Research Article

IGF-1R Expression in Egyptian Females with Type II D.M and Breast Cancers.

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Abstract

Introduction: Breast cancers are the most common cancers affecting females worldwide; the incidence of breast cancers new cases was more than 1,700,000 cases per year. Moreover**,** breast cancers are responsible for about 700,000 deaths worldwide every year, (Ferlay, et al., 2013). **Aim of work:** this study aimed to study the relationship between type II diabetes mellitus and breast cancers among Egyptian females. **Patient and methods:** Our study included 53 Egyptian females with breast cancers that prepared for surgical mastectomy either in Minia university hospital or Minia oncology center. Participants of this study were arranged into two groups according to D.M status: Group (I): Type II diabetic Egyptian females with breast cancer & Group (II): Non-diabetic Egyptian females with breast cancer. Multiple breast tissue biopsies obtained from malignant breast tissues and adjacent normal tissues obtained from free margins then fixed and stained with IHC stain to assess: Breast Tissue Estrogen Receptors (ER), Progesterone Receptors (PR), Chemerin expression and IGF-1 receptors expression **Results:** The current study disclosed that Egyptian diabetic females with breast cancers are pre-sented with higher breast cancers TNM scoring; larger tumor size (p value = 0.03), higher incidence of L.N metastasis (p value = 0.02), and higher incidence of distant metastasis (p value = 0.01) when compared with their non-diabetic counterparts. In addition, this study showed that Egyptian diabetic females with breast cancers are more frequently presented with more aggressive breast cancers phenotypes with significantly higher tumor grades (p value = 0.001), higher incidence of ER negative (p value = 0.01), and PR negative breast cancers (p value = 0.03) when compared with their non-diabetic counterparts. These data reflect the poor prognostic effect of type II diabetes mellitus on outcomes of breast cancers among Egyptian females. **Conclusions:** IGF-1R Over-expression was an independent risk factor for development and progression of breast cancers among Egyptian females.

Key words: Cancer breast, Diabetes Mellitus, IGF-1R Expression

Introduction

Breast cancers are the most common cancers affecting females worldwide; the incidence of breast cancers new cases was more than 1, 700,000 cases per year, (Ferlay, et al., 2013).

Moreover, breast cancers are responsible for about 700,000 deaths worldwide every year, (Ferlay, et al., 2013).

In addition, breast cancers are the most frequent cancers among Egyptian females accounting for 32.04 % of all incident cancers that affecting Egyptian females with crude incidence rate about 35.8 / 100,000 females, (Ibrahim, et al., 2014).

Approximately 16% of patients with breast cancers are suffering from type II diabetes mellitus, (Wolf, et al., 2011).

Patients & Methods

Our study included 53 Egyptian females with breast cancers that prepared for surgical mastectomy either in Minia university hospital or Minia oncology center. All participants were informed about the aim of this work and expressed their agreement for participation in this study.

Participants of this study were arranged into two groups according to D.M status: **Group (I):** Type II diabetic Egyptian females with breast cancer **& Group (II):** Non-diabetic Egyptian females with breast cancer**.**

All participants were subjected to the following measures: Height, Weight and Body Mass Index , Hemoglobin A_1C , Fasting blood glucose level, Complete blood counts, Liver and Renal function tests,

The diagnosis of breast cancer was confirmed by multiple breast tissue biopsies, while the staging of breast cancer was determined according to the TNM system based on definitions and recommendations, ESMO, (Senkus, et al., 2015). The diagnosis of Diabetes Mellitus was defined according to ADA, 2017 guidelines

The Immunohistochemistry Assay: Multiple breast tissue biopsies obtained from malignant breast tissues and adjacent normal tissues obtained from free margins then fixed and stained with IHC stain to assess: Breast Tissue Estrogen Receptors (ER), Progesterone Receptors (PR), Chemerin expression and IGF-1 receptors expression. Immediately after excision, tissue samples were fixed in 10% buffered formaldehyde solution; and embedded in paraffin blocks, which are then sectioned into slices (usually 4-10µm) using a microtome. These sections were transferred to glass slides for further processing. Histopathological examination of these sections was based on the WHO classification of breast cancers.

