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A Comparative Study of Some Biomarkers for Different Types of Cancers in Iraqi Patients

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ABSTRACT

Background: Breast cancer, the most frequently occurring cancer in women, is a major public health problem, with 1,384,155 estimated new cases worldwide with nearly 459,000 related deaths.

Methods: Forty subjects included in this study were represented by their archival formalin-fixed, paraffin embedded tissue blocks with breast tissue from the archives of Teaching Hospitals in Baghdad. Their age ranged from 35 to 70 years. This study was carried out in Department of patholohgy research of the National Cancer research Center and Forty subjects included in this study were represented by their archival formalin-fixed, paraffin embedded tissue blocks with cervical tissues collected from the archives of Teaching Hospitals in Baghdad and from private laboratories.

Result: Rusults obtained from this study indicate that most patients were positive for Twist2 expression and significant correlation of Twist 2 expression with breast cancer has been found , which indicates that the overexpression of Twist2 may contribute to breast cancer progression by activating the EMT program. we found that 33% of the positive cases shoed nuclear expression and 67% showed cytoplasmic localization which indicates the role of the Twist 2 with the loss of cell to cell junction metastasis of the breast cancer cells. Twist2 cellular localization determines the role of Twist 2 in the EMT, The cytoplasmic Twist2 in cancer cells at tumor center of primary carcinomas and lymph metastases contributes to the maintenance of epithelial cancer characteristics expressing E-cadherin in a noninvasive state, while the nuclear Twist2 at the cancer invasion front activates EMT to deprive epithelial property of neoplastic cells, thus facilitating invasion and metastasis.

Key words: Breast cancer, IHC, Twist 2.

INTRODUCTION

Breast cancer, the most frequently occurring cancer in women, is a major public health problem, with 1,384,155 estimated new cases worldwide with nearly 459,000 related deaths (Tao *et al*., 2015). There is a large variation in breast cancer survival rates around the world, with an estimated 5-year survival of 80% in high income countries to below 40% for low income countries (Coleman *et al*., 2008; Lamouilie *et al*., 2014). According to Iraqi Cancer Board as well as Iraqi Cancer Registry Center in Iraqi Ministry of Health has recently demonstrated that female breast cancers constituted about 25% out of the total registry of cancers in Iraqi patients (Alwan, 2010).

Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, prior history of breast cancer and family history (World Cancer Report, 2014).

A defining characteristic of tumor cells is the escape from regulatory mechanisms that normally restrain cell proliferation. This is accomplished through the accumulation of multiple genetic alterations. Among these are the inactivation of key tumor suppression pathways and the activation of oncogenes (Vogelstein and Kinzler, 1998; Li *et al*., 2013).

In spite of the fact that Papanicolaou (Pap) screening test is broadly utilized and prompts decrease in cervical malignant growth mortality, numerous patients with cervical carcinoma still died from metastasis. Accordingly, realization of the signaling pathways during cancer development is essential to understand cervical carcinoma (Lee and Shen, 2012).

Twist is a basic helix-loop-helix (bHLH) transcription factor that has been previously implicated in cell lineage determination and differentiation during embryogenesis. In recent years, Twist has also been shown to contribute to

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carcinogenesis through triggering epithelial to mesenchymal transition and down regulating E-cadherin expression, there by influencing tumor invasion, metastasis, adverse prognosis and drug resistance in multiple tumors (Yang *et al*., 2004., Vesuna *et al*., 2017). Such as such as breast (Martin *et al*., 2005), lung (Hung *et al*., 2009), prostate cancers (Yuen *et al*., 2007) and gastric carcinoma (Luo *et al*., 2008). Also Twist has already been used as a prognostic marker in cervical cancer (Shibata *et al*., 2008), bladder and prostate cancers (Wallerand *et al*., 2010; Kharat *et al*., 2024). And chronic kidney disease (Sun *et al*., 2012; Qiao *et al*., 2017). In particular, Yang *et al*. has shown that Twist overexpression in breast cancer can induce and promote tumorigenesis (Yang *et al*., 2004; Kiran *et al*., 2021). Since then, many studies have focused on the role of Twist on the progression and metastasis of malignancies. For instance, Martin *et al*. (2005) and Watanabe *et al*. (2004) have demonstrated the higher expression of this protein in human breast cancer tissues. However, the mechanism underlying how twist predicts prognosis is not well defined in breast cancers (Zhang *et al*., 2015; Qiao *et al*., 2017).

METERIALS AND METHODS

Twenty- five subjects included in this study were represented by their archival formalin-fixed, paraffin embedded tissue blocks with breast tissue from the archives of Teaching Hospitals in Baghdad. Their age ranged from 35 to 70 years. This study was carried out in Department of patholohgy research of the National Cancer research Center and Forty subjects included in this study were represented by their archival formalin-fixed, paraffin embedded tissue blocks with cervical tissues collected from the archives of Teaching Hospitals in Baghdad and from private laboratories. The sample was divided into two groups; 10 cases from apparently healthy women and Thirty cases from patients with cervical carcinoma..

