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Detection of HMGB1, RAGE and IL-33 Proinflammatory Cytokines in Serum of Breast Cancer Patients

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ABSTRACT

Breast cancer has been considered the most malignant neoplasm and the main cause of death among females worldwide. During 2020, there were approximately 2.26 million newly diagnosed breast cancer patients and 685 thousands deaths. This disease has revealed characteristics regarding role of HMGB1, RAGE and IL-33 cytokines in progression of breast cancer in patients. The present study aimed at investigating the levels of HMGB1, RAGE and IL-33 cytokines in patients serum. And evaluation their expression with the grad and stage of Breast cancer, and its role in the diagnosis and prognosis of Breast cancer in a group of 50 Iraqi women patients, in whom the Breast cancer has been already diagnosed and compared to a group of apparently healthy (control group N= 50) individuals. The by using ELISA technique was used for the HMGB1, RAGE and IL-33 cytokines in all members of the study. The median calculation of HMGB1 (pg/ml) were 434.99 and 132.27 among breast cancer patients and the healthy group respectively, with highly significant difference ($P<0.001$). The results indicated the mean of increased serum of HMGB1 in tumor growth of high levels in T4 size, compared to tumor sizes in patients' group, and it significantly increased with growth of tumor's grade and stage. The RAGE occurred in median concentration of 141.89 in patients' group and 61.73 in control group. It was significantly and highly different in the two groups when compared to each other ($P < 0.001$). There was a considerable association between mean levels of serum RAGE and tumor size and grade, which indicated that the mean of serum RAGE increased with the growth of tumor size by higher levels in T4 size, compared to T1, T2 and T3 size and higher levels in grade 3, compared to grade 1 and grade 2 in patients' group. However, there was an insignificant association between mean levels of serum RAGE and stage of cancer. For interleukin 33 (IL-33) assays, there was a median concentration of 9.74 in patients' group and 1.69 in control group. It was highly different in the two groups when compared to each other; with high significant difference ($P < 0.001$). The mean IL-33 serum levels increased significantly with the growth of tumor size, grade, and stage, compared to lower tumor sizes, grades and stages in breast cancer patients.

1. INTRODUCTION

Breast cancer (BC) is the most frequent cancer in women all around the world [1]. It is the most frequently diagnosed tumor and has the 6th highest malignancy-related mortality rate [2]. It is the second leading cause of cancer-related deaths amongst women in the United States, and morbidity and mortality of this disease increase each year [3]. The majority of deaths associated with the disease is due to metastases in the advanced stages, making early detection crucial. In last years, cancer biomarkers have become attractive due to their potential application in the early diagnosis of the cancer [4]. HMGB protein family contains three sub-types of proteins HMGB1, HMGB2 and HMGB3. HMGB1 protein was discovered in 1973 as DNA binding nuclear protein in calf thymus and all sub types of HMGB share common structure [5]. Clinically HMGB1 plays decisive role in various types of diseases such as autoimmune, infectious, and inflammatory such as cancer [6]. The high mobility group box 1 (HMGB1) protein is involved in apoptosis and immune responses because it can act as a ligand for the receptor for advanced glycation end products (RAGE) [7]. Some evidence suggests that DAMPs including HMGB1 may also have a key role in the development of cancer as well as in the host response to cytotoxic anti-tumor therapy. Moreover, it has been reported to work in the progression of inflammatory and autoimmune diseases [8]. It can play an important role as a cytokine which is involved in triggering the genesis of inflammation and inflammation related diseases including cancer by up-regulating the expression of other inflammatory cytokines [9]. HMGB1 promotes angiogenesis by binding to its receptor RAGE, activating NF-κB up-regulating leukocyte adhesion molecules and the production of proinflammatory cytokines and angiogenic factors in both hematopoietic and endothelial cells [10]. The HMGB1 levels in cancerous tissue could be correlated with lymphatic metastasis but were not associated with the age of patient and tumor size [11].

