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Gene expression of Epithelial Membrane Protein 2 gene and β1-Integrin gene in patients with breast cancer

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ABSTRACT

Background: Breast cancer is the most common invasive cancer and the leading cause of cancer death in women. The function of over a thousand genes is reported as affected by genetic modifications in breast cancer.
Objectives: To study the gene expression of Epithelial Membrane 2 (EMP2) and β1-Integrin genes in patients with breast cancer.
Subjects and methods: This study was carried out by cooperation between the Biochemistry Division Department of Chemistry, Faculty of Science and Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University. This study included 120 subjects divided into 2 groups **Group I**: Included 60 women with breast cancer undergoing modified radical mastectomy. Tissue specimens were taken from the cancerous breast tissue and from the marginal healthy breast tissues. **Group II**: Included 60 age and sex-matched apparently healthy women served as a control group. All patients participants were subjected to full history taking, general clinical examination, abdominal ultrasound, CT-scan for abdomen, mammography, fine needle biopsy, histopathological examination, immunostaining of tissues, metastatic work up (chest x-ray and bone scan) and laboratory investigations including: Complete blood count (patients and controls), serum carbohydrate antigen 15–3 (patients and controls), detection of EMP2 and β1-Integrin genes expression in the tissue samples by formation of cDNA by reverse transcription PCR after RNA extraction and real-time PCR using SYBR Green technique.
Results: Compared to healthy tissues, the breast cancer tissues had significant higher EMP2 and β1-Integringene expression levels. Also, there was a significant increase in CA15-3 in patients group as compared with the control group. It was found that EMP2 and β1-Integrin expression in malignant tissue samples correlates with advanced and metastatic disease.
Conclusion: The gene expression of EMP2 and β1-Integrin are important markers for the severity of breast cancer and they are good indicators of its prognosis.

1. Introduction

Breast cancer is the most common invasive cancer and the leading cause of cancer death in women. It is the result of dysregulation of gene networks that maintain normal cellular functions and identity [1].

Breast cancer originates from the transformation of breast epithelial cells found either lining the milk ducts or in the milk-producing lobules of the breast. Lobules and ducts are formed from three lineages of cells in two layers: the myoepithelial layer is common to both structures and forms the basal layer, while ductal epithelial cells line the ducts and alveolar epithelial cells synthesize the milk within the lobules [2,3].

Epithelial membrane protein-2 (EMP2) is a member of the growth arrest-specific gene 3/peripheral myelin protein-22 (GAS3/PMP22) subfamily, which together with tetraspanins and connexins comprise three subfamilies of the large 4-transmembrane family EMP2 was identified as a novel prognostic indicator in a number of gynecological cancers [4]. Its expression is increased in the breast, ovarian and endometrial cancers in which it has been shown to correlate with poor survival and/or advanced disease [5]. Practically, the best-known tetraspan proteins are connexins, which form the major structural element

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Table 1
Statistical Comparison between patients and controls regarding demographic and laboratory parameters.

	Patients Group I (N = 60)		Control Group II (N = 60)		Test of sig.	P
	No.	%	No.	%		
Age (years) ≤50	36	60.0	38	63.0	χ^2 = 0.205	0.651
> 50	24	40.0	22	37.0		
Age (years)	Mean ± SD.		Mean ± SD.		t = 0.340	0.735
	50.6 ± 11.8		49.9 ± 10.8			
RBCs (× 10^6/μl)	4.13 ± 0.3		4.38 ± 0.49		3.565	0.001*
Hb (g/dl)	11.41 ± 0.92		12.16 ± 1.02		4.291*	< 0.001*
HCT (%)	34.85 ± 2.74		36.1 ± 2.86		2.436	0.016*
MCV (fl)	83.12 ± 5.13		82.7 ± 4.32		0.492	0.642
MCH (pg)	31.04 ± 6.04		31.35 ± 6.14		0.273	0.786
MCHC (g/dl)	32.75 ± 1.3		33.7 ± 1.0		4.498*	< 0.001*
WBCs (× 10^3/μl)	6.39 ± 1.74		5.99 ± 1.58		1.313	0.192
Platelets (× 10^3/μl)	231.33 ± 41.64		227.92 ± 37.9		0.470	0.639
CA 15–3(IU/ml)	35.29 ± 6.1		12.29 ± 7.46		U = 50.0*	< 0.001*

χ^2, p: χ^2 and p values for **Chi square test** for comparing between the two groups.
t, p: t and p values for **Student t-test** for comparing between the two groups.
U, p: U and p values for **Mann Whitney test** for comparing between the two groups.
*: Statistically significant at p ≤ 0.05.