The Immunohistochemistry Staining: IHC staining of Chemerin, IGF-1R, ER, and PR expression was carried out using 5-µm consecutive tissue sections. These sections were de-waxed in xylene and rehydrated in graded alcohols, then a formalin fixed paraffin embedded breast tissue sections is heated for 7 min at high fire via microwave to retrieve in sodium citrate buffer (pH 6.0). Then the sections were incubated with the primary antibodies at 4 °C overnight, primary antibodies targeting the Chemerin, IGF-1R, ER, and PR expression.

After that, the streptavidin-biotin-peroxidase complex was applied to slides in order to reveal antibody-antigen reactions. Then the slides embedded in a solution containing the secondary antibodies which linked to horse-radish peroxidase enzyme that is capable of converting 3, 3' di-amino-benzidine (the coloring reagent which added later on) into brown precipitates that are deposited in the tissues at the site of the antigen antibodies reactions.

This brown stain was interpreted and scored under light-microscopy using a four-point scale based on intensity of the brawn stain as well as the percentage of cells that trapped this brawn stain. 0 (No Staining), 1+ (Weak Staining), 2+ (Moderate Staining) while 3+ (Strong Staining)

Statistical Analysis

The PASW version 24.0 software (IBM Co., Armonk, NY, USA) was used for the statistical analyses. Continuous variables were reported as means ± standard deviation. Comparisons between the diabetic and non-diabetic groups as regards continuous variables were performed using independed samples t-test. Comparisons between Chemerin and IGF-1R expression in normal versus malignant tissues were analyzed using chi-square test and mann-whitney test. P-value < 0.05 was considered as statistically significant".

Results:

Table-1.The Pathological Findings among Diabetic Versus Non-Diabetic Patients:

	D.M	N	Mean	Std. D	P-Value
Tumor Size	Non-Diabetic	28	4.321	3.6	0.004
	Diabetic	25	6.132	4.3	
L.N Mets	Non-Diabetic	28	10.57	10.189	0.006
	Diabetic	25	18.84	9.254	
Distant Mets	Non-Diabetic	28	1.12	0.8	0.001
	Diabetic	25	1.63	0.34	

Table-2, significant differences between diabetic & non-diabetic groups as regards Tumor Size, L.N and Distant Metastasis

Table (2) ER, PR Expression and tumor grading among Diabetic Versus Non-Diabetic Patients:

ANOVA-test Statistics		Non-Diabetic		Diabetic		P-value
		N	%	N	%	
ER Expression	(-) Expression	3	10.7 %	12	48.0%	0.045
	(+) Expression	25	89.3 %	13	52.0%	
PR Expression	(-) Expression	8	28.6 %	23	92.0%	0.001
	(+) Expression	20	71.4 %	10	40.0%	
Tumor Grading	Grade-II	27	96.4%	16	64.0%	0.02
	Grade-III	1	3.6%	9	36.0%	

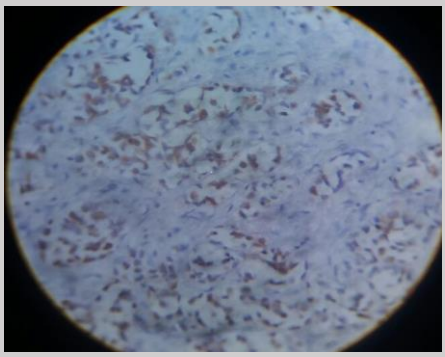
Table (3): disclosed significant differences between diabetic & non-diabetic groups as regards ER & PR Expression, Tumor Grading

Table (3): Comparison OF IHC findings among Diabetic & Non-Diabetic Patients:

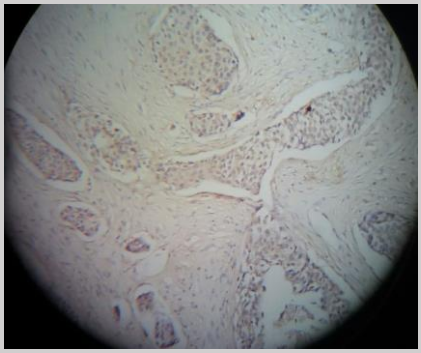
t-test Statistics	D.M	N	Mean	S.D	P-value
% IGF.1R (Nor mal Tissues)	Non-Diabetic	28	6.07	10.6	0.24
	Diabetic	25	12.0	17.7	
% IGF.1R (Malignant Tissues)	Non-Diabetic	28	49.1	34.1	0.03
	Diabetic	25	66.2	28.2	

Table: GF-1R Expression in malignant tissues were significantly different in both diabetic and non-diabetic groups of patients.