Receptor for advanced glycation end products (RAGE), member of the immunoglobulin superfamily, is normally involved in the regulation of tissue regeneration and resolution of inflammation. However, under pathological condition, activation of RAGE by some of its many ligands (not only advanced glycation end products, but also, for example, HMGB1 or S100 proteins) may induce diminished apoptosis, enhanced autophagy, and cell necrosis and, thus, contribute to the malignant transformation, cancer progression, and metastasis [12], [13]. The receptor for advanced glycation end products (RAGE) is highly expressed in various cancers and is correlated with poorer outcome in breast and other cancers [14]. RAGE has been implicated as a mechanism potentially driving breast cancer progression and metastasis [15]. Overexpression of RAGE in advanced-stage tumors may be a useful biomarker for diagnosis and the prediction of breast cancer progression [16]. High RAGE expression was observed in lymph node and distant metastases patient samples. In addition, high RAGE expression was associated with poor prognosis in BC [17]. It has also been well documented that RAGE ligands bind to RAGE and activate its downstream signaling mechanisms that sustain chronic inflammatory conditions, leading to neoplastic stage [18]. It is interesting to note that there is very low or no RAGE expression in normal tissues but enhanced expression in chronic inflammation and cancer. Although these features of RAGE make it an ideal candidate for therapeutic strategies against chronic inflammation, not much is known about its role in BC [19], [20].

Interleukin-33 (IL-33) is an important member of the IL-1 family, and in humans is expressed predominantly in skin, lung, adipocytes, and synovial fibroblasts [21]. IL-33 is constitutively expressed in many tissues and by a wide variety of cells. However, it is also induced in response to various stimuli in epithelial cells,

myofibroblasts, adipocytes, endothelial cells, smooth muscle cells, and macrophages predominantly as a pro-inflammatory cytokine [22], [23]. In response to cellular damage, tissue injury or viral infection, IL-33 is quickly released from the nucleus of necrotic cells and secreted into extracellular space where it can bind to the membrane bound ST2L receptor through its cytokine domain [24]. Several studies point a pro-tumorigenic role of IL-33 in breast cancer. IL-33 is elevated in human breast cancer tissue compared to normal breast tissue. In breast cancer patients, serum levels of IL-33 and of its decoy receptor sST2 were enhanced compared to healthy controls [25].

2. Materials and Methods

2.1 Subjects

Subjects that were enrolled in this study were categorized into two groups, first group composed of (50 females clinically diagnosed as Breast cancer at Specialized Oncology Center in Diwaniyah, with a range of (32) to (79) years, mean age is (49.66 ± 12.50) years whereas the healthy control group comprising of (50) females, with age range of (18-72) years, mean age is (46.96 ± 13.43) years. All patients were subject to a detailed history and clinical examination.

2.2 Blood Collection and DNA Extraction

Blood samples were collected by vein puncture. Three milliliters (ml) of venous blood, taken from each patient and controlled by vein puncture, using disposable syringes under aseptic condition. Three milliliters from each sample were transferred to 10 milliliters sterile Gel tube, and centrifuged at 3000 rpm for 5 minutes and the separated serum was divided into several Eppendorf tubes for the HMGB1, RAGE and IL-33.

2.3 The Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA assay was used for detection and measurement of concentrations in serum of HMGB1, IL-33 (Elabscience company/USA) and RAGE (BT LAB company/China) were used in this work. This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kits has been pre-coated with an antibody specific to HMGB1, IL-33 and RAGE.

2.4 Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation, range, median and interquartile range (IQR) after performance of Kolmogorov- Smirnov normality test and making decision about normally and non-normally distributed variables. Mann Whitney U test was used to study differences in mean rank between any two groups provided that the variable was non-parametric. On the other hand independent samples t-test was used to study the difference in mean between any two groups provided that the variable is normally distributed.

Chi-square test was used to study the association between any two categorical variables. Odds ratio and 95% confidence interval was estimated to measure risk. Pearson's correlation coefficient was used to study correlation between numeric variables. Receiver operator characteristic curve analysis was used to find out the proper cutoff values and further analysis of sensitivity and specificity was carried out accordingly. The level of significance was considered at P-value of 0.05 or less and highly significant level at 0.01 or less [26].

3. Results

The present study was designed to evaluate the role and expression of HMG1, RAGE and IL-33 in the diagnosis and prognosis of Breast cancer in Iraqi women patients compared to the normal population by using

ELISA.

3.1 Demographic characteristics Profile

The demographic characteristics of patients and control subjects are shown in table (1), there was no significant difference between patients and control subjects in mean age. According to weight, the present study indicate the weight mean of patients significantly higher than the weight mean of control subjects. The presence of family history is an important contributory factor in breast cancer Table (2). The comparison of some variables such as: menarche age, parity (parous) or (nulliparous), No of children among parous women and menopausal age, between patients and control groups had been carried out and the results were demonstrated in table (3). The clinical feature accompanying breast cancer are shown in table (4) include the frequency distribution of patients according to the location of breast cancer, the rates of chronic illnesses including diabetes mellitus and systemic hypertension. And estrogen receptor, progesterone receptor and HER 2.