Table 2
Comparison of relative quantitative (RQ) gene expression of EMP2 and β1-Integrin genes between healthy and malignant tissues.

genes	healthy tissue (no 60) Mean ± SD.	malignant tissue (no 60) Mean ± SD.	Z	P
RQ of EMP2	0.67 ± 1.36	8.59 ± 6.9	8.801	< 0.001*
RQ of β1-Integrin	0.81 ± 0.59	15.14 ± 12.34	9.45	< 0.001*

Z, p: Z and p values for **Wilcoxon signed ranks test** for comparing between normal and cancer.
*: Statistically significant at p ≤ 0.05.

Table 3
Correlation between different parameters for patients group (n = 60).

		Tumor Size	Grade	Stage	Carcinoma in situ	Multicentric
Age (years)	r	-0.211	-0.334	-0.173	-0.096	-0.147
	P	0.263	0.071	0.360	0.615	0.439
Affected lymph node	r	0.302	-0.083	0.236	-0.033	-0.042
	P	0.105	0.664	0.209	0.861	0.824
Molecular type	r	0.236	0.465*	0.000	0.136	0.000
	P	0.209	0.010*	1.000	0.472	1.000
Hb (g/dl)	r	-0.262	0.169	-0.436*	-0.035	0.440*
	P	0.163	0.372	0.016*	0.854	0.015*
WBCs (× 10^3/μl)	r	-0.245	-0.545*	-0.497*	0.085	-0.278
	P	0.193	0.002*	0.005*	0.657	0.137
Platelets (× 10^3/μl)	r	0.061	0.084	0.018	0.104	-0.042
	P	0.748	0.660	0.925	0.585	0.824
CA 15–3 (IU/ml)	r	0.279	-0.032	0.109	-0.391*	-0.003
	P	0.135	0.866	0.565	0.032*	0.988

r: Pearson coefficient, *: Statistically significant at p ≤ 0.05, molecular type (1:luminal,2:Triple negative,3:Her2 Positive).

of gap junctions. Connexins play vital parts in the regulation of cell growth and differentiation. Cancer cells generally have down-regulated levels of gap junctions. Moreover, numerous lines of evidence suggest that loss of gap junctional intercellular communication is an important step in carcinogenesis. Re-expression of connexins in cancer cells causes normalization of cell growth control and reduced tumor growth [6].

Integrins, which are transmembrane receptors in a large family of 18 α and 8 β heterodimeric transmembrane proteins, were well known as adhesion molecules in mediating cell-Extracellular matrix (ECM) interaction [7]. Integrins are important sensors of the cell micro-environment and regulate many intracellular and extracellular signaling pathways involving the organization of cells, tissues and organs during development in response to structural variations of the ECM. Integrins have been implicated in many processes associated with tumor cell adhesion to the ECM, including migration, invasion, and metastasis [8–10]. Integrins also play significant roles in regulating cell apoptosis-associated gene expression [11].

β1-Integrin is highly expressed in most tumors and is associated with a negative prognostic significance such as overall and disease-free survival, recurrence, and metastasis for head and neck and squamous cell carcinoma, melanoma, lung, breast, prostate, laryngeal and pancreatic cancers [12].

The aim of this work was to study the gene expression of Epithelial Membrane Protein 2 and β1-Integrin genes in patients with breast cancer.

