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# Immunohistochemical expression of Aromatase in malignant breast tumours

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## Abstract:

Twenty tumour biopsies of Iraqi breast cancer female patients were examined for immunohistochemical (IHC) expression of aromatase. The expression was correlated with age and histopathological type, grade and clinical staging of tumour. Results revealed that out of the 20 investigated cases, 75.0% showed positive IHC expression of aromatase, which was distributed as 35.0% for score 2+ and 40.0% for score 3+. The remaining five negative cases (25.0%) were observed to have the lowest age mean (38.6 ± 4.4 years), which was significantly different from the recorded age mean in patients with the score 2+ (53.6 ± 3.2 years; p = 0.008) or 3+ (49.4 ± 2.8; p = 0.04), but no significant difference was observed between age means of positive cases. A histpathological examination of tumours revealed that they were invasive ductal carcinoma (IDC) but of two types: tubular IDC (5 cases; 25.0%) and 15 cases (75.0%) were defined as not otherwise specified (IDC-NOS). In tubular IDC, most of cases (80.0%) had 3+ score, while in IDC-NOS, it was 26.7%. Such difference was significant (p = 0.023). Distributing patients by grade and stage of tumours revealed no significant difference in IHC scoring of aromatase or age. It is possible to conclude that positive IHC expression of aromatase is tended to occur in older ages (around 50 years) of breast cancer patients.

***Keywords:*** *Immunohistochemical expression, Aromatase, Breast cancer*

## Introduction:

The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women; comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality (Bray et al., 2012). In 2008, approximately 1.4 million women were diagnosed with breast cancer worldwide with a corresponding of 460,000 deaths (Ferlay et al., 2010). In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi Cancer Registry (Iraqi Cancer Registry, 2010), and is the second cause of cancer related deaths (Saaed et al., 2011). It is caused by the presence of malignant cells in the breast, which are characterized by uncontrolled division, leading to abnormal growth (in situ carcinoma), and their ability to invade normal tissue locally. The primary tumour begins in the breast, but once it becomes invasive, may progress to the regional lymph nodes (axillary/internal mammary) or metastasize (Davies, 2012).

No specific etiological factor has been documented, but different breast cancer-associated risk factors have been suggested by epidemiological studies; for instance, age, menarche, menopause, parity, breastfeeding, use of exogenous hormones or oral contraceptive, obesity, lack of exercise, diet, smoking, alcohol consumption and family history of breast cancer or other cancers (Davies, 2012). These risk factors have been shown to have different relations to breast cancer in different ethnic populations of the world (Abdulrahman and Rahman, 2012). However, oestrogen has been suggested to have a pathogenic role in breast cancer aetiology, and it has been demonstrated that aromatase is a major producer of oestrogen in post-menopausal women through converting androgen to oestrogen (Lipovka and Konhilas, 2016).

Aromatase is a principal enzyme involved in the catalytic conversion of adrenal androgens (testosterone and

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androstenedione) to aromatic oestrogens (estradiol-E2 and estrone-E1, respectively) (Subramanian et al., 2008). The enzyme belongs to the p450 superfamily of cytochrome, and is localized in the endoplasmic reticulum of oestrogen-producing cells (Ghosh et al., 2009). In this respect, studies have demonstrated that local production of oestrogen in breast cancer tissue is higher than in normal breast counterparts due to the presence of very high level of aromatase (Bagnoli et al., 2010). In a recent study, it has been further presented that an inhibition of aromatase activity may become a standard therapy for hormone-dependent breast cancer in women (Simeon et al., 2016). Accordingly, the present study was suggested to inspect the immunohistochemical (IHC) expression of aromatase in breast cancer tissues of Iraqi female patients, and correlate such expression with age and histopathological type, grade and clinical staging of tumour.

## Material and Methods:

**Subjects**

The study involved 20 Iraqi breast cancer female patients, whose age ranged between 25 and 62 years (mean ± standard error: 48.2 ± 2.2 years). The patients were referred to the Centre for Early Detection of Breast Tumour at Al-Alwayia Hospital for Gynaecology and Obstetrics (Baghdad) during the period June 2013 - October 2014. The diagnosis was made by the consultant medical staff, which was based on a Triple Assessment Technique (i.e. physical breast examination, ultrasonography, with or without mammography and fine needle aspiration cytology). According to histopathological examination, the patients were distributed into type, grade and clinical stage of breast cancer tumour.