The comparison of serum HMGB-1 level between patients with breast cancer and control groups has been carried out and the results shown in table (5), median levels of serum HMGB-1 in patients with breast cancer were higher in comparison with the median levels of control groups, the difference was highly significant ($P < 0.001$).

The mean levels of serum HMGB-1 according to tumor characteristics are shown in table (6). The results of present study indicated non-significant association between mean levels of serum HMGB-1 and tumor size, although the mean of serum HMGB-1 increased with increasing the size of tumor with high levels in T4 size in compared to T1, T2 and T3 size in patients group. The mean levels of serum HMGB-1 significantly increased with increasing tumor grade. Higher levels were in grade 3 compared with grade 1 and grade 2. The present results indicated highly significant association between mean levels of serum HMGB-1 and stage of cancer, were the levels increased with increasing the stage with high levels in stage 4 in compared to stage 1, stage 2 and stage 3.

The comparison of serum RAGE level between patients with breast cancer and control groups had been carried out and the results are shown in table (7), median levels of serum RAGE in patients with breast cancer were higher than in comparison the median levels of control groups, the difference was highly significant ($P < 0.001$).

The mean levels of serum RAGE according to tumor characteristics are shown in table (8). The results of present study indicated significant association between mean levels of serum RAGE and tumor size, and the mean of serum RAGE increased with increasing the size of tumor with higher levels in T4 size in compared to T1, T2 and T3 size in patients group. Also the mean levels of serum RAGE significantly increased with increasing tumor grade, were higher levels in grade 3 in compared to grade 1 and grade 2. But the present results indicated non-significant association between mean levels of serum RAGE and stage of cancer, although the levels increased with increasing the stage with higher levels in stage 4 in compared to stage 1, stage 2 and stage 3.

A comparison of serum IL-33 level between patients with breast cancer and control groups was carried out and the results are shown in table (9). Median levels of serum IL-33 in patients with breast cancer were higher than in comparison the median levels of control groups, the difference was highly significant ($P < 0.001$).

The mean levels of serum IL-33 according to tumor characteristics are shown in table (10). The results of

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present study indicated significant association between mean levels of serum IL-33 and tumor size, were the mean of serum IL-33 increased with increasing the size of tumor with higher levels in T4 size in compared to T1, T2 and T3 size in patients group (p= 0.003). Also the mean levels of serum IL-33 significantly increased with increasing tumor grade, were higher levels in grade 3 in compared to grade 1 and grade 2 (p= 0.001). Also the present results indicated significant association between mean levels of serum IL-33 and stage of cancer, were the levels increased with increasing the stage with higher levels in stage 4 in compared to stage 1, stage 2 and stage 3 (p= 0.008).

Table 1: Demographic characteristics of patients with breast cancer and control subjects.

Characteristic	Patients *n* =50	Control *n* = 50	*P*
Age (years)			
Mean ±SD	49.66 ± 12.50	46.96 ± 13.43	0.301 † NS
Range	32.00 – 79.00 years	18.00 – 72.00 years	
< 30, *n* (%)	1 (2.0 %)	3 (6.0 %)	0.292 ¥ NS
30-39, *n* (%)	7 (14.0 %)	13 (26.0 %)	
40-49, *n* (%)	20 (40.0 %)	15 (30.0 %)	
≥ 50, *n* (%)	22 (44.0 %)	19 (38.0%)	
Weight (kg)			
Mean ±SD	80.10 ± 9.34	74.66 ± 13.42	0.021 ¥ S
Range	60.00– 100.00 years	54.00 – 105.00 years	

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at P > 0.05.

Table 2: Distribution of patients and control subjects according to family history.

Family history	Cases *n* = 50		Control *n* = 50		χ^2	*P*
	N	%	*n*	%		
Positive	7	14.0	0	0.00	7.527	0.006 ¥ S
Negative	43	86.0	50	100.0		

n: number of cases; ¥: Chi-square test; S: significant at P > 0.05.

Table (3): Distribution of breast cancer patients and healthy controls according to the some variables.