2. Patients and methods

2.1. Patients

This study was carried out by cooperation between the Biochemistry

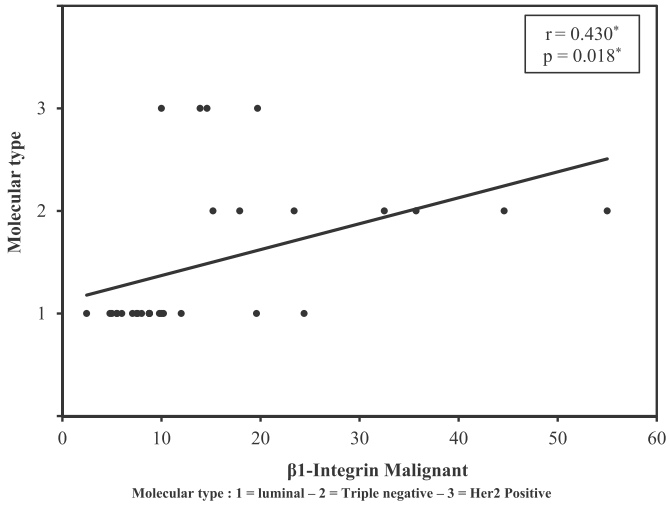

Fig. 1. Correlation between β1-Integrin in the malignant tissue and molecular type for patients (n = 60).

Division Department of Chemistry, Faculty of Science and Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University. It included 120 subjects divided into 2 groups **Group I:** Included 60 women with breast cancer undergoing modified radical mastectomy selected from the General Surgery Department, Menoufia University Hospital in the period from February to June 2017. Tissue specimens were taken from the cancerous breast tissue and from the marginal healthy breast tissues. **Group II**: Included 60 age and sex-matched apparently healthy women served as a control group.

2.2. Criteria of selection of patient

Proved breast cancer by mammography, ultrasound, breast MRI and fine Needle Aspiration cytology.

2.3. Exclusion criteria

Patients with preoperative chemo or radiotherapy. Patients diagnosed with previous breast tumors or with tumors located elsewhere. Informed written consent was obtained from all subjects who participated in this study. The protocol was approved by the Ethical Committee of Medical Research, Faculty of Science, Menoufia University.

2.4. All studied patients were subjected to the following

Full history taking: Including the family history of breast cancer, degree of relationship to a family member (first or greater), multiple cases in the family (particularly on one side): Age at the onset of breast cancer, bilateral disease, other related early-onset tumors (e.g. ovary, sarcoma). General clinical examination, abdominal ultrasound and CT-scan for the abdomen, mammography, fine needle biopsy and histopathological examination, metastatic workup: chest x-ray and bone scan. Laboratory investigations including: Complete Blood Count (CBC) (patients and controls), serum carbohydrate antigen 15-3 (CA15-3) level by enzyme-linked immunosorbent assay (ELISA) (patients and controls), pathological examination of tissue for grading, staging and detection of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu) and detection of EMP2 and β1-Integrin genes expression in cDNA samples prepared from RNA extracted from the malignant and the marginal benign tissue samples using real-time PCR using SYBR Green technique.

Complete blood count (CBC) was done Sysmex XN-1000 Automated Hematology Analyzer (Sysmex Corporation, Kobe 651–0073, Japan)

CA15-3 level was determined by ELISA, using. Human CA15-3 ELISA kit purchased from Monobind Company, China [13].

Two fresh parts of the excised mass were collected in 2 Eppendorf tubes one for malignant tissues and the other for adjacent healthy tissue and kept in −80 for further RNA extraction and an assay of EMP2 and β1-Integrin genes expression.

RNA Isolation from the tissue by (Direct –zol RNA Miniprep) kit Zymo Research.

Two-step RT–PCR was done as follows:

First Step - PCR: cDNA Synthesis (RT- Step): (QuantiTect Reverse Transcription Kit, Qiagen, Applied Biosystems, USA, 2012).

For reverse transcription step, samples were prepared in a final volume of 20 μl containing RT buffer, Multi scribe reverse transcriptase (PE Applied Biosystems), and 20 ng total RNA. Then the samples were incubated at 25 °C for 10 min and at 48 °C for 30 min. Heating to 95 °C for 5 min inactivated the reverse transcriptase on 2720 thermal cycler Singapore.

Second Step- PCR: cDNA Amplification with SYBR Green II with low ROX for detection of EMP2 and β1-Integrin genes expression: (QuantiTect SYBR Green PCR Kit, Applied Biosystems, USA).

