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ORIGINAL ARTICLE

Expression of IGFBP-rP1 in ovarian and breast cancers in association with diabetes mellitus status

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Abstract

Introduction: Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) is an important component of the IGF system that regulates insulin resistance-related to tumour development. The aim of this study is to investigate the expression of IGFBP-rP1 among female cancer patients who are known or not known to have Type 2 Diabetes Mellitus (T2DM). *Materials and Methods:* Using a cross-sectional design, cases of ovarian and breast cancer with clinical status of T2DM were selected over a 10-year period in Hospital Universiti Sains Malaysia. Immunohistochemical staining for IGFBP-rP1 was performed on paraffin-embedded tissues and the results were correlated with the patient's demographic and clinicopathological data. *Results:* A total of 152 breast cancer patients were recruited into the current study with 33.5% (51/152) patients were positive T2DM. Most of the breast cancer patients with T2DM were IGFBP-rP1-negative (66.7%, 34/51). The IGFBP-rP1 expression was significantly difference between breast cancer subjects with and without T2DM ($p<0.001$). There was no significant association of IGFBP-rP1 expression with data on the demographic and clinicopathological profiles of patients with breast cancer. Meanwhile, positive IGFBP-rP1 expression was evident in 44 out of 108 (40.74%) ovarian cancer cases. Among these cases, 36 were T2DM. In contrast to breast cancer cases, IGFBP-rP1 was mostly expressed among ovarian cancer patients with T2DM (66.7%, 24/36, $p < 0.001$). However, the -positive expression was not significantly associated with any sociodemographic and clinicopathological features of ovarian cancers. *Conclusions:* Majority of breast cancer patients with T2DM did not express IGFBP-rP1. In contrast, majority of the ovarian cancer patients with T2DM expressed IGFBP-rP1.

Keywords: Breast cancer, ovarian cancer, IGFBP-rP1, T2DM, insulin, IHC

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) and cancer are two common diseases worldwide. The prevalence of T2DM is increased over the years with 5.4% of world population with T2DM is projected in 2025.[1] Recent studies suggest that T2DM is positively correlated with both the risk of cancer and cancer-related mortality.[2-5] A significant association between female cancers such as breast, endometrial and ovarian carcinomas with T2DM has been reported.[6-11] The incidence of T2DM and cancer in developing countries are higher as compared to well developed countries.[12,13] Kelantan, a state in the north eastern part of Peninsular Malaysia is showing more than 100% increase in cancer prevalence, which includes female cancers over a period of 20 years.[14,15] The increasing trend in cancer incidence is in tandem with the increase in prevalence of diabetes in this region. It has been reported that Kelantan state has the highest prevalence of diabetes in Malaysia.[16] Our previous study has shown the relationship between T2DM and colorectal cancer among Kelantan population.[17] However, the prevalence of breast and ovarian carcinomas among Kelantanese women who are diabetics are still unknown.

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A mechanism known as insulin resistance has been studied to implicate diabetes associated increased risk and progression of cancer.[18] The mechanism is mediated by the insulin/ insulin-like growth factor (IGF) axis, which was overexpressed in cancer cells.[19] The role of hyperinsulinemia, a hallmark of insulin resistance has been extensively investigated as a key event for diabetes and cancer patients.[20] The persistence hyperinsulinemia has been hypothesised to be responsible for cancer formation by inhibiting apoptosis and increasing proliferation of damaged cells.[20] In addition, chronic hyperinsulinemia-related IGF-1 signalling leads to cancer formation.[21] However, the mechanisms by which this occurs are not well understood.