	Case – control comparison		
	Patients **n= 50**	***control subjects*** **n= 50**	***P***
Menarche age			
Mean± SD	12.78 ± 1.26	12.92 ± 1.14	**0.563 †** **NS**
Range	11.00 – 16.00	11.00 - 15.00	
SE	0.179	0.161	
Parity (Parous) or (Nulliparous)			
Parous, *n* (%)	45 (90%)	27 (54%)	**< 0.001 ¥** **HS**
Nulliparous, *n* (%)	5 (10%)	23 (46%)	
No of children among parous women			
≤ 3, n (%)	29 (64.5%)	16 (59.0%)	**0.909 ¥** **NS**
4-6, *n* (%)	10 (22.2%)	7 (26.0%)	
≥ 7, *n* (%)	6 (13.3%)	4 (15.0%)	
N	45 (100%)	27 (100%)	
Menopausal age			
Premenopausal, *n* (%)	33 (66.0%)	43 (86.0%)	**< 0.001 ¥** **HS**
Postmenopausal, *n* (%)	17 (34.0%)	7 (14.0 %)	

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; HS: Highly significant at P ≤ 0.001.

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Table (4): Frequency distribution of some associated Clinical features in patient with breast cancer.

Characteristic	Patients	
	N	%
Location of breast cancer		
Right, *n* (%)	27	54.0 %
Left, *n* (%)	23	46.0 %
Duration of breast cancer (Months)		
Mean± SD	4.98 ± 3.49	
Range	2.00 – 12.00	
SE	0.493	
Diabetic		
YES, n (%)	8	16.0 %
NO, *n* (%)	42	84.0 %
Hypertension		
YES, n (%)	3	6.0 %
NO, *n* (%)	47	94.0 %
Estrogen Receptor		
Positive, *n* (%)	32	64.0 %
Negative, *n* (%)	18	36.0 %
Progesterone Receptor		

Positive, *n* (%)	31	62.0 %
Negative, *n* (%)	19	38.0 %
HER 2		
Positive, *n* (%)	17	34.0 %
Negative, *n* (%)	33	66.0 %

n: number of cases.

Table (5): Median levels of Serum HMGB-1 in patients with breast cancer and control subjects.

	Case – control comparison		
HMGB-1 (pg/ml)	**Patients n = 50**	**Control n = 50**	***P***
Range	155.31– 538.32	37.02 – 277.57	< 0.001 † HS
Median (IQR)	434.99 (90.98)	132.27 (98.94)	

n: number of cases; IQR: inter-quartile range; †: Mann Whitney U test; HS: Highly significant at P ≤ 0.001

Table (6): Distribution of mean serum HMGB-1 level according to Tumor characteristics

HMGB-1 levels					
Tumor Size					
	T1	**T2**	**T3**	**T4**	***P***
Mean± SD	372.26 ± 122.44	390.6 ± 81.11	433.9 ± 60.75	467.17± 38.2	**0.115 † NS**
Range	155.31-500.34	205.45- 493.06	271.60 – 538.32	435.3- 515.81	
N	9	20	16	5	
Tumor Grade					
	G1	**G2**	**G3**		***P***
Mean± SD	335.39 ± 131.46	415.72 ± 73.55	432.68 ± 55.63		**0.028 † S**
Range	155.31-500.34	205.45 -538.32	328.23 -515.81		
N	8	30	12		
Stage of cancer					
	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	***P***

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Mean± SD	249.72 ± 88.05	386.24 ± 106.08	417.92 ± 53.95	481.40 ± 45.89	**< 0.001 † HS**
Range	155.31 - 363.51	201.24 - 500.34	271.60 -480.80	435.33 - 480.8	
N	4	11	31	4	

†: Anova test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

Table (7): Median levels of Serum RAGE in patients with breast cancer and control subjects.

	Case – control comparison		
***RAGE* (pg/ml)**	**Patients n = 50**	**Control n = 50**	***P***
Range	64.64 – 223.21	4.79 – 139.74	< 0.001 † HS
Median (IQR)	141.89 (49.61)	61.73 (27.10)	

n: number of cases; IQR: inter-quartile range; †: Mann Whitney U test; HS: Highly significant at P ≤ 0.001

Table (8): Distribution of mean serum RAGE level according to Tumor characteristics.