Forward primers and reverse primers were used with SensiFAST™ SYBR® Lo-ROX Kit, nuclease-free water, cDNA in a total reaction volume 25 μl and using GAPDH as endogenous control using the 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA).

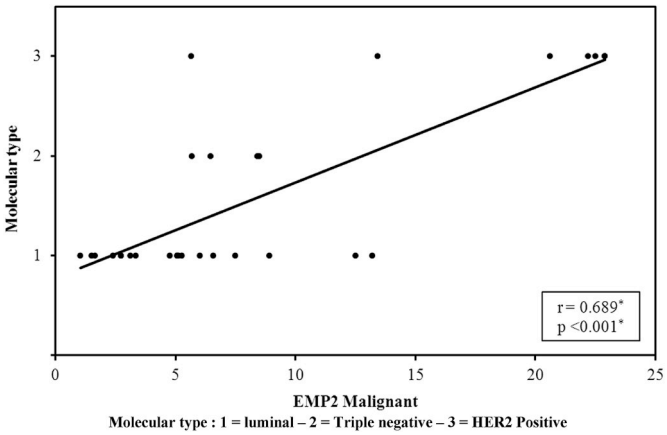

Fig. 2. Correlation between EMP2 in the malignant tissue and molecular type for patients (n = 60).

Forward and reverse primers of EMP2 were (5′-TCCTCTCCACCATTCTCT-3′) and (5′-AAACCTCTCTCCCTGCTTCA-3′) respectively [14].

Forward and reverse primers of β1-Integrin were (5′- TCACCACCCTTCGTGACAC-3′) and (5′-GAGATCCTGCATCTCGGAAG-3′) respectively [12].

Forward and reverse primers of GAPDH (endogenous control) were (5′-CCACTCCTCCACCTTTGAC-3′) and (5′-ACCCTGTTGCTGTAGCCA-3′) respectively [15].

Each primer was reconstituted by the addition of the labeled amount of TE buffer after centrifugation, the contents of the vial were mixed by vortexing. 2x QuantiTect SYBR Green PCR Master Mix, cDNA, primers, and RNase-free water were thawed and the individual solutions were mixed. The reaction mix was prepared for each primer in a separate well each with a total reaction volume of 25 μl.

Data analysis using Applied Biosystems 7500, software version 2.0.1. Using the comparative CT method.

Slices from the tumor mass were then immersed in formalin and were submitted to routine tissue processing ending with paraffin-embedded blocks formation. Tumors were graded according to the criteria of Nottingham modification in the Bloom-Richardson system [16]. Tumor staging was performed according to Tumor Node Metastasis (TNM) staging system [17].

2.5. Immunohistochemical (IHC) Staining

The method used for immunostaining was a streptavidin-biotin–amplified system. From each block, 4 μm thick sections were cut on positively charged slides, which were subjected to subsequent steps of deparaffinization, rehydration and antigen retrieval by boiling in citrate buffer saline (pH 6) followed by cooling at room temperature. The primary antibodies were incubated overnight at room temperature and they included ER (clone 1D5; Dilution, 1:50). (DakoCytomation), PR (clone IA6; Dilution, 1:50) (DakoCytomation) and HER2/neu (clone 250, Dilution, 1:100) (DakoCytomation). Breast cancer cases positive for ER, PR and HER2/neu were used as positive control slides. Negative control slides were also included in each run and prepared by the replacement of primary antibodies by the buffer solution. The secondary antibody was applied with diaminobenzidine as a chromogen substrate and Mayer's hematoxylin as a counterstain.

2.6. Immunostaining Interpretation

ER and PR were considered positive if ≥ 1% of tumor cell nuclei are immunoreactive [18]. HER2/neu immunoreactivity was evaluated

Table 4
Correlation between different parameters for patients group (n = 60).