**Immunohistochemical (IHC) expression of aromatase**

Twenty paraffin embedded sections of breast cancer tumours were cut into 4 µm thicknesses using a microtome. The sections were applied on Fisher-brand positively charged slides and left overnight to dry at room temperature, and then processed for the tissue expression of aromatase by means of IHC staining, which was based on using a specific monoclonal antibody that detect aromatase. The reaction was detected by NovolinkTM polymer detection system (Envision technique) using a commercial kit from NovocastraTM (NovocastraTM Laboratories Ltd, Newcastle, UK, RE7150-K).

The aromatase IHC expression was scored according to the score presented by the manufacturer, which ranged from 0 to 3+. Score 0 is negative with no stained cells; score 1+ shows diffuse and weak staining in cytoplasm and cell membrane (less than 10% of cells is stained); score 2+ shows moderate to strong granular cytoplasmic and cell membrane staining (10 – 90% of cells is stained); while in score 3+, over 90% of cells is strongly stained.

**Statistical analysis**

Age was given as mean ± standard error (SE) and significant differences between means were assessed by analysis of variance (ANOVA) followed by either least significant difference (LSD) or Duncan test. For aromatase IHC expression, number and percentage frequencies of cases in each score were presented, and significant differences between the percentages were assessed by Pearson's Chi-square or Overall's Continuity-Corrected Probability. These analyses were carried out using the statistical package SPSS version 13.

## Results:

**Total Cases**

Out of the 20 investigated cases, 75.0% showed positive IHC expression of aromatase, which was distributed as 35.0% for score 2+ and 40.0% for score 3+. The remaining five negative cases (25.0%) were observed to have the lowest age mean (38.6 ± 4.4 years), which was significantly different from the recorded age mean in patients with the score 2+ (53.6 ± 3.2 years; $p = 0.008$) or 3+ (49.4 ± 2.8; $p = 0.04$), but no significant difference was observed between age means of positive cases (Table 1).

**Table 1: Age mean in breast cancer patients distributed by immunohistochemical expression of aromatase.**

| Aromatase Expression Score | Breast Cancer Patients (No. = 20) | | Age (Years) | | |
|---|---|---|---|---|---|
| | No. | % | Mean ± SE | Minimum | Maximum |
| 0 | 5 | 25.0 | $38.6 \pm 4.4^{B}$ | 25 | 47 |
| 2+ | 7 | 35.0 | $53.6 \pm 3.2^{A}$ | 38 | 62 |
| 3+ | 8 | 40.0 | $49.4 \pm 2.8^{A}$ | 36 | 60 |

Different superscript letters: Significant difference ($p \leq 0.05$) between means (Duncan test).

**Histopathological classification of breast cancer**

A histpathological examination of 20 breast cancer biopsies revealed that they were invasive ductal carcinoma (IDC) but of two types: tubular IDC (5 cases; 25.0%) and 15 cases (75.0%) were defined as not otherwise specified (IDC-NOS). Both groups showed approximated age (49.4 ± 3.9 and 47.7 ± 2.7 years, respectively), and no significant difference between their means was recorded (Table 2).

**Table 2: Histopathological types of breast cancer biopsies and age of patients.**

| Histopathological Type | Breast Cancer Biopsies (No. = 20) | | Age (Mean ± SE; Years) | Probability |
|---|---|---|---|---|
| | No. | % | | |
| Tubular IDC | 5 | 25.0 | 49.4 ± 3.9 | Not Significant |
| IDC-NOS | 15 | 75.0 | 47.7 ± 2.7 | |

The IHC expression of aromatase showed different profiles in tubular IDC and IDC-NOS. In the first, most of cases (80.0%) had 3+ score, while in the latter type, it was 26.7%. Such difference was significant at p = 0.023 (Table 3).

**Table 3: Immunohistochemical expression of aromatase in breast cancer biopsies distributed by histpathological type.**

| Aromatase Expression Score | Histopathological Type | | | | Overall's Continuity-Corrected Probability |
|---|---|---|---|---|---|
| | Tubular IDC (No. = 5) | | IDC-NOS (No. = 15) | | |
| | No. | % | No. | % | |
| 0 | 1 | 20.0 | 4 | 26.7 | Not Significant |
| 2+ | 0 | 0.0 | 7 | 46.6 | Not Significant |
| 3+ | 4 | 80.0 | 4 | 26.7 | 0.023 (Significant) |

**Grade**

Three grades were recognized in the investigated 20 cases; low, intermediate and high grades. Twelve cases (60.0%) were observed in intermediate grade, while low and high grades shared a similar frequency (20.0% each). Intermediate grade patients were observed to have the lowest age mean (46.2 ± 3.3 years) compared to low (51.8 ± 3.7 years) or high (50.5 ± 2.2 years) grade, but the difference was not significant between the three means (Table 4).