RAGE levels					
Tumor Size					
	T1	**T2**	**T3**	**T4**	***P***
Mean± SD	100.99 ± 30.15	135.21± 39.68	145.59 ± 39.26	147.76 ± 48.69	**0.047 † S**
Range	64.64-160.68	90.83 – 223.21	78.36 – 218.34	78.63 - 223.21	
N	9	20	16	5	
Tumor Grade					
	G1	**G2**	**G3**		***P***
Mean± SD	104.38 ± 29.79	134.35 ± 54.14	149.15 ± 36.21		**0.014 † S**
Range	64.64 -160.68	78.36 -223.21	88.71 -223.21		
N	8	30	12		
Stage of cancer					
	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	***P***
Mean± SD	114.51± 31.26	129.07 ± 26.28	143.96 ± 58.69	144.76± 47.74	**0.301 † NS**
Range	64.64 -160.68	78.36- 148.03	78.36 -223.21	79.54- 223.21	
N	4	11	31	4	

†: Anova test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

Table (9): Median levels of Serum IL-33 in patients with breast cancer and control subjects.

	Case – control comparison		
***IL-33* (pg/ml)**	**Patients n = 50**	**Control n = 50**	***P***
Range	1.69– 29.43	0.24 – 10.78	< 0.001 † HS
Median (IQR)	9.74 (10.09)	1.69 (1.21)	

n: number of cases; IQR: inter-quartile range; †: Mann Whitney U test; HS: Highly significant at P ≤ 0.001

Table (10): Distribution of mean serum IL-33 level according to Tumor characteristics.

IL-33 levels					
Tumor Size					
	T1	**T2**	**T3**	**T4**	***P***
Mean± SD	5.97 ± 2.64	11.34 ± 6.30	11.56 ± 8.35	21.94 ± 7.51	**0.003** † **S**
Range	3.13-11.03	1.69– 22.49	1.76– 29.43	11.51- 29.43	
N	9	20	16	5	
Tumor Grade					
	G1	**G2**	**G3**		***P***
Mean± SD	7.25 ± 6.06	10.32 ± 7.47	18.85 ± 12.94		**0.001** † **S**
Range	2.60-21.66	1.69– 29.43	5.79-29.43		
N	8	30	12		
Stage of cancer					
	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	***P***
Mean± SD	4.42 ± 1.08	10.59 ± 7.99	10.91 ± 6.9	21.93 ± 7.57	**0.008** † **S**
Range	3.13-5.33	1.76- 21.66	1.69-29.43	11.51- 29.43	
N	4	11	31	4	

†: Anova test; HS: Highly significant at P ≤ 0.001; NS: not significant at P ≤ 0.05.

4. Discussion

The immune response play an important role in prevention of both early and metastatic of breast cancer. Lymphocytes, including T cells, T reg cells and NK cells and their cytokine release patterns are implicated in both primary and secondary prevention (relapse or recurrence) of breast cancer. Several studies have

indicated that cancer prognosis in patients with breast cancer are related to immune system functional status [27]. The present study revealed that the level of immune marker, HMGB1 showed significantly increased among cases with breast cancer as median serum was (434.99 (90.98) pg/ml) when compared with healthy controls (132.27 (98.94) pg/ml), these results indicate significant association between HMGB1 and breast cancer ($p<0.001$). HMGB1 is a factor regulating malignant tumorigenesis, proliferation, and metastasis, and is associated with poor clinical pathology in various human cancers, and HMGB1 levels in the tissues and sera of patients with breast cancer were significantly higher than those in patients with benign breast disease or normal individuals [11]. Both pre-clinical and clinical studies have suggested that HMGB1 might be a useful target in the management of breast cancer [28]. HMGB1 can play an important role as a cytokine which involved in triggering the genesis of inflammation and inflammation related diseases including cancer by up-regulating the expression of other inflammatory cytokines.

In this study the serum HMGB1 levels were significantly higher in patients with breast cancer than normal healthy subjects. As findings suggest that serum HMGB1 levels can be used as a novel diagnostic marker because of HMGB1 is translocated to the cytoplasm and secreted by cancer cells. Consistence with this results, [11], documented that the HMGB1 levels in serum of the 55 patients with breast cancer, 25 patients with benign breast disease, and 30 healthy subjects were mean serum HMGB1 concentration in breast cancer patients was significantly higher than in healthy subjects. The results in table (6) suggested significant association between HMGB1 levels and tumor characteristics. The HMGB1 levels in cancerous tissue could be correlated with lymphatic metastasis but were not associated with the age of patient and tumour size [11]. The involvement of HMGB1 in cancer is complex, and intracellular/nuclear and extracellular forms of HMGB1 have been implicated in tumor formation, progression, and metastasis and in the response to chemotherapeutics [29]. And [30], who indicated High HMGB1 expression is connected to all the hallmarks of malignancy, such as unlimited replicative potential, ability to form vessels, avoiding apoptosis, tolerance towards growth-suppressors, invasion, inflammation, and metastasis, this suggests that HMGB1 may be a promising target for BC therapeutics. Also this results agree with [31], who reported that, HMGB1 is a promising candidate for diagnostic/prognostic marker and is innovative target for treating BC.