		ER	PR	HER2neu	EMP2 Normal	EMP2 Malignant	β1- integrin Normal	β1- integrin Malignant
Age (years)	r	0.211	0.211	-0.246	0.285	-0.276	0.012	-0.252
	P	0.264	0.264	0.190	0.126	0.140	0.951	0.178
Affected lymph node	r	0.350	0.350	-0.205	0.178	-0.225	0.171	-0.028
	P	0.058	0.058	0.278	0.347	0.231	0.366	0.882
Molecular type	r	-0.914*	-0.914*	0.241	-0.397*	0.689*	-0.275	0.430*
	P	< 0.001*	< 0.001*	0.200	0.030*	< 0.001*	0.142	0.018*
Hb (g/dl)	r	0.154	0.154	0.198	-0.289	-0.144	0.072	-0.009
	P	0.415	0.415	0.295	0.122	0.449	0.704	0.963
WBCs (× 10^3/μl)	r	0.153	0.153	-0.072	0.264	-0.024	0.256	-0.006
	P	0.419	0.419	0.706	0.158	0.899	0.172	0.973
Platelets (× 10^3/μl)	r	-0.222	-0.222	-0.075	-0.079	0.269	0.066	0.180
	P	0.239	0.239	0.694	0.678	0.151	0.728	0.341
CA 15–3 (IU/ml)	r	-0.009	-0.009	-0.071	0.150	-0.197	0.050	0.037
	P	0.964	0.964	0.708	0.430	0.297	0.792	0.844

r: Pearson coefficient, *: Statistically significant at p ≤ 0.05.

Table 5
Univariate and multivariate analysis for the parameters affecting grade in patients group (n = 60).

Grade	Univariate		#Multivariate	
	P	OR (95%C.I)	P	OR (95%C.I)
Age (years)	0.072	0.90 (0.80–1.009)		
Stage$ (III)	0.616	1.40 (0.11–16.45)		
Affected lymph node	0.328	0.950 (0.811–1.11)		
Molecular type				
Luminal	0.023*	0.067 (0.006–0.690)	0.027*	0.043*(0.003–0.702)
Triple negative	0.102	5.0 (0.728–34.3)		
HER2 Positive	0.134	5.50 (0.59–51.2)		
Multicentric	0.999	–		
Carcinoma in situ	0.999	–		
ER	0.023*	15.0*(1.44–155.3)	0.027*	0.043*(0.003–0.702)
PR	0.023*	15.0*(1.44–155.3)	0.027*	0.043*(0.003–0.702)
HER2/neu	0.850	1.20 (0.182–7.9)		
Hb (g/dl)	0.652	1.299 (0.59–2.82)		
WBCs (× 10^3/μl)	0.004*	0.492 (0.23–1.01)	0.061	0.394 (0.149–1.042)
Platelets (× 10^3/μl)	0.560	0.999 (0.977–1.02)		
CA 15–3 (IU/ml)	0.978	0.984 (0.9–1.13)		
EMP2 (Normal)(> 0.22)	0.097	0.143 (0.014–1.41)		
EMP2 (Malignant) (> 5.84)	1.000	1.00 (0.167–5.98)		
β1-Integrin (Normal) (> 0.70)	0.998	–		
β1-Integrin (Malignant) (≥10)	0.998	–		

$: Reference (I + II), OR: Odd's ratio, C.I: Confidence interval.
#: All variables with p < 0.05 was included in the multivariate.
*: Statistically significant at p ≤ 0.05.

according to the American Society of Clinical Oncology guideline recommendations [19]. Positive HER2/neu cases were defined as 3 positivity (> 10% intense and complete staining); however, score 0 or 1 was considered negative.

According to the IHC results of ER, PR and HER2/neu, the cases were classified into;

- Luminal subtype: positive ER and/or PR and negative HER2/neu.
- HER2/neu positive subtype: negative ER, negative PR and positive HER2/neu.
- Triple-negative subtype: negative ER, negative PR and negative HER2/neu [20].

2.7. Statistical analysis

Results were collected, tabulated, statistically analyzed by IBM's personal computer and statistical package SPSS version 20. Two types of statistics were done. Chi-square test (x^2) is a test of significance used to study the association between two qualitative variables. Odd ratio, describes the probability that people who are exposed to a certain factor will have a disease compared between the two groups. Mann-Whitney test for abnormally distributed quantitative variables comparing between two groups.

Kruskal-Wallis test for abnormally distributed quantitative variables, to compare between more than two studied groups, P-value $^{<}$0.05 was considered statistically significant.