The upregulation of IGF-1 has been shown to increase cell proliferation and inhibit apoptosis of damaged cell via stimulation of Bcl-2 gene.[22] The activation of IGF receptors is ligand dependent, which is facilitated by IGF binding proteins.[23,24] Among the IGF binding proteins, the insulin-like growth factor binding protein related protein 1 (IGFBP-rP1) has relatively high affinity to bind with insulin receptor.[25] It competes with insulin to bind to insulin receptor and limits the response of insulin-mediated glucose uptake.[26] Decreased level of IGFBP-rP1 is associated with hyperinsulinemia, which is one of the major features of insulin resistance.[27]

In this study, we investigated the hypothesis that IGFBP-rP1-associated insulin resistance plays a key role in the development of female carcinomas by determining the expression of IGFBP-rP1 in breast and ovarian cancer patients with and without T2DM.

MATERIALS AND METHODS

Patients

Using a cross-sectional design, the ovarian and breast cancer cases with clinical status of T2DM were selected over a 10-year period in Hospital Universiti Sains Malaysia. Diabetic status and other clinical information were retrieved from the patients' medical records. Paraffin embedded tissue blocks of ovarian and breast cancers were archived in the Department of Pathology, Hospital USM. Cases with inadequate clinical history or sections were excluded. To protect confidentiality of patients, all tissue block and clinicopathological data were assigned with pathological number. The research was approved by the ethics committee of Universiti Sains Malaysia in compliance of the Declaration of Helsinki (USM/JEPeM/249.4 (4.4)).

Immunohistochemical staining for IGFBP-rP1

The paraffin blocks were sectioned at 3-5 μm of thickness and subjected to IHC staining using standard protocols. For antigen retrieval, the tissue sections were heated in Tris-EDTA, pH 9 (Dako, USA) using a pressure cooker for 3 minutes. The sections were cooled down gradually to room temperature and washed with running water. The sections were then incubated with 0.3% hydrogen peroxide (H202) (Dako, USA) for 5 minutes to neutralise the endogenous peroxidase activity and subsequently rinsed in distilled water. The sections were then incubated for an hour with a 1:200 dilution of mouse monoclonal against human IGFBP-rP1 (s-6064, Santa Cruz Biotechnology, USA) at room temperature. The sections were also applied with LSAB detection system to detect the bound primary antibody (Dako, USA) according to the manufacturer's instructions. Reaction products were visualised using 3, 3-diamiobenzidine (DAB) (Dako, USA). The sections were then washed in running water twice, and counterstained with 10-second dips in haematoxylin (BDH Chemical Ltd, England) followed by bluing in the ammonia water. They were then washed in running water for 3 to 5 minutes before commencement of dehydrating procedure. The sections were immersed in alcohol with gradual increased in concentration from 50 to 95%. Once ready, the sections were immersed in 3 changes of xylene and finally mounted with DPX (Sigma-Aldrich, USA). The sections were examined under light microscope (Nikon Eclipse E600, Japan).

Staining characteristic and scoring

The IHC scoring was from 0 (negative) to 3+ (high positivity) of cytoplasmic staining based on previous published study.[28] Score 0 and 1+ were considered as negative or low expression whereby score 2+ and 3+ were considered as positive or high expression.[28] Human kidney tissue was used as positive control.

Statistical Analysis

Data entry and analyses were done using SPSS version 22.0. For descriptive statistics, numerical data were reported as mean and SD while categorical data was reported as frequency and percentage. Chi square test, simple and multiple logistic regressions were used to determine the

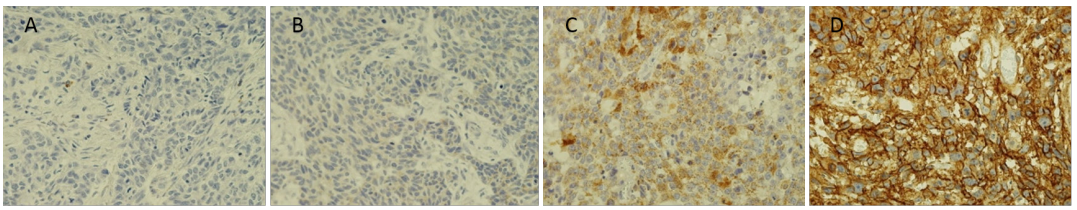

FIG. 1. IGFBP-rP1 IHC staining in breast carcinomas. (A-D) IGFBP-rP1 IHC staining showed negative to positive cytoplasmic reactivity in tumour cells (x200). The scoring for IGFBP-rP1 was given as 0 and 1+ for negative staining and 2+ and 3+ for positive staining.

association of sociodemographic and clinical characteristics with IGFBP-rP1 expression. P value of <0.05 was considered statistically significant.

