (Hx & E x100)

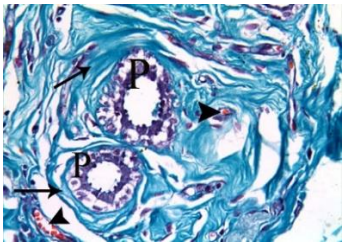

Figure 10. A higher magnification of the inset in figure 9 showing collagen fibers (arrows) surrounding the parenchymatous elements (P) inside a breast lobule. Intralobular capillaries can also be seen (arrowheads). (Masson's trichrome x400)

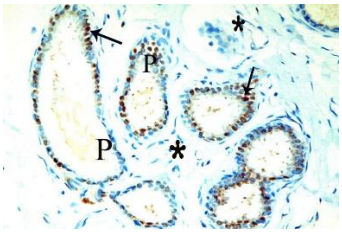

Figure 12. A photomicrograph of normal non-neoplastic tissue illustrating the AR nuclear staining (arrows) which is exhibiting moderate intensity. Parenchymatous elements of the breast (P) are surrounded by stroma (asterisks). (AR x 400)

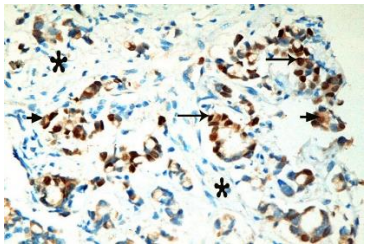

Figure 13. AR nuclear staining of the malignant cells exhibiting mild (short arrows) to moderate (long arrows) intensity. The surrounding stroma can also be visualized (asterisks). (AR x400)

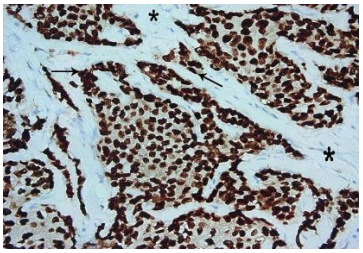

Figure 23. A higher magnification of the inset in figure 22 showing the AR nuclear staining of the malignant cells (arrows) which exhibited marked intensity. The surrounding stroma can be also visualized (asterisks).
(AR x 400)

DISCUSSION

The present work elucidated the AR expression in normal tissue of the four breast quadrants as well as in invasive duct carcinoma NOS. Examination of the normal tissue for AR expression demonstrated that 84% of the cases were AR positive. The mean area percentage of AR expression in the normal tissue ranged between 8.0-26.6% with a mean 19.9% in the UO quadrant, between 7.9-26% with a mean 19.7% in LO, between 7-23.5% with a mean 17.3% in LI and in the UI ranged between 4.3-12% with a mean 7.8%.

Monifar . (2003) recorded results higher than those of the present work; 100% of the cases examined for normal tissues were AR positive and the AR expression ranged between 10-70% with a mean 40%. Unlike the methodology adopted in the present study, these authors did not collect normal tissue of the four breast quadrants but only from the normal tissue adjacent to the tumor.

Evaluation of the difference in the AR expression between each pair of the four quadrants in the present study showed that AR expression of the UO quadrant was the highest. The difference was statistically significant compared to both UI and LI quadrants, while the difference between the UO and the LO quadrants was statistically non significant. The LI quadrant was the lowest in AR expression. The difference was statistically significant compared to each of the other three quadrants.

Examination of the tumors in the present work for AR expression demonstrated that 84% of the cases were AR positive. These results are in agreement with findings of previous studies which demonstrated that AR is expressed in considerable proportions of invasive breast carcinomas. Monifar . (2003) reported that AR expression ranged between 47 - 88% of different grades of invasive duct carcinomas. Riva . (2005) investigated AR expression in both invasive duct and lobular carcinoma and found it to be 56% and 87% respectively.

Gonzalez and coworkers obtained a result of 74.8% expression of AR in invasive breast carcinomas (Gonzalez ., 2008). Another study reported the AR expression in high grade invasive duct carcinoma as 55% and in non high grade invasive carcinoma as 92% (Hanley ., 2008).

In the present study, the area percentage of AR expression of the tumor ranged between 8.6 - 35.1% with a mean 28.1%. There was a statistically significant increase in AR of the tumor compared to that of each of the four quadrants.

In the present work, a statistically significant positive correlation was obtained between the AR expression of the tumor and that of the normal tissue adjacent to the tumor; the increase in AR expression of the normal tissue adjacent to the tumor was associated with increase in the AR expression of the tumor.

In the present study, the AR nuclear staining of the normal breast tissue as well as the tumor tissue exhibited variable degrees of intensity; mild, moderate and marked. These results are in agreement with those of Narita . (2008) who recorded variable degrees of AR expression in their work with prevalence of the mild and moderate intensity with heterogenous distribution.