3. Results

There was no significant statistical difference in the age of patients group as compared with the control group. There was significant decrease in RBCs, Hb, HCT and MCHC in patients group as compared with the control group. While the non-significant decrease in MCH and non-significant increase in MCV, WBCs and platelets count. A significant increase in CA15-3 in patients group as compared with the control group (Table 1).

Regarding the EMP2 gene and β1-Integrin gene expression, there was a significant statistical increase of EMP2 gene and β1-Integrin gene expression in malignant tissues compared to healthy tissues (Table 2).

There was a significant positive correlation between grade and the molecular type and between multicentric and Hb, while there was a significant negative correlation between grade and WBCs, between

Table 6
Univariate and multivariate analysis for the parameters affecting EMP2 expression in malignant tissue samples (n = 60).

EMP2 malignant	Univariate		#Multivariate	
	P	OR (95%C.I)	P	OR (95%C.I)
Age (years)	0.152	0.953 (0.892–1.018)		
Affected lymph node	0.146	0.911 (0.803–1.033)		
Molecular type				
Luminal	0.014*	0.103 (0.017–0.628)	0.112	0.148 (0.014–1.56)
Triple negative	0.054	9.33 (0.958–90.94)		
Her2 Positive	0.304	3.50 (0.320–38.23)		
Multicentric	1.000	1.00 (0.167–5.98)		
Carcinoma in situ	0.999	–		
ER	0.014*	0.103 (0.017–0.628)	0.112	0.148 (0.014–1.56)
PR	0.014*	0.103 (0.017–0.628)	0.112	0.148 (0.014–1.56)
HER2/neu	0.705	1.333 (0.301–5.91)		
Hb (g/dl)	0.676	0.878 (0.477–1.617)		
WBCs ($\times 10^3$/μl)	0.923	0.980 (0.646–1.48)		
Platelets ($\times 10^3$/μl)	0.791	1.002 (0.985–1.02)		
CA 15–3 (IU/ml)	0.458	0.953 (0.841–1.08)		
EMP2 (Normal) (> 0.22)	0.715	0.766 (0.182–3.21)		
β1-Integrin (Normal) (> 0.70)	0.715	1.306 (0.311–5.48)		
β1-Integrin (Malignant) (≥10)	0.033*	5.50*(1.145–26.41)	0.639	1.667 (0.198–14.054)

$: Reference (I + II), OR: Odd's ratio, C.I: Confidence interval.
#: All variables with p < 0.1 was included in the multivariate.
*: Statistically significant at p ≤ 0.05.

Table 7
Univariate and multivariate analysis for the parameters affecting β1-Integrin expression in malignant tissue samples (n = 60).

β1-Integrin malignant	Univariate		#Multivariate	
	P	OR (95%C.I)	P	OR (95%C.I)
Age (years)	0.040*	0.927*(0.86–0.99)	0.084	0.934 (0.864–1.009)
Affected lymph node	0.655	1.025 (0.91–1.14)		
Molecular type				
Luminal	0.999	–		
Triple negative	0.999	–		
HER2 Positive	0.919	1.08 (0.24–4.79)		
Metacentric	0.283	0.357 (0.054–2.34)		
Carcinoma in situ	0.368	3.0 (0.27–32.7)		
ER	0.999	–		
PR	0.999	–		
HER2/neu	0.919	1.08 (0.24–4.79)		
Hb (g/dl)	0.202	0.658 (0.346–1.25)		
WBCs ($\times 10^3$/μl)	0.806	1.054 (0.69–1.60)		
Platelets ($\times 10^3$/μl)	0.534	1.006 (0.988–1.02)		
CA 15–3 (IU/ml)	0.221	1.09 (0.948–1.25)		
EMP2 (Normal)(> 0.22)	0.466	0.583 (0.137–2.48)		
EMP2 (Malignant) (> 5.84)	0.033*	5.50*(1.145–26.41)	0.076	4.522 (0.852–23.98)
β1-Integrin (Benign) (> 0.70)	0.466	0.58 (0.13–2.48)		

$: Reference (I + II), OR: Odd's ratio, C.I: Confidence interval.
#: All variables with p < 0.05 was included in the multivariate.
*: Statistically significant at p ≤ 0.05.

stage and Hb, between stage and WBCs and between carcinoma in situ and CA15-3 (Table 3).

