

Predictability of the Expression of ER, PR Receptors, HER2 and AgNORs in Breast Carcinoma among Iraqi Women in Thi - Qar Province: Immunohistochemical study

Hayfaa Abid Ali

Doha Kadhim Najam*

Biology Departement - College of Science - University of Thi-Qar

* E-mail:dhohakazem@gmail.com

Abstract

This study is descriptive exploratory in Thi – Qar providence for some breast cancer patients .The study aims of the study to assess the relationship between ER,PR,HER2 and the nuclear regulatory regions Argyrophilic nucleolar organizer region (AgNOR) in breast cancer.

The present study addressed the identification the nuclear organization region in the breast cancer patients in Thi-Qar providence and the extent of their association with ER, PR and HER2 in the occurrence of breast cancer. Fifty tissue samples collected from Hussein Teaching Hospital in Thi Qar and some private laboratories in the province with the province of each case data included the most important factor is age, found that the highest percentage of injuries, age (55-46 years) increased by 38%. And mixed samples. Either partial removal of the tumor or whole breast. The results of the current study showed that there was a clear positive relationship between nuclear organization regions and breast cancer, as it was noted the presence of many of the nuclear regions of the dye-derived tissue sections of tumors while nuclear chromosomal regions did not appear in the sections derived from tumors with negative expression of estrogen receptor and progesterone hormone.

Key words: ER, PR, HER2, AgNOR, Breast cancer.

إمكانية التنبؤ عن التعبير الجيني لمستقبلات الاستروجين والبروجيستيرون ومُوسمات الورم ومناطق التنظيم النووي لسرطان الثدي بين النساء العراقيات في محافظة ذي قار (دراسة نسجية مناعية)

ضحى كاظم نجم

هيفاء عبد علي

قسم علوم الحياة – كلية العلوم – جامعة ذي قار

الخلاصة

أجريت هذه الدراسة الوصفية الاستطلاعية في محافظة ذي قار لبعض مرضى سرطان الثدي , اذ هدفت هذه الدراسة الى تقييم العلاقة بين التعبير الجيني الايجابي لكل من مستقبلات الاستروجين ER- Estrogen-receptor والبروجيستيرون PR –Progesterone receptor و مُوسمات الورم(HER2 (Human epidermal growth factor receptor باستخدام تقنية كيميائية الاصبغ المناعية وبين مناطق التنظيم النووي Argyrophilic nucleolar organzier region (AgNOR) . تناولت الدراسة الحالية تحديد العلاقة بين مناطق تنظيم النوى والمستقبلات الهرمونية

الاستروجين والبروجيستيرون والعامل الوراثي ومدى ارتباطهن بحدوث سرطان الثدي. خمسون عينة نسيجية تم جمعها من مستشفى الحسين التعليمي في محافظة ذي قار وبعض المختبرات الخاصة في المحافظة مع البيانات الخاصة بكل حالة حيث تضمنت اهم عامل وهو العمر، حيث وجد ان اعلى نسبة اصابات بعمر (46-55) سنة بنسبة 38% . وتباينت العينات، إما باستئصال جزئي للورم او استئصال كلي للثدي. وأظهرت نتائج الدراسة الحالية أيضاً أن هناك علاقة إيجابية واضحة بين مناطق التنظيم النووية وسرطان الثدي، كما لوحظ وجود العديد من المناطق النووية من المقاطع الأنسجة المشتقة من صلب الأورام في حين لا تظهر المناطق تنظيم النوى في المقاطع المأخوذة من الأورام ذات التعبير السليبي لمستقبلات هرمون الاستروجين وهرمون البروجيستيرون.

1.Introduction

The Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way, sometimes, to metastasize. Cancer is not one specific type of diseases. It is in fact over 100 different types of distinctive diseases. Cancer can be in any tissue of the body and have many forms in each area in the body (National Cancer Institute, 2014).

Breast cancer is the most common cancer among women worldwide, comprising 23% of the 1.1 million female cancers that newly diagnosed each year. (Parkin and Fernandez, 2006) The Iraqi Cancer Registry (ICR) considered breast cancer is popular type of Iraqi female malignancy, accounting approximately one-third of the registered female cancers in 2008. (Iraqi Cancer Registry Center, Ministry of Health, 2007), to understanding what meaning by AgNORs explanation by defining as following: The Ag NOR is the nucleolus organizer region (NOR) a loop of nucleolar DNA which encodes rRNA .Ag NOR reflect proliferation, The number of AgNors may correlate with tumor behavior .AgNor staining in tissue increase as the tumors become more aggressive . (Schliephake, 2003) .It is the particular part of a chromosome that is associated with a nucleolus after the nucleus divides. Schliephake, (2003) mention that the AgNORs reflect proliferation; the number of AgNORspots.