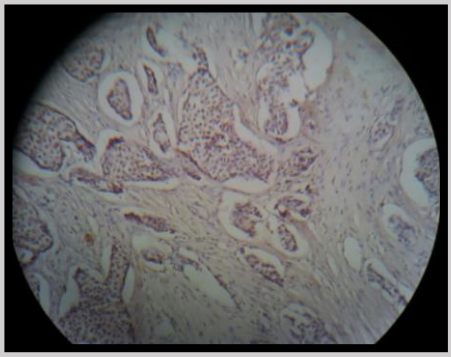
Fig 1: IHC Scoring of Chemerin (cytoplasmic staining) Vs. IGF-1R (membranous staining)



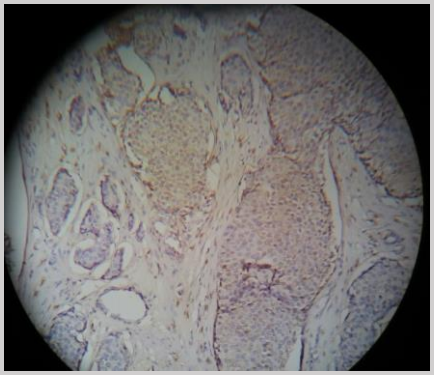
(+) CHEMERIN EXPRESSION



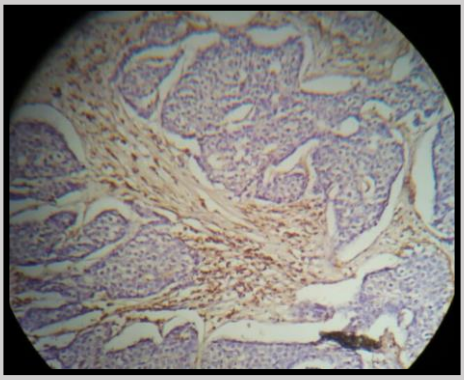
(++) CHEMERIN EXPRESSION



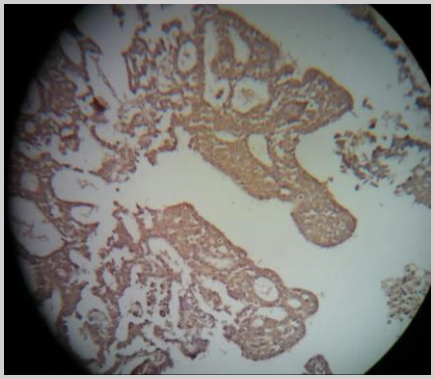
(+++) CHEMERIN EXPRESSION



(+) IGF-1R EXPRESSION



(++) IGF-1R EXPRESSION



(+++) IGF-1R EXPRESSION

Discussion

IGF-I stimulation contributes to breast cancers progression via its mitogenic and anti-apoptotic effects on the mammary epithelial cells and additionally protects breast cancers cells from the toxic effects of radio- and chemotherapy (Hankinson, 2008).

Women with high cytoplasmic levels of IGF-1R in epithelial cells found in benign terminal duct lobular units (TDLU's) biopsies have up to 15 times increased Breast cancers incidence (Tamimi, et al., 2011). Insulin-like growth factor-1 receptor (IGF-1R) activation results in apoptosis blockade and tumor growth, (Xiao, et al., 2016).

Our results found that there was significant increase in expression of IGF-1R in malignant breast tissues compared with non-malignant tissues with p value (0.01).

Also, malignant breast tissues in diabetic patients significantly express more IGF-1 receptors compared with non-diabetic patients, (p = 0.03).

The IGF-1 receptors overexpression are significantly associated with higher breast cancer TNM staging; larger tumor size (P-value = 0.004), higher rate of L.N metastasis (P-value = 0.006), higher incidence of distant metastasis (P-value < 0.001).

This study explored that IGF-1R overexpression are significantly associated with higher breast cancer grades (P = 0.02), and higher incidence of ER negative (P value = 0.045) and PR negative breast cancers (P-value = 0.01).

In our work, the multiple logistic regression analysis identified IGF-1R over-expression as an independent risk factor for development and progression of breast cancers among Egyptian females with (P-value < 0.001).

These results signify that overexpression of IGF-1 receptors in malignant tissues conveys poorer prognosis in patients with breast cancers even after omission of the effect of type II diabetes mellitus.