The present study adopted the image analysis computer system in estimating the area percentage of AR stained nuclei in immunohistochemical sections in order to report the AR expression. Contradictory to this non-subjective methodology, Narita (2008) counted on the degree of the intensity of nuclear staining added to the number of the AR stained nuclei in an equation to calculate the degree of AR expression. Their method depended more on the subjectivity in estimating the degree of the color of AR stained nuclei.

In the present study, the prevailing breast quadrant regarding the occurrence of the primary tumor was the upper outer quadrant (38%) followed by the lower outer quadrant (24%), the lower inner quadrant (20%) and lastly the upper inner quadrant (18%). The UO quadrant also exhibited statistically significant higher AR expression compared to the both the UI and LI quadrants. However, this difference was statistically non significant when compared to LO quadrant. These results may explain the high prevalence of the tumor in the UO quadrant detected in the present work.

Ellsworth . (2004) reported that the incidence of the primary tumor was most frequent in the lower outer quadrant (30.9%); both the upper outer and lower inner quadrant were next in order with a frequency of 16.7%. These authors attributed the prevalence

of the primary tumors in the outer quadrants to the greater genomic instability they found in such quadrants compared to the inner quadrants.

Dehm and Tindall (2005) elucidated the mechanism of activation of androgen receptor and its relationship to DNA and genes. Androgen receptor is a nuclear transcriptional factor located primarily in the cytoplasm. After binding to dihydrotestosterone, an active form of androgen, AR dimerizes, translocates into the nucleus then binds to the androgen-response elements on DNA and activates certain genes responsible for the execution of the role of androgen.

An earlier study proposed another hypothesis for the propensity of tumors in the UO quadrant. Haagensen (1986) claimed that the high incidence of occurrence of cancer in the UO quadrant was due to the presence of more breast tissue in such quadrant. However, the results reported by Thomas . (1997) could support the hypothesis adopted by the present work. These authors concluded that AR expression is necessary for the androgenic effect on breast cancer cell proliferation. Lillie . (2003) found that increased level of androgen in serum was associated with an increased risk for breast carcinoma in postmenopausal women.

Furthermore, Tamimi . (2006) concluded that the hormonal replacement therapy containing both testosterone and estrogen was associated with a significantly higher risk of breast cancer than that containing estrogen alone, in post menopausal women. Comparing the mean area percentage of collagen fibers of the normal tissue of the four quadrants, in the present study, that of the UO quadrant was higher than those of the other three quadrants and the difference was statistically significant. On the other hand, the mean area percentage in the UI quadrant was lower than that of each of the three quadrants and the difference was statistically significant.

The mean ± SD of the area percentage of collagen fibers of tumors among all cases in the present study was 44.75 ± 2.2. The mean area percentage of the tumor demonstrated statistically significant increase compared to the normal tissue of each of the four quadrants.

These results could be explained by the findings of Lester (2005) who reported that invasive carcinomas of breast are usually surrounded by intense non-neoplastic host fibrous tissue reaction composed of fibroblasts, collagen fibers, lymphocytes and extracellular matrix; a desmoplastic response giving the tumor a hard consistency on palpation and replacing the fat of the breast.

Evaluation of the relationships between the clinicopathological prognostic parameters and the AR expression of the tumor (and its adjacent normal breast tissue), in the present study, revealed a statistically negative significant correlation between the size of the tumor (with its corresponding T staging) and AR expression of both the tumor and the normal tissue adjacent to the tumor.

Also a statistically significant negative correlation was found between the number of affected LN by tumor spread (with its corresponding N stage) and AR expression of both the tumor and the normal tissue adjacent to the tumor. Therefore, AR expression could be associated with good prognosis of invasive duct carcinoma and could also be associated with better survival and longer disease free periods.

These results are in concordance with those recorded by Honma . (2005) and Narita . (2008) who found that AR expression was significantly associated with small sized tumors and negative lymph nodes status. Other results revealed positive correlation between AR expression and parameters predisposing cancer invasion but, at the same time, demonstrated positive correlation between AR expression and good prognosis and longer disease free periods (Gonzalez ., 2008; Hanley ., 2008).

In conclusion The UO quadrant exhibited the highest percentage of AR expression among the four quadrants while tumor AR expression was higher than that of any of the four quadrants. There were negative correlations between the increase in AR expression of the tumor and the clinicopathological parameters of tumor prognosis such as tumor size and number of affected LN by tumor spread; AR can be used as prognostic indicator and a therapeutic target for hormonal treatment of breast cancer.

REFERENCES

AJCC (American Joint Committee on Cancer). 2009. AJCC Cancer Staging Atlas, 7th ed. Springer, pp. 419-440.

Bevilacqua JLB, Cody HS III, MacDonald KA, Tan LK, Borgen PI and Van Zee KJ. 2002. A model for predicting axillary node metastases based on 2000 sentinel node procedures and tumour position. Eur. J. Surg. Oncol., 28: 490–500.