RESULTS

IGFBP-rP1 expression in breast cancer

The scoring for IGFBP-rP1 expression of breast cancers ranging from 0 to 3 was designed according to its staining intensity (Fig. 1). A total of 152 breast cancer patients were recruited into this study with 33.5% (51/152) patients were positive T2DM. Overall, 11.2% (17/152) of breast cancer patients demonstrated positive expression. Majority of the breast cancer patients diagnosed with T2DM had more IGFBP-rP1-negative (66.7%, 34/51) than IGFBP-rP1-positive (33.3%, 17/51) (Table 1). The IGFBP-rP1 expression was significantly difference between breast cancer subjects with and without T2DM (p<0.001, Table 1).

In Table 1, the mean age of breast cancer that had positive IGFBP-rP1 expression was 54.82 years old. Unfortunately, we were unable to demonstrate any significant association of IGFBP-rP1 expression with other patients' outcomes e.g. menopausal status, histological type, tumour grade and tumour stage of breast cancers. In addition, the oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER2) and lymph nodes status of breast cancer were also not significantly associated with IGFBP-rP1 expression (Table 1).

IGFBP-rP1 expression in ovarian cancer

IGFBP-rP1 staining was performed in a total of 108 ovarian cancer cases. A similar scoring pattern of IGFBP-rP1 for breast cancer cases was applied to ovarian cancer cases (Fig. 2). 40.7% (44/108) ovarian cancer patients demonstrated positive IGFBP-rP1 expression. In our study, thirty six out of 108 (33.3%) ovarian cancer patients were positive T2DM (Table 2). IGFBP-rP1 was mostly expressed at 66.7% (24/36) of diabetic ovarian cancer patients (p<0.001, Table 2).

It was noted that the mean age of ovarian cancer with positive IGFBP-rP1 expression was 54.42 years old (Table 2). There was no significant association of IGFBP-rP1 expression with menopausal status, histological type, tumour grade and tumour stage of both ovarian and breast cancers. The association between IGFBP-rP1 expression with other ovarian cancer pathological features e.g. ovarian capsule and peritoneal metastases were also noted to be not significant.

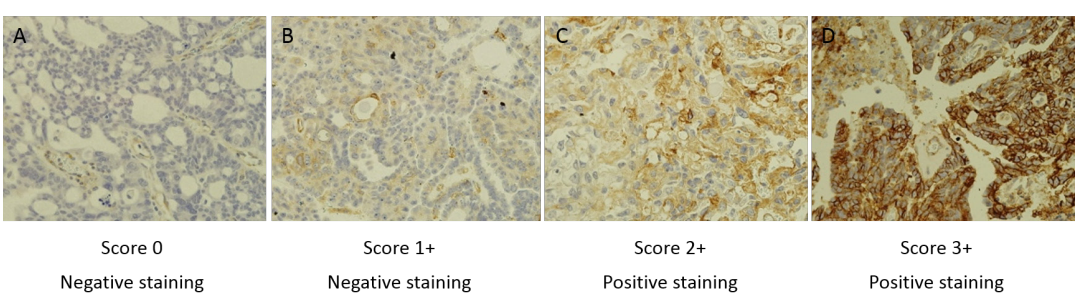

FIG. 2. IGFBP-rP1 IHC staining in ovarian carcinomas. (A-D) IGFBP-rP1 IHC staining showed negative to positive cytoplasmic reactivity in ovarian tumour cells (x200). The scoring for IGFBP-rP1 was given as 0 and 1+ for negative staining and 2+ and 3+ for positive staining.