Previous studies have implicated importance of receptor for advanced glycation end products (RAGE) in the pathogenesis of various human disorders including cancers [19]. Table (7) showed that mean serum concentration of RAGE was highly significantly increased among cases with BC compared to apparently healthy controls. Higher serum concentration of RAGE may be due to its important role in pathophysiology of Breast cancer [32]. A study by [33], indicated that RAGE ligands are overexpressed in breast cancer. Meanwhile, a study by [17], demonstrated that RAGE plays a critical role in promoting breast cancer growth and metastasis, and RAGE is highly expressed in human TNBC and murine breast cancer cell. The results in table (8) showed positive correlation association between mean levels of serum RAGE and tumor size and grade, and Consistent with this results, [14] showed, RAGE drives tumor cell invasiveness and metastasis in human breast cancer models through tumor-intrinsic and non-tumor cell effects, and there is strong evidence that RAGE is a key mediator of breast cancer metastasis in the models evaluated and strongly implicate it as a mediator of metastasis in vivo, The present result consistent with [34], who reported that, high RAGE expression was observed in lymph node and distant metastases patients samples of BC. Also agree with [17], which investigated the correlation between RAGE level and BC score, who demonstrated that RAGE is highly expressed in basal-type breast cancer, especially TNBC, and is preferentially expressed in invasive and lymph node metastasis tissues. But this result disagree with the results of [32], who studied serum levels of sRAGE during the study were significantly lower in (only 9) patients who died of breast cancer compared to the patients in remission.

The present study showed in table (9) revealed that the median level of immune marker, IL-33 showed significantly increased among cases with BC when compared with healthy controls, these results indicate significant association between IL-33 and BC. IL-33 may exert a dual function, as damage-associated molecular pattern (DAMP) and cytokine or as nuclear factor modulating gene expression [35]. Previous studies demonstrated in breast cancer patients, serum levels of IL-33 and of its decoy receptor sST2 were enhanced compared to healthy controls [25]. Several studies point toward a pro-tumorigenic role of IL-33/ST2 signaling in breast cancer. IL-33 and ST2 expression are elevated in human breast cancer tissue compared to normal breast tissue [36]. The present results are similar to results of [37], which revealed that the concentrations of IL-33 were nearly twofold higher in the patients with BC, compared with patients with benign breast disease (BBD), and higher expression of IL-33 in carcinomas and adjacent tissues to tumors, compared with normal breast tissue from the same patients. The results of the study also agree with those of [38], who studied 100 cases diagnosed with BC, which showed that high levels of IL-33 are reported in patients with BC compared to healthy control.

Table (10) suggested significant correlation between IL-33 levels and tumor characteristics such as, tumor size, grade. The present results also indicated significant association between mean levels of serum IL-33 and stage of cancer. The results in this study Consistence with this results of [37], who found, the higher expression of IL-33 association with the IL-33/ST2 axis in progression and metastasis of BC, and the local expression of IL-33 may be an important marker for differentiating malignant from normal/benign tissues. IL-33 expression in adjacent tissues also tends to be higher compared to normal tissues. The serum levels of IL-33 increased with advanced stages. This results also agree with [38], who reported the mean serum levels of IL-33 in patients with stages I, II and III were also higher than the healthy control groups and the mean serum level of IL-33 in patients with tumor stage IV was significantly higher than controls and other stages. Moreover, IL-33 has been shown to be a potential prognostic biomarker and target for new therapeutic strategies [39].

5. Conclusion

In this study the presence of HMGB-1, RAGE and IL-33 assay yields a sensitive breast cancer marker as diagnostic methods for detection of breast cancer in apparently healthy subjects and monitor treatment response in patients, and also are associated with risk and prognosis of breast cancer. There is significantly a higher concentration of HMGB1 in breast cancer patients compared to control group were associated with increased risk of breast cancer and highly correlate with TNM stage of breast cancer, and consider as diagnostic marker. The RAGE significant increase was observed in breast cancer patients than healthy controls and also closely related to TNM stage, differentiation, and metastasis of breast cancer. High proportion of IL-33 level was found in the patients with breast cancer than control group and increased with advanced stages of breast cancer, which was significantly higher in patients with tumor stage four.

