Research Article

Impact of type II Diabetes Mellitus on Outcomes of Breast Cancers among Egyptian Females.

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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:** our results clarified that there were significant differences between diabetic and non-diabetic groups as regards Tumor Size (p= 0.004), L.N Metastasis (p= 0.006), Distant Metastasis (p= 0.003) which signifying a worse TNM score among diabetic patients compared with non-diabetic patients. ER Expression in Malignant Tissues (p= 0.045), PR Expression In Malignant Tissues (p= 0.001), Tumor Grading (p= 0.02). **Conclusions:** the preexisting D.M coveys a worse prognostic outcomes among diabetic compared with non-diabetic egyptia females with breast cancers.

Key Words: Cancer breast, Diabeties Mellitus, IHC

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 obtained from breast tissue samples. 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 (0, 1+, 2+ and 3+) 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):demographic & clinical data of studied groups				
	D.M	N	Mean	P-value
Age	Non-Diabetic	28	47.7	0.04
	Diabetic	25	56.2	
Weight	Non-Diabetic	28	70.5	0.01
	Diabetic	25	78.4	
Height	Non-Diabetic	28	157.1	0.21
	Diabetic	25	155.3	
BMI	Non-Diabetic	28	28.6	0.03
	Diabetic	25	32.5	
Fasting blood sugar	Non-Diabetic	28	95.3	0.01
	Diabetic	25	215.9	
Table (1): shows that diabetic patients presented with significantly higher age Age (P = 0.04), higher Weight (P = 0.01), higher BMI (P = 0.03), higher fasting blood sugar (P = 0.01), compared with non-diabetic patient's.				

Table(2): Pathological Findings among Diabetic Vs 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, revealed significant differences between diabetic and non-diabetic groups as regards Tumor Size (p= 0.004), L.N Metastasis (p= 0.006), Distant Metastasis (p= 0.003) which signifying a worse TNM score among diabetic patients compared with non-diabetic patients.

Table (13) ER, PR Expression and tumor grading in Diabetic Vs 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 regards ER expression (p= 0.045), PR expression (p= 0.001), tumor grading (p= 0.02).						

Discussion

This prospective case controlled study was designed to clarify the associations between type II D.M, Chemerin expression, IGF-1R expression with occurrence and progression of breast cancers among Egyptian females.

Our results revealed that Egyptian females with breast cancer and type II diabetes mellitus were presented with higher TNM scoring of breast cancer with larger tumor size (p value < 0.008), higher incidence of L.N metastasis (p value < 0.006), higher incidence of distant metastasis (p value < 0.001), higher tumor grading (p value < 0.02), higher incidence of ER negative (p value < 0.045), and higher incidence of PR negative breast cancers (p value < 0.001) when compared with those without D.M.

These data reflect the poorer prognostic effect of type II diabetes mellitus on outcomes of breast cancers among Egyptian females.

These results are in consistence with Hardefeldt, et al., (2012) who confirmed that diabetes mellitus has been identified as an independent risk factor for development and progression of breast cancers.

Similarly, Zhihua, et al., (2011) disclosed that diabetes mellitus is a risk factor for poor prognosis of breast cancer patients.

In addition, De Bruijn, et al., (2013) conducted a well-designed meta-analysis revealing that women with diabetes mellitus had a 23% greater risk of subsequent breast cancers compared with those without diabetes**.**

Likewise, Larsson, et al., (2007) concluded that patients with breast cancer and diabetes has been associated with adverse outcomes throughout the full course of disease i.e., initial presentation, treatment, recurrence patterns, and mortality**.**

Also, Zhihua, et al., (2011) diclosed that diabetes mellitus was a poor prognostic factor in women with breast cancers, and hyperinsulinemia might be an important reason for poor prognosis of breast cancer in such patients.

However, Novosyadly, et al., (2010) released that hyperinsulinemia and insulin-like growth factors, even without diabetes mellitus, may play roles in promoting breast cancer development and progression,

Kimberly, et al., 2011 supposed that the poor influences of type II diabetes mellitus on outcomes breast cancers were attributed to:

1. Hyperinsulinaemia related to underlying insulin resistance might stimulate tumor growth. Insulin may work directly on epithelial cells or indirectly by activating insulin-like growth factor pathways.
2. Women with diabetes may receive less aggressive treatment, including chemotherapy, radiotherapy, and/or surgery. This may be related to their underlying comorbidities precluding treatment options or a perceived risk of toxicity from therapy in patients with diabetes.
3. Women with pre-existing diabetes may have a greater risk of chemotherapy-related toxicity (e.g., infection, fever, and neutropenia); such risk might explain and justify less aggressive treatment.

Type II D.M induces meaningful changes in several hormonal systems such as IGFs, estrogens and other cytokines that may affect breast cancers risk, (Lawlor, et al., 2004).

Diabetes can directly influence breast cancer prognosis by altering hyperinsulinemia and insulin-like growth factors, endogenous sex hormones, and inflammatory markers, (Erickson, et al., 2011).

In addition, breast cancer patients who are diabetics have a 32% increased risk of chemotherapy-related complications and a 24–61% increased risk of all-cause mortality compared to breast cancer patients without D.M, (Srokowski, et al., 2009).

Lorincz and Sukumar, (2006), have proposed several hypotheses to explain the association between type II diabetes mellitus and breast cancers:

1. The underlying obesity (most type II D.M patients are actually obese) which results in hyper-estrogenemia via peripheral aromatization of androgens in adipose tissue,

2. The metabolic syndrome, results in hyper-insulinemia with relatively overexpression of insulin receptors and insulin-like growth factor (IGF-1), which act as mitogens,

3. Adipokines such as leptin, chemerin and Adiponectin with their autocrine, paracrine, and endocrine roles that associated with initiation and progression of breast cancer.

Limitations of the present study:

- First, our findings were based tissue findings and IHC only, it was better to reinforce this work with serum assay of IGF-1, Chemerin, and insulin level applied to larger number of patients for better correlation between tissues and serum findings.
- Further multicenter researches with longer follow-up interval are needed to assess the impact of diabetes on survival outcomes among Egyptian females with breast cancers.
- Finally, this study did not adjust for some confounding factors such as duration of D.M, the use of insulin versus other oral anti-diabetic medication and intensive glycemic control was not taken into account to analyze the impact of diabetes on breast cancer prognosis.

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