TABLE 1: Association of demographic and clinical profile with IGFBP-rP1 expression among breast cancer patients using Chi-square test (n=152)

Demographic and clinical profile	IGFBP-rP1 staining		OR (95% CI)	X^2 value	P value
	Negative n (%)	Positive n (%)			
Age (years)	55.07 (10.64)	54.82 (8.70)			
Diabetes mellitus					
No	101 (100.0)	0 (0.0)	-	37.91	< 0.001*
Yes	34 (66.7)	17 (33.3)			
Menopausal status			1.47	0.55	0.457
Pre	68 (90.7)	7 (9.3)	(0.53, 4.10)		
Post	66 (86.8)	10 (13.2)			
Type of tumour					
IDC	11 (88.1)	16 (11.9)			
ILC	14 (100.0)	0 (0.0)	-	-	0.271
Other	3 (75.0)	1(25.0)			
Grade					
1	20 (90.5)	2 (9.1)			
2	49 (83.1)	10 (16.9)	-	3.30	0.192
3	66 (93.0)	5 (7.0)			
ER			1.01	0.00	0.990
-ve	57 (90.5)	6 (9.5)	(0.32, 3.17)		
+ve	66 (90.4)	7 (9.6)			
PR			0.96	0.00	0.948
-ve	64 (90.1)	7 (9.9)	(0.31, 3.03)		
+ve	57 (90.5)	6 (9.5)			
HER2					
0-1+	39 (86.7)	6 (13.3)			
2+	41 (95.3)	2 (4.7)	-	-	0.392
3+	42 (89.4)	5 (10.6)			
Stage					
1	12 (80.0)	3 (20.0)			
2	43 (87.8)	6 (12.2)	-	-	0.652
3	28 (90.3)	3 (9.7)			
4	52 (91.2)	5 (8.8)			
LN			1.51	-	0.744
No	35 (92.1)	3 (7.9)	(0.38, 6.04)		
Yes	62 (88.6)	8 (11.4)			

Note: Invasive lobular carcinoma (ILC), Invasive ductal carcinoma (IDC), estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER2), lymph nodes (LN), odd ratio (OR), confidence interval (CI), Chi-Square test (X^2), a significant P value (*).

DISCUSSION

The breast and ovarian cancers are the two commonest cancer diagnosed in Hospital USM.[14,15] Even though the state of Kelantan has the highest prevalence of diabetes in Malaysia,[16] the prevalence of cancer incidence among Kelantanese women who are diabetics are still unknown. To determine the association between T2DM status with breast and ovarian cancer cases, this study investigated the expression of IGFBP-rP1, an important IGF binding protein that plays a role in mediating insulin resistance and involves in cancer progression.

Of all breast cancer cases examined, 88.8% showed negative IGFBP-rP1 staining. This study is in agreement with Park et al.[29] that demonstrated low levels of the IGFBP-rP1 expression in breast cancer, suggesting that IGFBP-rP1 might play a role as a tumour suppressor gene. Furthermore, IGFBP-rP1 also showed low expression in several other cancer subtypes.[30-35] In this study, the lack of IGFBP-

TABLE 2: Association of demographic and clinical profile with IGFBP-rP1 expression among ovarian cancer patients using simple logistic regression (n=108)