There was a significant positive correlation between β1-Integrin in malignant tissue samples and molecular type (Fig. 1), and between EMP2 expression in malignant tissue samples and molecular type (Fig. 2), while there was a significant negative correlation between ER and molecular type, between PR and molecular type, and between EMP2 expression in healthy tissue samples and molecular type (Table 4).

Univariate analysis of the studied parameters the breast cancer grade was significantly increased with each of positive ER, PR, luminal type and WBCs, and the grade of breast cancer was non significantly increased with each of stage, triple-negative type, HER2 positive type, HER2/neu, Hb and EMP2 expression in malignant tissue samples. While in multivariate analysis of the studied parameters the breast cancer grade was significantly increased with each of positive luminal type, ER and PR (Table 5).

Univariate analysis of the studied parameters the EMP2 expression in malignant tissue samples of breast cancer was significantly increased with each of positive ER, PR, luminal type and β1-Integrin expression in malignant tissue samples, and the EMP2 expression in malignant tissue samples of breast cancer was non significantly increased with each of triple-negative type, HER2 positive type, HER2/neu, multicentric, platelets, and β1-Integrin expression in healthy tissue samples. While in multivariate analysis of the studied parameters the EMP2 expression in malignant tissue samples of breast cancer was non significantly increased with each of positive luminal type, ER, PR and β1-Integrin expression in malignant tissue samples (Table 6).

Univariate analysis of the studied parameters the β1-Integrin expression in malignant tissue samples of breast cancer was significantly increased with each of positive age and EMP2 expression in malignant tissue samples, and the β1-Integrin expression in malignant tissue samples of breast cancer was non significantly increased with each of carcinoma in situ, HER2 positive type, HER2/neu, WBCs, platelets and CA 15–3. While in multivariate analysis of the studied parameters the β1-Integrin expression in malignant tissue samples of breast cancer was non significantly increased with each of positive age and EMP2 expression in malignant tissue samples of breast cancer (Table 7).

4. Discussion

Breast cancer is characterized by molecular and histological heterogeneity. Although the diagnostic and prognostic factors related to breast cancer outcomes are being increasingly refined, there remains a need to improve on the specificity and sensitivity of prognostic markers which may impact the quality of life for breast cancer patients [21].

In the current study, there was a significant statistical difference between the two cancerous and normal studied groups as regards to the Hb level, HCT and MCHC, they were decreased in the cancer patients. While a non-significant difference existed as regards to RBC, MCV, MCH, WBC and platelets count.

This was in agreement with results reported by previous other studies of **Leonard et al. Chaumard et al. and Macciò et al.** [22–24].

A variety of factors are known to be involved in anemia development, and these relate directly to the tumor itself (blood loss, bone marrow infiltration or nutritional deficiencies) or to anticancer treatment [22].

In the current study, there was a significant statistical difference between the two studied groups as regards to the CA15-3 as it was elevated in cancer breast patients.

This result was in agreement with **Muthuswamy and Raste, (2000) and Hashim et al. (2014)** who reported an increased level of CA15-3 in breast cancer patients when compared to both women with benign tumor and healthy controls [25,26]. In addition, **Gautam et al. (2015)** found that there was a significant difference between benign and malignant breast lesions patients regarding serum CA 15-3 level, while there was a non-significant difference between benign breast lesions patients and controls [27]. Similarly, **Alobaidi et al. (2015)** reported that serum mean values of CA 15–3 were significantly higher in women with breast cancer than in controls [28].

On the other side, the studies of **Duffy et al., Kucera et al. and Daniele et al.** [29–31] found that CA15-3 lacked sensitivity for in situ or low stage invasive disease and lacked specificity for breast cancer preclude its use for detecting early breast cancer. Indeed, in patients with early or localized breast cancer, serum CA15-3 levels largely overlap those found in healthy women or those with benign breast disease.

