ORIGINAL ARTICLE

Immunological Status in breast Cancer Women's in Erbil Province-Iraq

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ABSTRACT

Breast cancer is cancer that forms in the cells of the breasts.Most women diagnosed with breast cancer are over the age of 50, but younger women can also get breast cancer. A total of 115 women patients with breast tumor (55 malignant and 60 benign) and 40 females as a control group. Epidemiology and risk factors were determined using questionnaire form. Different blood and immunological parameters were evaluated. Results indicated obvious relation between risk factor and breast cancer (BC), among these factors is the age; the most frequent age for breast cancer was that at age group (38- 47). The study showed, there was a marked increase in the total mean of WBC count, granulocytes, lymphocytes and monocytes in breast cancer and benign breast disease when compared with control group ($P < 0.05$). There was a significant increase in the concentrations of all the immunological parameters (IL- 6, IL- 8, IFN- γ) and tumor marker CA15- 3, the oxidative stress (Adenosine Deaminase) activity, IgG, IgM, IgA, C3 and C4 in patients in comparison with the control group.
Key words: Prevalence, Risk factor, Cytokine, CA15.3, ADA activity, breast cancer

INTRODUCTION

Cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018. Globally, about 1 in 6 deaths is due to cancer. Approximately 70% of deaths from cancer occur in low- and middle-income countries. (WHO, 2018). Breast cancer is the second most common type of cancer after lung cancer. One woman in ten will develop the disease and one in 29 will die as a direct result of it (Wernberg*et al.,* 2009). Generally, breast cancer refers to cancer originating from breast tissue (Martini *et al.,* 2006). In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one- third of the registered female cancers according to the latest Iraqi Cancer Registry (Alwan, 2016). The incidence of breast cancer in Erbil city was more common than the other type of cancers (Iraqi Cancer Registry, 2005). Breast cancer is a multifactorial disease and every woman is at risk for developing breast cancer. Several relatively strong risk factors for breast cancer have been known which include female gender, age, previous breast cancer, hereditary factors, early age at menarche, late age at menopause etc……) (Stuart, 1998; Ahmed *et al.,* 2006; Kamińska*et al.,* 2015).

During the development of cancer, the ability of the immune system to identify and destroy nascent tumors and thereby to function as a primary defense against cancer has been debated for many years. The functioning of both innate and the adaptive immune system plays a role in preventing relapse in women with breast cancer (Pandya*et al.,* 2016). Cytokines, signaling molecules that mediate and regulate immunity and inflammation, are an important component of the biological milieu associated with breast cancer. They have been used as biomarkers in research for prognosis and have been associated with symptoms and adverse outcomes in multiple conditions, including breast cancer (Ahmed *et al*., 2006 and Abbas *et al.*, 2015). Tumor markers are substances that can be found in the body when cancer is present. Elevated tumor markers level in blood are found in less than 10% of patients with early disease and in about 70% of patients with advanced disease (Duffy, 2006).

Adipose tissue is abundant in the breast cancer microenvironment; interactions with cancer cells create cancer-associated adipocytes which produce a variety of adipokines that influence breast cancer initiation, metastasis, angiogenesis, and cachexia. Interleukin (IL)-6 has emerged as key compound significantly produced by breast cancer cells and adipocytes, with the potential of inducing proliferation, epithelial-mesenchymal phenotype, stem cell phenotype, angiogenesis, cachexia, and therapeutic resistance in breast cancer cells (Gyamfi*et al.,* 2018). In breast cancer, the increase in adipocyte IL-6 secretion is regulated by paracrine interaction with tumor cells. The increased secretion of IL-6 in CAAs and the pleiotropic roles associated with IL-6 make the potential effects of adipocyte-derived IL-6 on breast cancer cells an interesting area of research (Lee *et al.,* 2017). Interleukin-8 (IL-8) is a chemokine that has an autocrine and/or paracrine tumor-promoting role and significant potential as a prognostic and/or predictive cancer biomarker (Todorović-Raković and Milovanović, 2013). IL-8 is highly expressed in ER− breast cancers, but it increases invasiveness and metastatic potential of both ER− and ER+ breast cancer cells. It is also highly expressed in HER2+ breast cancers. In addition to being elevated in the sera of cancer patients. Higher IL-8 levels are typically found in high-grade peritumoral fluids rather than low-grade tumors and benign conditions, with the exception of inflammatory processes indicating that local IL-8 production is related to malignant processes (Kotyza, 2012). The role of IFN-γ has been documented in both breast cancer pathogenesis and patients' response to treatments. IFN-γ has been initially recognized for its role in antitumor host immunity which is exerted through induction of Th1 polarization and activation of both cytotoxic T cells (CTLs) and dendritic cells (Mandai*et al.,* 2016). An ideal cancer marker for breast cancer would be clinically useful in many ways and, therefore, has been searched for decades (Fineke*t al.,* 2007). CA 15-3 is regarded as the most suitable cancer marker and therefore became established in the clinical routine worldwide. However, its sensitivity is still unsatisfactory in the early stages of primary breast cancer (Laessig*et al.,* 2007). The significance of ADA in the breast cancer development seems to be particularly important as its activity regulates the pool of intra- and extracellular adenosine, a key modulator of a cell function via adenosine receptor-dependentand independent mechanisms. It has been shown that both ADA isoenzymes were elevated in