Our results are in consistency with Shimizu, et al., (2004) observed that IGF-IR overexpression was more predominant in malignant breast biopsies compared with benign breast biopsies. Furthermore, upon cancer progression, metastatic stages associated with higher levels of IGF-1R, (Panagiotis, et al., 2015).
Also, we are in agreement with Yue, et al., (2015); identified IGF-1R overexpression as a marker of an aggressive breast cancers phenotype.

Also, high levels of IGF-1R were identified as independent predictors for poor overall survival, high rate of lymph nodes invasion and high incidences of triple negative breast cancers, (Yue, et al., 2015).

This study was in agreement with (Pollak, et al., 2008); found that IGF-1R is implicated in tumor-genesis of breast cancers. IGF-1R is often expressed in breast cancers, and their overexpression has been associated with worse prognosis and shorter disease-free survival.

In the same way, we are in agreement with Sachdev, (2008) confirmed that IGF-1R activation increases motility and metastasis of breast cancers independently of tumor size.

Similarly, we are in consistency with Yue, et al., (2015); disclosed that IGF-IR stimulation leads to aggressive, fast growing, metastasizing tumors.

Pizon, et al., (2013) and Munagala, et al., (2011) demonstrated that IGF-IR play an important role in determining how aggressive circulating tumor cells are and their ability to grow with subsequent adhesion to form metastatic deposits.

Malignant breast tissues are significantly express higher levels of IGF-1R. Activation of IGF-1R plays a crucial role in breast cancer progression and metastasis, (Aaltonen, et al., 2013).

High levels of IGF-IR were detected in 100% of the triple negative breast cancers (Sarfstein, et al., 2006). In addition, high serum IGF-I/IGF-IR levels were significantly associated with reduced overall survival independent of other clinicopathological features, (Yue, et al., 2015).

Serum IGF-IR levels were demonstrated to be significantly lower in patients who experienced complete or partial responses to breast cancer treatment compared with non-responders, (Yue, et al., 2015).

Nevertheless, Targeting IGF-IR expression and degradation is therefore a potential therapeutic strategy for the treatment of breast cancer, (Pizon, et al., 2013).

Also, Gibson, et al., (2007) and Jackson et al., (2001) observed that IGF-1 signaling enhances motility of cancer cells in vitro and increases tumors capability for metastasis.

Indeed, this is also true and observed in other malignancies such as neuroblastomas and rhabdomyosarcomas, (Van Golen, et al., 2006).

There were numerous lines of evidence supporting our results (Sachdev , 2008), (Dearth, et al., 2006) & (Burtrum, et al., 2003) divulged that blockade of IGF-1 and insulin signaling pathways inhibit growth and metastasis in multiple cancers including breast cancers, both in vitro and in vivo.
On the other hand, some studies demonstrate that IGF-I is also able to increase cell differentiation in certain cancer cell lines that are associated with less aggressive types of cancer and hence improved prognosis, (Panagiotis, et al., 2015).
Whereas IGF-1R is highly expressed in patients with early Breast cancers and overall

positively associated with good prognostic variables, (Yerushalmi, et al., 2012).

Additionally, (Mountzios, et al., 2013) found a good positive association between IGF-1R and better clinical outcomes in ER positive, HER2 negative tumors.

Whereas IGF-1R is highly expressed in patients with early breast cancers and overall positively associated with good prognostic variables, (Yerushalmi, et al., 2012).

The inconsistency of results between these studies and our study could be explained by the following:

- IGF-1R may have different prognostic impact in different Breast cancers subtypes; (Panagiotis, et al., 2015).
- IGF-1R has been associated with favorable outcome in patients with the luminal B Breast cancers molecular subtype, in contrast to HER2 enriched patients, (Yerushalmi, et al., 2012).
- IGF-1R has been associated with favorable outcomes in patients with ER positive breast cancers compared with poor outcomes in patients with ER negative breast cancers.

LIMITATIONS OF THIS STUDY:

- Multicenter researches with longer follow-up interval are needed to assess the impact of diabetes on survival outcomes among Egyptian females with breast cancers.
- This study did not adjust for some confounding factors such as duration of D.M, the use of insulin versus other oral antidiabetic medication and intensive glycemic control was not taken into account to analyze the impact of diabetes on breast cancer prognosis.