**Table 4: Breast cancer biopsies distributed by grade and age of patients.**

| Grade | Breast Cancer Biopsies (No. = 20) | | Age (Mean ± SE; Years) |
|---|---|---|---|
| | No. | % | |
| Low | 4 | 20.0 | 51.8 ± 3.7[A] |
| Intermediate | 12 | 60.0 | 46.2 ± 3.3[A] |
| High | 4 | 20.0 | 50.5 ± 2.2[A] |

Similar superscript letters: No significant difference (p > 0.05) between means (Duncan test).

was observed that all high grade biopsies (100%) were positive for aromatase IHC expression and their score was 3+. With respect to low grade biopsies, 25.0% had 0 score (negative expression), while 2+ and 3+ scores represented 50.0 and 25.0%, respectively. These frequencies were not much different in intermediate grade biopsies, and the corresponding percentages were 33.3, 41.7 and 25.0%, respectively for scores 0, 2+ and 3+.. Overall comparison between the three scores in the three types of grade revealed no significant difference (Table 5).

**Table 5: Immunohistochemical expression of aromatase in breast cancer biopsies distributed by grading.**

| Aromatase Expression Score | Grade | | | | | | Statistical Analysis |
|---|---|---|---|---|---|---|---|
| | Low (No. = 4) | | Intermediate (No. = 12) | | High (No. = 4) | | |
| | .No | % | .No | % | .No | % | |
| 0 | 1 | 25.0 | 4 | 33.3 | 0 | 0.0 | $X^2 = 7.643$; D.F. = 4; $p > 0.05$ (Not Significant) |
| 2+ | 2 | 50.0 | 5 | 41.7 | 0 | 0.0 | |
| 3+ | 1 | 25.0 | 3 | 25.0 | 4 | 100.0 | |

**Clinical Stage**

Clinical staging is based on assessment of three tumour criteria; T (tumour size), N (regional lymph node) and M (distant metastasis), and accordingly, the stages can range for zero to IV (Benson *et al*., 2003). The 20 investigated breast cancer tumours were classified under I, II and III clinical staging with frequencies of 40.0, 40.0 and 20.0%, respectively. Age distribution revealed that stage II had the lowest age mean (45.6 ± 4.2 years) compared to stage I (49.5 ± 3.3 years) and stage III (50.5 ± 4.2 years); however, the difference was not significant between the three means (Table 6).

**Table 6: Breast cancer biopsies distributed by clinical stage and age of patients**

| Clinical Stage | Breast Cancer Tumours (No. = 20) | | Age (Mean ± SE; Years) |
|---|---|---|---|
| | No. | % | |
| I | 8 | 40.0 | $49.5 \pm 3.3^{A}$ |
| II | 8 | 40.0 | $45.6 \pm 4.2^{A}$ |
| III | 4 | 20.0 | $50.5 \pm 4.2^{A}$ |

Similar superscript letters: No significant difference ($p > 0.05$) between means (Duncan test).

Scoring of aromatase IHC expression revealed no significant variation in the three observed clinical stages of breast cancer tumours; although 100.0% of stage III tumours had the positive score 3+ (Table 7).

**Table 7: Immunohistochemical expression of aromatase in breast cancer biopsies distributed by clinical stage.**

| Aromatase Expression Score | Clinical Stage | | | | | | Statistical Analysis |
|---|---|---|---|---|---|---|---|
| | I (No. = 8) | | II (No. = 8) | | III (No. = 4) | | |
| | No. | % | No. | % | No. | % | |
| 0 | 3 | 37.5 | 2 | 25.0 | 0 | 0.0 | $X^2 = 7.929$; D.F. = 4; $p > 0.05$ (Not Significant) |
| 2+ | 3 | 37.5 | 4 | 50.0 | 0 | 0.0 | |
| 3+ | 2 | 25.0 | 2 | 25.0 | 4 | 100.0 | |