All breast tissue sections were cut at 4 µm and placed on positively-charged slides; one section was stained with hematoxylin and eosin while other use for anti-TWIST (Abcam,UK)

Immunohistochemcial staining was performed using anti-TWIST Negative and positive control slides were included in each IHC run (as recommended by the manufacturer). Immunodetection was performed according to manufacture instructions of Cambridge Science Company using EXPOSE Mouse and Rabbit Specific HRP/ DAB Detection IHC kit (ab80436). All sections were dewaxed and rehydrated then subjected to antigen retrieval. Endogenous peroxidase activity and non-specific binding were blocked by incubation with 3% hydrogen peroxide and protein block, respectively. heat mediated antigen retrieving was performed with citrate buffer pH 6 before commencing with IHC staining protocol. Slides were then incubated sequentially with primary antibodies using a dilution of (1/100) for 1hour at 37°C and secondary antibody was applied for 10 minutes at room temperature followed by incubation with Streptavidine-HRP for 10 minutes at 37°C. Diaminobenzidinehydrochloride (DAB) was used as the chromogen to visualize peroxidase activity. Sections were counterstained with Mayer's hematoxylin for 30 seconds, dehydrated and mounted (Khshman, 2017).

Statistical analysis

Statistical analysis was done using Social Science Statistic (http://www.socscistatistics.com) and Excel application. Fisher exact test was used to find out the relation between Twist 2 expression with the breast cancer and normal groups. P value (<0.05) was considered statistically significant.

RESULTS AND DISCUSSION

The immunohistochemical expression for Twist 2 showed significant correlation in the breast carcinoma tissues, as shown in (Table 1 and Fig 1) in which the patients with age above 50 years represent 57% while those with age less than 50 years constitutes 43% of the samples.

The present study were designed to involve the cervical cancer tissues with HPV16 positive reaction (Fig 1) to be evaluated for the immunohistochemical expression of Twist 2 (Figs 1 C, D). The result revealed that there is a significant correlation in the cervical cancer tissues when compared with normal cervical tissues, as shown in Table 2.

Breast cancer has become a major threat to female health in Iraq, where it is the leading cause of death after cardiovascular diseases among women, with a cancer-related mortality rate of 23%. The cellular interaction paly critical role in development the disease, so the current study, considered the first attempt to study the role of Twist expression in the development of breast cancer in Iraq.

According to the age, has been found that age of patients in current study was above 50 years more than less 50 years, it is seems to be similar with those participated in other studies done in different area such as El-Zaemey *et al*. (2012).Due to most frequently complications of this age group as well as high rates of breast cancer are the result of a several-fold increase in the age specific incidence of ER+/HER2- tumors in post-menopausal women (WHO,2010).

Table 1: The immunohistochemical expression of Twist 2 in the studied groups.

IHC expression of Twist 2	Breast cancer	Normal breast tissue	Marginal row totals
Twist 2 postive	18	0	18
Twist 2 negative	2	10	12
Marginal column totals	20	10	30 (Grand total)

The Fisher exact test statistic value is <0.00001. The result is significant at p<0.05.

Data obtained from this study indicate that most patients were positive for Twist2 expression and significant correlation of Twist 2 expression with breast cancer has been found as shown in Table 1, which indicates that the overexpression of Twist2 may contribute to breast cancer progression by activating the EMT program, these results are in agreement with study done by Fang *et al*. (2011). Also agreed with several study achievement about breast cancer metastasis. It is well accepted that the mesenchymal-to-epithelial transition plays an important role in cancer metastasis. It has been considered that the non-motile epithelial cancer cells at the primary site first acquire the migratory characteristics of mesenchymal cells and then undergo a reverse mesenchymal- to-epithelial transition when they seed at a secondary site (Wang *et al*., 2016). Epitheliale mesenchymal transition, a critical step in the acquisition of metastatic state (Avtanski *et al*., 2014; El-Megharbel *et al*., 2025). Twist 2 (Dermo1) has been shown to mediate the epithelial-mesenchymal transition to promote tumor invasion and even metastasis (Mao *et al*., 2012; Li *et al*., 2013). According to Iraqi cancer registry, the distribution of female breast cancers showed that 64.18% were infiltrating duct carcinoma, this made it is rationale to study the role of Twist 2 in a group of patients with infiltrative ductal carcinoma .

According to Fig 1, we found that 33% of the positive cases shoed nuclear expression and 67% showed cytoplasmic localization which indicates the role of the Twist 2 with the loss of cell to cell junction metastasis of the breast cancer cells. Twist2 cellular localization determines the role of Twist 2 in the EMT, The cytoplasmic Twist 2 in cancer cells at tumor center of primary carcinomas and lymph metastases contributes to the maintenance of epithelial cancer characteristics expressing E-cadherin in a noninvasive state, while the nuclear Twist 2 at the cancer invasion front activates EMT to deprive epithelial property of neoplastic cells, thus facilitating invasion and metastasis (Mao *et al.,* 2012).