In the present study, there was a significant increase in both EMP2 gene and β1-Integrin gene expression in malignant tissues as compared with healthy tissues. EMP2 is a 167-amino acid multi-pass membrane protein that contains four-transmembrane domains. EMP2 is a cancer-promoting protein, which is highly expressed in the malignancies derived from the epithelium and associated with tumorigenesis, in which triple-negative breast cancer (TNBC) is highly prominent**. Obermayr et al. (2010)** identified an EMP2 gene that can be used as one of the potential markers for the detection of circulating tumor cells (CTC) in the peripheral blood of patients with breast cancer [32]. **Fu et al. (2014)** discovered that EMP2 is a novel target in human breast cancer, and it was upregulated in 63% of invasive breast cancer and in 73% of TNBC tested. Similar to another study was found that EMP2 was highly expressed in over 70% of serous and endometrioid ovarian tumors compared to non-malignant ovarian epithelium using a human ovarian cancer tissue microarray [33]. Also **Chen et al. (2019)** reported that EMP2 is a potentially novel biomarker that can be used for capturing breast cancer cells and CTC in patient blood samples [34].

In the present study, there was a significant positive relation between the molecular type and each of EMP2 expression in malignant tissues and β1-Integrin expression in malignant tissue samples and a non-significant positive relation between the molecular type and each of EMP2 and β1-Integrin expression in healthy tissue samples.

Goodglick et al. (2012) EMP2 expression that is highly expressed in the majority of breast cancer tumors examined compared to the healthy mammary epithelium. In particular, high levels of EMP2 have observed in over 70% of TNBC cases examined [35].

Fu et al. (2014) examined the expression levels of EMP2 in breast cancer and found that it was expressed in 63% of invasive ductal carcinomas tested with low to minimal expression in normal mammary glandular and ductal cells. Of significance, greater than 70% of TNBC cases from two independent cohorts of patients expressed EMP2. Importantly, our results are concordant with several studies showing that EMP2 mRNA is upregulated in breast cancer and that its expression correlates with advanced and metastatic disease. Thus, its expression profile and localization on the plasma membrane make EMP2 an attractive target for passive immunotherapy with recombinant monoclonal antibodies [33].

β1-Integrin is a potential candidate biomarker of TNBC patients [36]. Reported that high β1-Integrin expression had a significant high metastatic stage, significant-high tumor recurrent rate, and significant low survival rate, compared to patients with low β1-Integrin expression. In addition, the average disease-specific survival time in patients with high β1-Integrin expression was significantly lower than that in patients with low β1-Integrin expression [37].

In the current study, univariate analysis of the studied parameters the β1-Integrin expression in malignant tissue samples of breast cancer was significantly increased with positive age and EMP2 expression in malignant tissue samples, and the β1-Integrin expression in malignant tissue samples of breast cancer was non significantly increased with Carcinoma in situ, HER2 positive type, HER2/neu, WBCs, platelets and CA 15–3. While in multivariate analysis of the studied parameters the β1-Integrin expression in malignant tissue samples of breast cancer was non significantly increased with positive age and EMP2 expression in malignant tissue samples of breast cancer.

Integrin expression modulates cell invasion and migration properties, which can enhance tumor aggression and growth [38,39]. Studies have found that integrins are partially controlled by members of the tetraspanin family [40]. Given that EMP2's amino acid sequence is 33–43% similar to that of the tetraspanins, it is possible that EMP2 may also influence integrin expression [41]. **Wadehra et al. (2002)** found that EMP2 and β1-Integrins expression are simultaneously expressed in 60% of NIHT3 fibroblast cells; thus, one well-supported hypothesis proposes that EMP2 regulates cell migration and invasion through β1-Integrins [42]. The influence of EMP2 on integrins has been validated in several studies including those by **Morales et al. (2009)** which showed a similar influence of EMP2 within ARPE-19 cells, a retinal pigmented epithelial cell line, and in studies by **Lesko et al. (2017)** in Madin-Darby Canine Kidney cells, a canine kidney epithelial cell line [43,44]. The presence of β1-Integrin residing on the cell surface has been observed to lead to changes in the surrounding ECM that further promotes tumor progression [42].

5. Conclusion

The gene expression of EMP2 and β1-Integrin are important markers for the severity of breast cancer and they are good indicators of its prognosis. When the gene expression of the EMP2 elevated in breast cancer tissue it is usually accompanied with an increase in β1-Integrin and CA 15-3.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2019.100708.

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