Dehm SM and Tindall DJ. 2005. Regulation of androgen receptor signaling in prostate cancer. Exp. Rev. Anticancer Ther., 5: 63-74.

Dimitrakakis C, Zhou J and Bondy CA. 2002. Androgens and mammary growth and neoplasia. Fertil. Steril., 77: 26-33.

Ellsworth DL, Ellsworth RE, Love B, Deyarmin B, Lubert SM, Mittal V, Hooke JA and Shriver CD. 2004. Outer breast quadrants demonstrate increased levels of genomic instability. Ann. Surg. Oncol., 11(9): 861-868.

Gonzalez LO, Corte1 MD, Vazquez J, Junquera1 S, Sanchez R, Alvarez AC, Rodriguez JC, Lamelas ML and Vizoso FJ. 2008. Androgen receptor expresion in breast cancer: Relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. Cancer, 8:149-158.

Haagensen CD. 1986. Anatomy of the mammary gland. In: Diseases of the Breast. 3rd ed., by Haagensen, C.D., W.B. Saunders, Philadelphia, pp. 1-46.

Hall RE, Aspinall JO, Horsfall DJ, Birrell SN, Bentel JM, Sutherland RL and Tilley WD. 1996. Expression of the androgen receptor and an androgen-responsive protein, apolipoprotein D, in human breast cancer. Br. J. Cancer, 74(8): 1175-1180.

Hanley K, Wang J, Bourne P, Yang Q, Gao AC, Lyman G and Tang P. 2008. Lack of expression of androgen receptor may play a critical role in transformation from in situ to invasive basal subtype of high-grade ductal carcinoma of the breast. Human Pathol., 39: 386-392.

Honma N, Takubo K, Akiyama F, Sawabe M, Arai T, Younes M, Kasumi F and Sakamoto G. 2005. Expression of GCDFP-15 and AR decreases in larger or node-positive apocrine carcinomas of the breast. Histopathol., 47:195-201.

Janni W, Rack B, Sommer H, Schmidt M, Strobl B, Rjosk D, Klanner E, Thieleke W, Gerber B, Friese K and Dimpfl T. 2003. Intra-mammary tumor location does not influence prognosis but influences the prevalence of axillary lymph-node metastases. J. Cancer. Res. Clin. Oncol., 129: 503–510.

Lester SC. 2005. The Breast. In: Robbin and Cotran Pathologic basis of diseases. 7th ed., by Kumar, V.; Abbas, A.K. and Fausto, N., Elsevier Saunders, pp. 1119-1154.

Lillie EO, Bernstein L and Ursin G. 2003. The role of androgens and polymorphisms in the androgen receptor in the epidemiology of breast cancer. Breast Cancer Res., 5(3): 164-173.

Moinfar F, Okcu M, Tsybrovsky O, Regiting P, Lax SF, Weybora W, Ratschek M, Tavassoli FA and Denk H. 2003. Androgen receptors are frequently expressed in breast carcinomas. Potential relevance to new therapeutic strategies. Cancer, 98: 703-711.

Narita D, Pribac GC and Ardelean A. 2008. The role of androgen receptors for new therapeutic stratigies and the prognostic outcome in patients with breast cancer. Stud. Univers., 18: 259-264.

Park S, Koo J, Park HS, Kim JH, Choi SY, Lee JH, Park BW and Lee KS. 2010. Expression of androgen receptor in primary breast cancer. Ann. Oncol., 21: 488-492.

Rakha EA, El-Sayed ME, Green AR, Lee AHS, Robertson JF and Ellis IO. 2007. Prognostic markers in triple negative breast cancer. Cancer, 109: 25-32.

Riva C, Dainese E, Caprara G, Rocca PC, Massarelli G, Tot T, Capella C and Eusebi V. 2005. Immunohistochemical study of androgen receptors in breast carcinoma. Evidence of their frequent expression in lobular carcinoma. Virchows Arch., 447(4): 695-700.

Tamimi RM, Hankinson SE, Chen WY, Rosner B and Colditz GA. 2006. Combined estrogen and testosterone use and risk of breast cancer in postmenopausal women. Arch. Intern. Med., 166: 1483-1489.

Tavassoli FA and Devilee P. 2003. Tumors of the breast. In: World Health Organization classification of tumors. 2nd ed., IARC Press, Lyon, France, pp. 9-110.

Thomas HV, Key TJ, Allen DS, Moor JW, Dowsett M, Fentiman IS and Wang DY. 1997. A prospective study of endogenous serum hormone concentrations and breast cancer risk in postmenopausal women on the island of Guernsey. Br. J. Cancer, 76: 401-405.

Zhou J, Ng S, Adesanya-Famuiya O, Anderson K and Bondy CA. 2000. Testosterone inhibits estrogen-induced mammary epithelial proliferation and suppresses estrogen receptor expression. FASEB. J., 14: 1725-1730.