Demographic and clinical profile	IGFBP-rP1 staining		B	OR (95% CI)	P value
	Negative n (%)	Positive n (%)			
Age (years)	52.95 (11.06)	50.45 (14.17)			
Diabetes mellitus					
No	52 (72.2)	20 (27.8)			
Yes	12 (33.3)	24 (66.7)	1.65	5.20 (2.19, 12.34)	< 0.001*
Menopausal status					
Pre	23 (52.3)	21 (47.7)			
Post	41 (64.1)	23 (35.9)	-0.49	0.6 (0.28, 1.34)	0.222
Type of tumour					
Serous adenocarcinoma	29 (52.7)	26 (47.3)			
Mucinous adenocarcinoma	10 (62.5)	6 (37.5)	-0.40	0.67 (0.21, 2.10)	0.491
Other	25 (67.6)	12 (32.4)	-0.63	0.54 (0.23, 1.28)	0.158
Grade					
1	8 (50.0)	8 (50.0)			
2	28 (66.7)	14 (33.3)	-0.69	0.50 (0.16, 1.61)	0.246
3	28 (56.0)	22 (44.0)	-0.24	0.79 (0.25, 2.43)	0.675
Stage					
1	14 (63.6)	8 (36.4)			
2	13 (56.5)	10 (43.5)	0.30	1.35 (0.41, 4.46)	0.627
3	24 (57.1)	18 (42.9)	0.27	1.31 (0.45, 3.80)	0.616
4	13 (61.9)	8 (38.1)	0.07	1.08 (0.31, 3.71)	0.907
Ovarian capsule					
Intact	22 (56.4)	17 (43.6)			
Ruptured	42 (60.9)	27 (39.1)	-0.18	0.83 (0.38, 1.85)	0.651
Peritoneal metastases					
No	29 (59.2)	20 (40.8)			
Yes	35 (59.3)	24 (40.7)	-0.01	0.99 (0.46, 2.15)	0.988

Note: Regression coefficients (B), Odd ratio (OR), confidence interval (CI), a significant P value (*).

rP1 expression was observed in majority of breast cancer patients with positive T2DM status (p<0.001, Table 1). This study showed that the IGFBP-rP1 expression was negatively expressed in all cases of invasive lobular carcinoma (ILC), a low-grade breast carcinoma subtype that generally has a favourable prognosis as compared to the IDC. In addition, negative IGFBP-rP1 expression was observed to be associated with higher histopathological grade and stage of invasive breast carcinoma as well as positive lymph node involvement. However, the statistical analysis failed to establish any significant association between IGFBP-rP1

expressions with those histopathological features of breast cancer.

The IGFBP-rP1 was positively expressed in 40.7% of ovarian cancer cases, which is comparable to those that have negative IGFBP-rP1 expression (59.3%, Table 2). Previously, increased expression of IGFBP-rP1 were demonstrated in women with ovarian clear cell adenocarcinoma (CCA)[36] and endometrial cancer[37], indicating a controversial role of IGFBP-rP1 among various type of cancer. In contrast to that observed in breast cancer cases, 66.7% of ovarian cancer cases with known positive for T2DM showed significant association with positive expression of IGFBP-rP1 ($p<0.001$).

In conclusion, the number of IGFBP-rP1 negative staining cases was more than positive staining cases in DM2-positive breast cancer patients. In contrast, majority of the ovarian cancer patients with positive T2DM were more often IGFBP-rP1-positive. Our findings suggest a possibility that IGFBP-rP1-associated insulin resistance may have important implications in tumour development of ovarian cancer following T2DM. Further investigations are required to investigate on how IGFBP-rP1-associated insulin resistance modulates tumour growth of ovarian cancer.

The limitation of this study is that only two sets of clinical ovarian cancer and breast cancer samples were available for IGFBP-rP1 immunostaining with the small number of patients involved. Therefore, it is hoped that a future study could be done in a larger cohort of tumour samples to further validate IGFBP-rP1 as a potential prognostic biomarker for ovarian and breast cancer associated with T2DM status. Our findings may contribute some inputs on the impact of insulin-associated diabetes in female cancer progression. However, this study is limited by possible confounding factors i.e. obesity, life style, exercise, food, parity and others.

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Conflict of interest: The authors declared no conflict of interest.

Authors' contribution statement: SNMN, NACJ and AHSA involved in data analysis and prepared the draft of manuscript. AAMN and NHO participated in study design. All authors agree with the final version of the article.

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