In Kaposi sarcoma make more aggressive. The role of estrogen and progesterone receptors in breast cancer is well established, it is receptors promoted us to evaluate the role of estrogen in the breast cancer. Also of progesterone receptors measurement the predictive value further by defining ER-positive/PR-negative tumor type, which is likely to respond to therapy than tumors that are positive for both receptors (Locker, 1998).

The results of the current study showed that there is a clear positive relationship between nuclear organization regions and Argyrophilic nucleolar organiser region (AgNOR) Fairs oncogene HER2, as it was noted the presence of many of the nuclear regions of the dye-

derived tissue sections of tumors with positive gene expression of the HER2 oncogene fairs, while nuclear chromosomal regions did not appear in the sections derived from tumors with negative expression of estrogen receptor and progesterone hormone.

2. Materials and Methods

2.1 Subject

This study designs as prospective study, all samples including (50) samples from patients with breast cancer who undergoing surgical resection (mastectomy).

2.2 Histological preparations

The histological technique was used in the preparation of tissue according to Luna, 1986. Microscopic examination including:

2.3.1Grossin

In all grossing, the part which was cut from each specimen was in thick between 3-4 mm, which makes the Fixation satisfactory.

2.3.2 Histological Technique

2.3.2.1 Fixation

2.3.2.2 Dehydration

2.3.2.3 Clearing

2.3.2.4 Impregnation

2.3.2.5 Embedding

2.3.2.6 Sectioning

2.3.2.7 Mounting and Staining

2.3.3 Immunohistochemistry for Estrogen and Progesterone Hormone receptor

2.3.3.1 Principle

Immunohistochemistry (IHC) was a process to detecting antigens (e.g. Proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. Immunohistochemical staining is widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue

2.3.4 Silver Staining for Nucleolar Organizer Regions

Evaluation of nucleolar organizer region-associated proteins in breast malignancy Nucleolus organizer regions (NORs) have been identified by means of an argyrophilic technique (Ag-NOR) in routinely processed, formalin-fixed paraffin sections of breast lesions. This method reveals NORs as black dots in the nuclei of cells, by virtue of the argyrophilia of NOR-associated proteins. The number of Ag-NORs has been thought to be related to cellular activation and has recently been applied to non-Hodgkin's lymphomas and melanocytic Skin lesions (Smith, Crocker, 2014).

Solutions:

Solution 1: 50% silver nitrate (AgNO₃ 50g+ distilled water 100 ml)

Solution 2: Gelatin 2g+formic acid 1ml+distilled water 100ml

Work solution: Silver nitrate solution 2 part by volume with gelatin solution 1 part by volume. Mix in above preparation immediately before use, the volume of working solution used depends on the number of slides.

1. De wax section in xylene hydrate through ethanol to water.
2. Rinse in D.W.
3. Incubate freshly working solution for (45 min.) at room temperature.
4. Wash in D.W for 1min.
5. Dehydrate clear and amount in aqueous mounting media (John and et al., 2012).

The Results

3.1 Age Distribution

In this study the distributing of patients into age groups revealed that most patients 50 cases were at the age group (26-35) years, 5 cases (10%) were in the age group (36-45) years, 16 cases (32%) were in the age group (46-55) years, 19 cases (38%) were in the age group (56-65) years, 10 cases (20%) .

Maximum percents appear in age (46-55) year while showed minimum percents in age (26-35) year as following in Table (1).

Table (1): Distribution of breast carcinoma patients according to age groups

Age of groups (years)	Number of Patients	%
26-35	5	10
36-45	16	32
46-55	19	38
56-65	10	20

Carcinoma is divided into 44 cases (88%) of ductal carcinoma. invasive ductal carcinoma characteristic of (IDC) : Large pleomorphic tumor cells combined by abundant mitotic activity, solid, with permanent tissue (adipose tissue) and association with necrosis and 6 cases (12%) were invasive lobular carcinoma, features of these type : The tumor cells are small and uniform with round nuclei.

3.3 Histology of normal breast

In the present study, the histology investigation of the control sections of normal breast tissue was shown there are a lot of lobes which are tubuloalveolar type, each lobe, separated from the others by dense connective tissue with adipose tissue. The alveolar or acinar surround by the basement membrane Fig (1)

3.4 Histology of breast cancer

The histological structure in breast cancer was shown there are lacking regular and uniform pattern. anaplastic tumor cells forming solid (high aggressive) nest, cords, poorly formed glandular structure and some intraductal foci Fig (2,4,6). infiltration by these pattern of tumor cells into diffuse fibrous stroma and fats.

3.5 The relationship between Agnor and PR, ER, HER2

In this study, there is a inverse relationship between the present adots of AgNORs and negative or positive of ER, PR and HER 2 as shown in the figures.