## Discussion:

The presented results suggest that aromatase expression in breast cancer tumors might have a pathogenic role in progression of breast cancer in women, as 75.0% of patients showed a positive expression. These findings in agreement with previous investigations, in which a higher activity of aromatase was reported in tumours of breast cancer patients compared to fat tissue of normal breast (Ghosh *et al*., 2009; Bagnoli *et al*., 2010). In addition, molecular analysis of adipose stromal cells that surround breast cancer cells revealed that they contain a higher level of aromatase mRNA than in adipose stromal cells of noncancerous areas in the tumour (Suzuki *et al*., 2009). Such increased activity has also been correlated with increased concentrations of oestrogens in breast cancer tumour tissues; an observation that confirm the correlation between breast cancer tumour accumulation and *in situ* or local synthesis of oestrogens (Sasano *et al*., 2010). Accordingly, the present results confirm the aetiological role of aromatase activity in breast cancer progression. However, in present study, there were still 25.0% of breast cancer patients whose biopsies showed negative IHC expression of

aromatase, but such patients were observed to have an age mean below 40 years, and the difference was significant compared to IHC-positive cases of scores 2+ and 3+; in which the age was around 50 years. Such observation may suggest that there is a correlation between age, aromatase activity and breast cancer progression. Investigations in this context have reported that postmenopausal women (their ages exceeded 50 years) had higher concentrations of estradiol in breast cancer tissue than in plasma and normal breast tissue, and such increased concentration of estradiol might be be due to *in situ* synthesis of oestrogen by breast tissues, which is supposed to be catalyzed mainly by aromatase that has increased activity (Brueggemeier *et al*., 2003; Yamaguchi, 2007). Further investigations confirmed that aromatase mRNA showed a significantly increased level of expression in patients older than 50 years (Zhang *et al*., 2003; Licznerska *et al*., 2008). Accordingly, inhibition of aromatase pathway has been clinically suggested to be a useful strategy that controls the progression of breast cancer tumours in postmenopausal women (Simeon *et al*., 2016).

Distributing breast cancer patients by histopathological type of tumour revealed that IHC expression of aromatase showed a significant increased frequency in patients with tubular IDC (80.0%) at 3+ score compared to IDC-NOS patients (26.7%) of the same score. Tubular breast carcinoma of the breast is an uncommon histologic subtype of IDC, and epidemiological reviews have reported the favourable prognosis (i.e. low incidence of metastases and recurrences and a recognized overall survival) (Rakha *et al*., 2010), but the significance of aromatase activity in such type of IDC has not be determined. However, the present results suggest an important role of aromatase expression in tubular IDC; although the observation was limited by the small sample size. Therefore, further investigations are certainly required to inspect the role of aromatase expression in breast tumours of tubular IDC type.

When grade and clinical stage of investigated breast cancer tumours were inspected, no significant variation in aromatase IHC expression between grades or stages and the IHC expression scores was noticed. However, it is worth to mention that 100% of tumours in clinical stage III showed a strong positive expression of aromatase (score 3+). Again, the sample size may limit the significance of such observation.

In conclusion, the present results confirm the pathogenic role of aromatase activity in aetiology and progression of breast cancer. In addition, the age might be a critical factor in mediating the pathogenic role of aromatase in breast cancer.

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# التعبيرالنسجي-الكيميائي-المناعي للأرومتيز في أورام الثدي الخبيثة

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**الخلاصه:**

اجريت الدراسة على عشرين مريضة عراقية مصابة بسرطان الثدي. اخذت مقاطع نسجية للورم واجري التقييم النسجي-الكيميائي-المناعي للارومتيز. ارتبط التعبير للارومتيز مع التقدم في العمر ونوع النسيج والرتبة والتدريج السريري للورم. واظهرت النتائج ان ٪75.0 من المريضات كانت موجبة لفحص الارومتيز، والتي كانت موزعة على النحو) ٪35.0 للـ +score2 و٪40.0 للـ +score 3)، وقد لوحظت خمس حالات سالبة لفحص الارومتيز (٪25.0) وكان لديهم أقل معدل العمر (38.6 ± 4.4 سنة)، واختلف معنويا عن المريضات الموجبة للفحص (53.6 ± 3.2 score 2+ سنوات؛ P = 0.008) و± 49.4) score 3+ 2.8؛ P = 0.04)، ولكن لم يلاحظ أي اختلاف معنوي بين عمر العينات الموجبة للفحص. وكشف الفحص النسجي المرضي للمريضات انه من نوع سرطان القنوات الغازي، ولكن بنوعين: الانبوبي 5 tubular IDC حالات (٪25.0) و15 حالة (٪75.0) تم تعريفها بأنها (IDC-NOS). في النوع الانبوبي وأن معظم الحالات (٪80.0) موجبة +score 3، بينما في IDC-NOS، كان ٪26.7. وهنا الفرق معنويا (p = 0.023). فمن الممكن أن نستنتج ان التعبير IHC الايجابي للارومتيز ويميل للحدوث في الأعمار المتقدمة (حوالي 50 عاما) من مريضات سرطان الثدي.