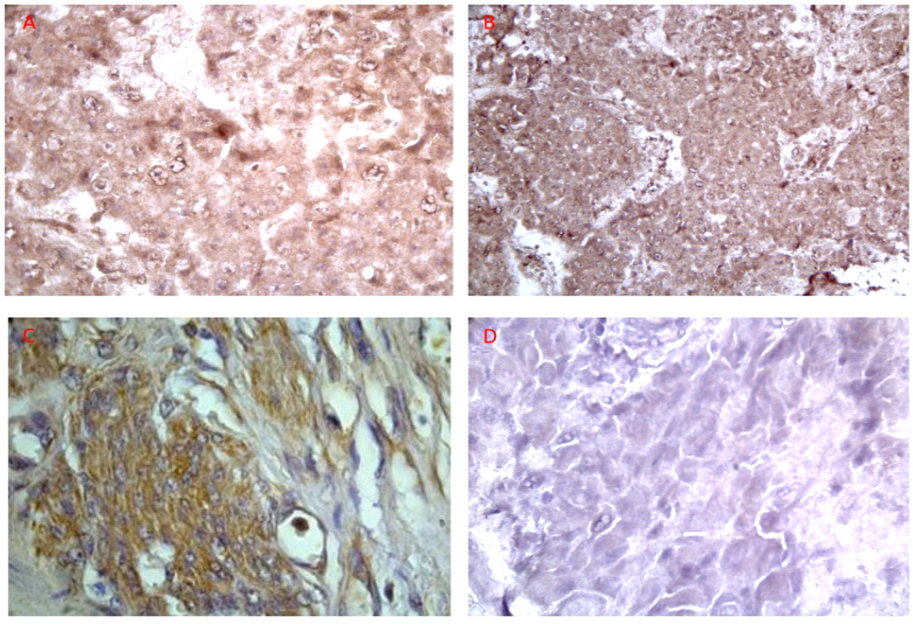
In conclusion, there are increased evidences of the contribution of Twaist 2 in the development of breast cancer makes it very interesting protein to become an important target for cancer treatment. Further studies are required to study the correlation of twist on the expression of both epithelial and mesenchymal markers are required to determine.

The crucial role of TWIST involvement in regulation cancer metastasis pathway have been demonstrated by different researchers (Shibata *et al*., 2008).

Table 2: The immunohistochemical expression of Twist2 in the studied groups.

IHC expression	Cervical cancer	Normal tissue	Total
Twist 2 positive	26	0	26
Twist 2 Negative	4	10	4
Total	30	10	40

*The Fisher exact test statistic value is <0.00001. The result is significant at p<.05.



The DAB produced (Brown) signals while the counter staining by Harris Hematoxylin produced (purple) color. (A and B): Nuclear positive results (100×). (C): cytoplasmic positive results (100×). (D) : Immunohistochemstrypositive results for detection nuclear and cytoplasmic TWIST 2 expression in cervical cancer tissues. The DAB produced (Brown) signals while the counter staining by Harris Hematoxylin produced (purple) color (100×).

Fig 1: Immunohistochemstry results for detection nuclear TWIST 2 in breast cancer tissues.

The cancer still a serious global public health dilemma with increasing incidence rate in spite of new therapeutic and diagnostic methods (Araldi *et al.*, 2018).

Since the first discovery of cervical screening test "Pap smear", there was an enormous effect on decreasing the disease mortality and morbidity rates.The discovery of human papillomavirus (HPV), the main causative agent of cervical cancer which is responsible of 95% of this cancer, make this tool more valuable in the cervical cancer screening (Rizzo and Feldman, 2018).

Although, Human Papillomavirus (HPV) infections are established as the main causative agent of cervical cancer worldwide, there is limited studies available regarding the virus prevalence,distributionof onocogenic types and viral risk factors among Arab countries (Elmi *et al.*, 2017).

In the present study, twist was over-expressed in 86.6% (26 out of 30) of the cervical cancer cases with a significant correlation P<0.00001 (Table 1, Fig 1). This results indicates the importance role of twist on the pathogenesis. Since all the samples in the current study were squamous cell carcinoma, the current findings coincide with Shibata *et al.* (2008), who found a significant correlation between The expression of Twist with the squamous type of cervical cancer, while it isnot in the adenocarcinoma type (Shibata *et al.*, 2008; Lamouilie *et al.*, 2014).

CONCLUSION

According to the study, there is a strong link between overexpression of Twist2 and breast cancer, indicating that Twist2 may contribute to the development of cancer by triggering the epithelial-mesenchymal transition (EMT). Twist2's cellular location is crucial; cytoplasmic Twist2 (67% of cases) may aid in maintaining epithelial traits in non-invasive cancer cells, but nuclear Twist2 (33% of positive cases) is linked to EMT activation and cancer cell invasion. According to these results, Twist2 may be a biomarker for the development and metastasis of breast cancer.

Conflict of Interest

The authors declare that they have no conflict of interest.

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