3.6 The AgNORs

In this study, the AgNORs dots were a present in all cases with positive HER2 in spite of the positive or negative of PR and ER. The cases with negative HER2 haven't AgNORs dots, Figs (3, 5, 7), (Table 2)

Table (2): The relationship between the ER, PR, HER2 and AGNOR in breast cancer in Iraqi women.

Factors	AGNOR
ER- ,PR-, HER2+	+
ER- ,PR+, HER2+	+
ER+ , PR+, HER2-	-

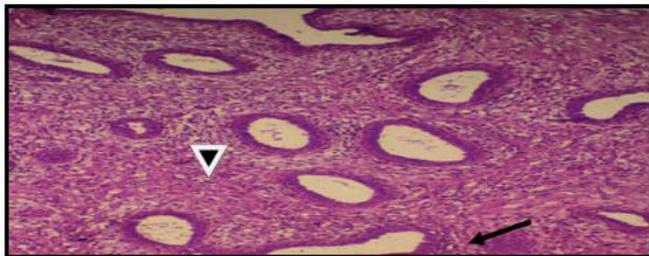


Fig (1) Photomicrographs, Normal breast appears the ducts (arrow) and stroma (head arrow) 10X (E&H)

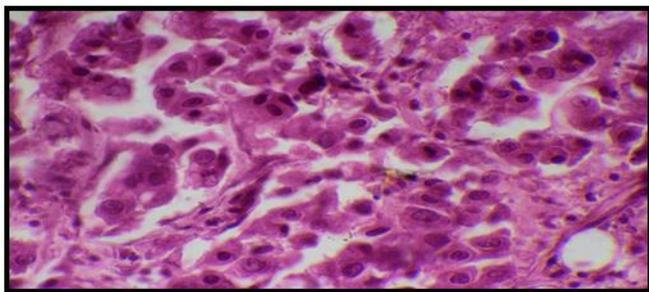


Fig. (2) Photomicrographs , Ductal carcinoma ,show the invasive tumor cells,(E&H).40x

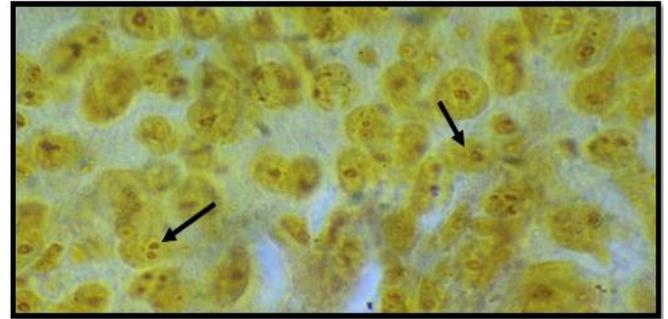


Fig (3)Photomicrograph showing AgNORs in the intra ductal carcinoma breast .

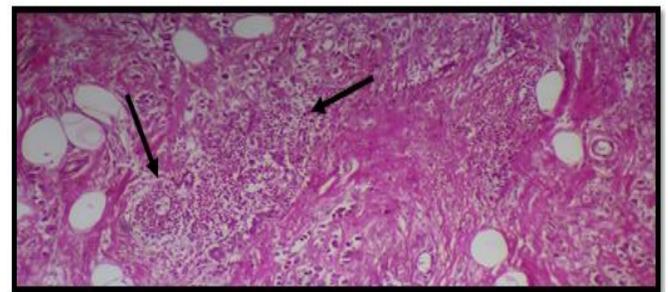


Fig. (4) Photomicrographs , Intra ductal carcinoma ,shows the invasive tumor cells , H&E(40 X).

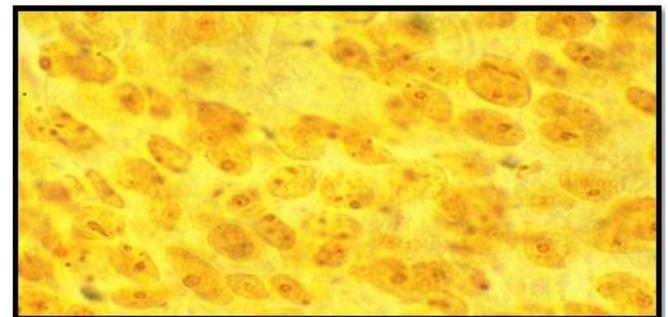


Fig (5) Photomicrograph showing AgNORs in the intra ductal carcinoma breast (100x)

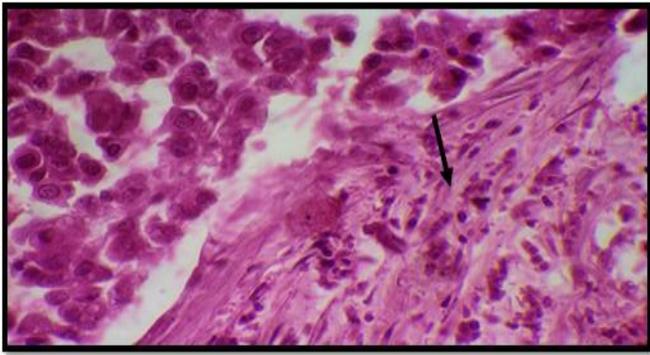


Fig. (6) Photomicrographs ,Intraductal carcinoma ,shows the invasive tumor cells , H&E(40 X).