References

1. Aaltonen KE, Rosendahl AH, Olsson H, Malmström P, Hartman L, Fernö M. Association between insulin-like growth factor-1 receptor (IGF1R) negativity and poor prognosis in a cohort of women with primary breast cancer. BMC Cancer. 2014;14:794.
2. Farabaugh M. Susan, Boone N. David, Lee V. Adrian: Role of IGF1R in Breast Cancer Subtypes, Stemness, and Lineage Differentiation. J Front Endocrinol (Lausanne). 2015; 6: 59.
3. Fleming ST, Rastogi A, Dmitrienko A, et al. A comprehensive prognostic index to predict survival based on multiple comorbidities: A focus on breast cancer. Med Care. 1999;37:601–614.
4. Fu P, Ibusuki M, Yamamoto Y, Hayashi M, Murakami K, Zheng S, et al., Insulin-like growth factor-1 receptor gene expression is associated with survival in breast cancer: a comprehensive analysis of gene copy number, mRNA and protein expression. Breast Cancer Res Treat. 2011;130:307–17.
5. Hankinson SE. Circulating levels of sex steroids and prolactin in premenopausal women and risk of breast cancer. Adv Exp Med Biol. 2008; 617: 161–169.
6. Kimberly S. Peairs, Bethany B. Barone, Claire F. Snyder, et al., Diabetes Mellitus and Breast Cancer Outcomes: A Systematic Review and Meta-Analysis. J Clin Oncol. 2011; 29(1): 40–46.
7. Liao S. , Li J , Wang L., Zhang Y., et al., Type 2 Diabetes Mellitus and Characteristics of Breast Cancer in China Asian Pacific J Cancer, 2011, 11, 933-937.
8. Lipscombe LL, Goodwin PJ, Zinman B, et al., The impact of diabetes on survival following breast cancer. Breast Cancer Res Treat. 2008; 109: 389–395.
9. Mountzios G, Aivazi D, Kostopoulos I, Munagala R, Aqil F, Gupta RC. Promising molecular targeted therapies in breast cancer. Indian J Pharmacol. 2011;43:236–245. doi: 10.4103/0253-7613.81497.
10. Panagiotis F Christopoulos, Pavlos Msaouel, Michael Koutsilieris: The role of the insulin-like growth factor-1 system in breast cancer. Mol Cancer. 2015; 14: 43.
11. Peairs K, Barone B, Snyder C, Yeh H, Stein K, Derr R., et al., Diabetes

mellitus and breast cancer outcomes: a systematic review and meta-analysis. J Clin Oncol 2011; 29: 40–46.
12. Pizon M, Zimon DS, Pachmann U, Pachmann K. Insulin-like growth factor receptor I (IGF-IR) and vascular endothelial growth factor receptor 2 (VEGFR-2) are expressed on the circulating epithelial tumor cells of breast cancer patients. PLoS One. 2013;8: e56836. doi: 10.1371/journal.pone. 0056836.
13. Shimizu C, Hasegawa T, Tani Y, Takahashi F, Takeuchi M, et al., Expression of insulin-like growth factor 1 receptor in primary breast cancer: immunohistochemical analysis. Hum Pathol. 2004;35:1537–1542. doi: 10. 1016/j.humpath.2004.09.005.
14. Tamimi RM, Colditz GA, Wang Y, Collins LC, Hu R, Rosner B, et al., Expression of IGF1R in normal breast tissue and subsequent risk of breast cancer. Breast Cancer Res Treat. 2011; 128:243–50.
15. Vermeulen JF, Kornegoor R, van der Wall E, van der Groep P, van Diest PJ. Differential expression of growth factor receptors and membrane-bound tumor markers for imaging in male and female breast cancer. PLoS One. 2013; 8:e53353.
16. Xiao-Bo Zhao, and Guo-Sheng Ren; Diabetes mellitus and prognosis in women with breast cancer; A systematic review and meta-analysis. J Medicine (Baltimore). 2016; 95(49): e5602.
17. Yerushalmi R, Gelmon KA, Leung S, Gao D, Cheang M, Pollak M, et al., Insulin-like growth factor receptor (IGF-1R) in breast cancer subtypes. Breast Cancer Res Treat. 2012;132:131–42.
18. Yue Mei Xu, Hong-Jiang Wang, Fang Chen, Wan-Hua Guo, et al., HRD1 suppresses the growth and metastasis of breast cancer cells by promoting IGF-1R degradation. J Oncotarget. 2015; 6(40): 42854–42867.