6. References

[1] Yoo J., Kim BS., Chung J., Yoon HJ., (2018). Clinical value of delayed 18F-FDG PET/CT for predicting nipple-areolar complex involvement in breast cancer: A comparison with clinical symptoms and breast MRI. PLoS One. 13: e0203649.

[2] van Rooijen JM., Qiu SQ., Timmer-Bosscha H., et al., (2018). Androgen receptor ex¬pression inversely correlates with immune cell infiltration in human epi¬dermal growth factor receptor 2-positive breast cancer. Eur J Cancer. 103: 52–60.

[3] Siegel R., Naishadham D., Jemal A., (2013). Cancer statistics. CA Cancer J Clin. 63(1): 11–30.

[4] Zhang Y., Jiao J., Yang P., Lu H., (2014). Mass spectrometry-based N-glycoproteomics for cancer biomarker discovery. Clin Proteomics. 11(1): 18.

[5] Tripathi A., Shrinet K., Kumar A., (2019). HMGB1 protein as a novel target for cancer. Toxicology Reports 6: 253–261.

[6] Harris H., Andersson U. and Pisetsky D., (2012). HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease, Nat. Rev. Rheumatol. 8: 195–202.

[7] Eisenberg T., Carmona-Gutierrez D., Büttner S., et al., (2010). Necrosis in yeast. Apoptosis.15: 257-68.

[8] Willis W.L., Wang L., Wada T.T., Gardner M., Abdouni O., Hampton J., Valiente G., Young N., Ardoin S., Agarwal S., Freitas M.A., Wu L.C., Jarjour W.N., (2018). The proinflammatory protein HMGB1 is a substrate of transglutaminase-2 and forms highmolecular weight complexes with autoantigens, J. Biol. Chem. 293: 8394–8409.

[9] Alok T., Kriti S. and Arvind K., (2019). HMGB1 protein as a novel target for cancer. Toxicology Reports 6:253–261.

[10] Van Beijnum JR., Buurman WA., Griffioen AW., (2008). Convergence and amplification of toll-like receptor (TLR) and receptor for advanced glycation end products (RAGE) signaling pathways via high mobility group B1 (HMGB1). Angiogenesis. 11: 91–99.

[11] Sun S., Zhang W., Cui Z., Chen Q., Xie P., Zhou C., Liu B., Peng X., Zhang Y., (2015). High mobility group box-1 and its clinical value in breast cancer, Oncol. Ther. 8: 413–419.

[12] Fritz G., (2011). RAGE: a single receptor fits multiple ligands. Cell. 36: 625–632.

[13] Ibrahim ZA., Armour CL., Phipps S., et al. (2013). RAGE and TLRs: relatives, friends or neighbours? Mol Immunol. 56: 739–744.

[14] Kwak T., Drews-Elger K., Ergonul A., et al., (2017). Targeting of RAGE-ligand signaling impairs breast cancer cell invasion and metastasis. Oncogene. 36(11):1559-1572.

[15] Kalea AZ., See F., Harja E., et al, (2010). Alternatively spliced RAGEv1 inhibits tumorigenesis through suppression of JNK signaling. Cancer Res. 70: 5628–5638.

[16] Nankali M., Karimi J., Goodarzi M., et al., (2016). Increased Expression of the Receptor for Advanced Glycation End-Products (RAGE) Is Associated with Advanced Breast Cancer Stage. Oncol Res Treat. 39:622-628.

[17] Nasser MW., Wani NA., Ahirwar DK., et al., (2015). RAGE mediates S100A7-induced breast cancer growth and metastasis by modulating the tumor microenvironment. Cancer Res. 75(6):974-85.

[18] Wang H., Zhang L., Zhang IY., Chen X., Da Fonseca A., Wu S., et al., (2013). S100B promotes glioma growth through chemoattraction of myeloid-derived Macr phages. Clin Cancer Res. 19: 3764–75.

[19] Rojas A., Figueroa H., Morales E., (2010). Fueling inflammation at tumor microenvironment: the role of multiligand/RAGE axis. Carcinogenesis. 31: 334–41.