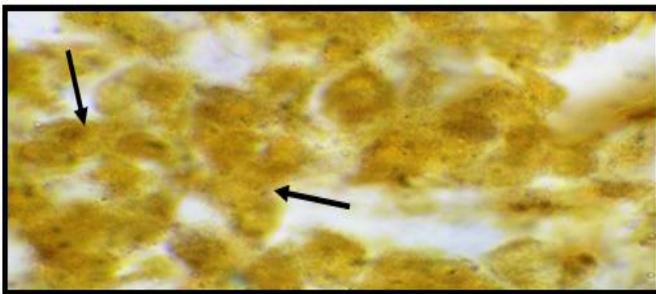


Fig. (7) Photomicrograph showing AgNORs in intraductal carcinoma breast , there are no dots per

4. Discussion

The management of breast carcinoma depends on a number of biological and morphological factors. These include the degree and proportion of nuclei showing atypia, the presence or absence of particular tumor markers (e.g., ER and PR receptor status) and tumor type and grade the presence of local invasion and lymph node metastases (Russo et al., 1987; Martin *et al.*, 1987; Rank *et al.*; 1987 and Alexieva-Figusch, 1987). In this current study, we focus on the relationship between the multi factors ER ,PR , HER2 and AGNOR area in breast carcinomas in Iraqi women.

4.1 The relationship between Agnor and PR, ER, HER2.

The results of this study refer that there are relationships between the study factors .The Nucleoli became an important biomarker and therapeutic target. The recent researches are enabling nucleoli to be used

effectively as an important biomarker and therapeutic target.

The increase of AgNORs applied in histopathology research following suggestions that there may be a possible relationship between high AgNOR counts and malignant tumors (Ploton *et al.*, 1986; Fallowfield *et al.*, 1988).

It is interesting to hypothesize, that the numbers, the shape, and the distribution of AgNORs within the nucleus might be potential use in predicting behavior in breast carcinomas.

The AgNORs staining in the present study was found to be a very useful prognostic marker of cell proliferation and it has correlation with ER and PR receptors, it has been shown that there are significantly inversely associated between the AgNORs counts with PR and PR expression, our result agreed with Ahmed *et al.* (2011).

This study agrees with Ahmed *et al.* (2013) shown in his study that the mean of AgNORs counts were high in hormonal receptors negative (ER+ve = 2.45 vs. ER-ve= 2.71 and PR+ve= 2.36 vs. PR-ve= 2.71) and p53 positive cases.

AgNORs is correlate with the proliferation tumor activity; the high rate of AgNOR counts may reflect increase proliferative activity of cells (Mourad *et al.*, 1992; Dasgupta *et al.*, 1997).

The counts of AgNOR have been studied in breast carcinomas, but the outcomes have been inconsistent. Some studies have shown that quantitative analysis of AgNORs yields a prognostic factor in malignancy breast (Rzymowska, 1997), but in the findings of the study of Derenzini *et al.*, (2004) indicate that the prognostic value of the AgNOR parameter cannot be ascribed to the relation between AgNORs and the cell proliferation rate also it depends on the status of the tumor suppressor proteins pRb and p53.

In the present study, significant inverse correlations were found between proliferation markers and ER and PR receptor status this result agreed with Guski *et al.* (2000) result ,which refers that the AgNOR number and total AgNOR area haven't correlation not with ER/PR status.

Alot of factors effecting on the AgNORs scores variation on histological sections and cytological smears, This variation can be accounte for by the choice of the length of incubation in silver nitrate solution, fixative used, subjective impression of dot distinction and subjective variation in counting technique (Meehan *et al.*,1994). Agarwal *et al.*, (1995) mentioned that the standardization of section thickness and Heterogeneity

of tumors with regard to cell proliferation can affect on the AgNOR counts.

our result could be comes from the individually effecte of the sex hormone on the breast cells and the extracellular matrix , the Heredity and gene mutation , The AgNORs is a simple and inexpensive method for analysis of cell proliferation compare with the more expensive and complicated procedures like immunohistochemistry and thymidine labeling index.

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