[20] Riehl A., Nemeth J., Angel P., Hess J., (2009). The receptor RAGE: Bridging inflammation and cancer. Cell Commun Signal. 7: 12.

[21] Wood IS., Wang B., Trayhurn P., (2009). IL-33,arecentlyidentifiedinterleukin-1gene family member, is expressed in human adipocytes. Biochem Biophys Res Commun. 384(1): 105–9.

[22] Moussion C., Ortega N., Girard J., (2008). The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: A novel 'alarmin'? PLoS ONE 3:e3331.

[23] Haraldsen G., Balogh J., Pollheimer J., et al., (2009). Interleukin-33–cytokine of dual function or novel alarmin? Trends Immunol. 30: 227–233.

[24] Zhao W. and Hu Z., (2010). The enigmatic processing and secretion of interleukin-33. Cell. Mol. Immunol. 7: 260–262.

[25] Yang ZP., Ling DY., Xie YH., Wu WX., Li JR., Jiang J., et al., (2015). The association of serum IL-33 and sST2 with breast cancer. Dis Markers. 516895.

[26] Daniel W.W., (2009). Biostatistics: A Foundation for Analysis in the Health Sciences, John Wiley & Sons New York.

[27] Standish LJ., Sweet ES., Novack J., Wenner CA., Bridge C., Nelson A., Martzen M., Torkelson C., (2008). Breast cancer and the immune system. J Soc Integr Oncol. 6(4): 158-68.

[28] Sohun M. and Shen H., (2016). The implication and potential applications of high-mobility group box 1 protein in breast cancer. Ann Transl Med. 4(11): 217.

[29] Sparvero LJ., Asafu-Adjei D., Kang R., Tang D., Amin N., et al., (2009). RAGE (receptor for advanced glycation end products), RAGE ligands, and their role in cancer and inflammation. J. Transl. Med. 7:17.

[30] Han B., Jiang P., Li Z., et al., (2018). Coptisine-induced apoptosis in human colon can¬cer cells (HCT-116) is mediated by PI3K/Akt and mitochondrial-associated apoptotic pathway. Phytomedicine. 48: 152–60.

[31] He H., Wang X., Chen J., Sun L., Sun H., Xie K., (2019). High-Mobility Group Box 1 (HMGB1) Promotes Angiogenesis and Tumor Migration by Regulating Hypoxia-Inducible Factor 1 (HIF-1α) Expression via the Phosphatidylinositol 3-Kinase (PI3K)/AKT Signaling Pathway in Breast Cancer Cells. Med Sci Monit. 25: 2352-2360.

[32] Tesarova P., Zima T., Kubena AA., Kalousova M., (2017). Polymorphisms of the receptor for advanced glycation end products and glyoxalase I and long-term outcome in patients with breast cancer. Tumor Biology. 1–7.

[33] Kostova N., Zlateva S., Ugrinova I., et al., (2010). The expression of HMGB1 protein and its receptor RAGE in human malignant tumors. Mol Cell Biochem. 337: 251–258.

[34] Yin C., Li H., Zhang B., Liu Y., Lu G., Lu S., et al., (2013). RAGE-binding S100A8/A9 promotes themigration and invasion of human breast cancer cells through actin polymerization and epithelial-mesenchymal transition. Breast Cancer Res Treat. 142: 297–309.

[35] Cayrol C. and Girard J., (2014). IL-33: An alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. Curr. Opin. Immunol. 31: 31–37.

[36] Kim JY., Lim SC., Kim G., et al., (2015). Interleukin-33/ST2 axis promotes epithelial cell transformation and breast tumorigenesis via upregulation of COT activity. Oncogene. 34(38):4928–38.

[37] Liu J., Shen JX., Hu JL., et al., (2014). Significance of interleukin-33 and its related cytokines in patients with breast cancers. Front Immunol. 5:141.

[38] Jafarzadeh A., Minaee K., Farsinejad AR., et al., (2015). Evaluation of the circulating levels of IL-12 and IL-33 in patients with breast cancer: influences of the tumor stages and cytokine gene polymorphisms. Iran J Basic Med Sci. (12): 1189-98.

[39] Jorns JM., Healy P., Zhao L., (2013). Review of estrogen receptor, progesterone receptor, and HER-2/neu immunohistochemistry impact son treatment for a small subset of breast cancer patients transferring care to another institution. Arch Pathol Lab Med. 137(11):1660–3.