tumor tissues of patients with breast cancer correlating with tumor grade, size and lymph node involvement (Mahajan*et al.,* 2013;Jafari*et al.,* 2017).

The present study was aimed to shed light on the prevalence, epidemiology, risk factors and immunological profile of breast tumor (malignant and benign), in female patients through the following parameters. White blood cell (WBC) and differential leukocyte count (DLC). Cytokines including: (Interleukin-6, Interleukin-8 and Interferon-gamma). Serum level of CA15-3.Serum adenosine deaminase activity.Immunoglobulins (IgG, IgA and IgM). Complement components (C3 and C4).

SUBJECTS AND MATERIALS

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Patients and Control group: The study was involved 115 patients women with breast mass (55 malignant which confirmed by biopsy report and 60 benign) attending Rizgary and Hawler Teaching Hospital in Erbil City. The age of patients was ranged from 18- 71 years and the mean was (44.5) years. Total of 40 women selected as control group, they were matched with patients by age group without any history of breast problem or neoplastic disease. Data were collected by interview with patients and control group, a questioner form were provided to each patients which include (age, age at menarche, regularity of menstruation, marital status, age at marriage, age at first pregnancy etc.) and clinical file were created for each patient who attends to these two hospitals for the first time, in spite of histopathological diagnosis for each patients.

Twenty-four hours before operation of breast mass samples of venous blood (seven ml) were collected from each patient and control group by sterile disposable syringes. The blood was divided into two aliquot 3ml into EDTA tube for complete and differential leucocyte count and the second into gel tube to obtain serum for determination of the immunological parameters by ELISA, which include (IL-6, IL-8 and IFN-Y) the study also include tumor marker CA15.3 and adenosine deaminase activity investigation. The serum level of immunoglobulins (IgA, IgG and IgM) complement protiens (C3 and C4) were evaluated by immunodiffusion plate method.

Analysis of data was performed by using (statistical package for social science (SPSS) version 18). Results were expressed as (Mean± S. E). Statistical differences were determined by LSD and Duncan test for multiple comparisons after analysis of variance (Two Way ANOVA). P< 0.05 was considered statistically significant (Petrie and Sabin, 2000).

RESULTS

Results indicated obvious relation between risk factor and breast cancer (BC), the highest number of breast cancer patients were found at age interval (38- 47) year, while the lower or minimum record was at aged 58 and older, while the higher number about benign breast disease patients (BBD) was found at age interval (28- 37). Table 1.summarize the demographic characteristic and major risk factors among BC and BBD. Percentage of BC patients starting age menarche after 12 years (54.5%) was higher when compared with patients starting at or before 12 year (45.4%). Age of first pregnancy in 20 years and greater which was higher than other group (56.3%). Nearly 16% of BC patients were nulliparous while 61.8% of patients have 1-6 children. The results indicated that the percentage of BBD patients which diagnosed premenopausal was higher than postmenopausal (80%). BC Patients which not used hormone replacement therapy has higher percentage (83.6%) when compared with patients used HRT (16.3%). About half of the patients recorded positive family history.

Table 1: Demographic characteristic and major risk factors among breast cancer (BC) and benign breast disease (BBD).

Characters	**Malignant**	**Benign**
	N=55 %	**N=60 %**
Age groups		
18-27	14.54%	20%
28-37	23.66%	26.66%
38-47	27.27%	23.23%
48-57	20%	16.66%
≥58	14.54%	13.33%
Age of menarche		
≤12	%45.45 25	%16.66 10
>12	%54.54 30	%83.33 50
Body mass index:		
<20	%7.27 4	% 3.33 2
20-25	%18.18 10	%55 33
>25	%74.54 41	%41.66 25
Age of first pregnancy:		
None(single or infertile)	%16.36 9	%16.66 10
Before 20	%27.27 15	%36.66 22
20 and greater	%56.36 31	%46.66 28
Age of diagnosis		
Premenopausal	%65.45 36	%80 48
postmenopausal	%34.54 19	%20 12
Type of diseases		
	Invasive Lobular carcinoma = 0	Lipoma = 20 33.33%
	Invasive Ductal carcinoma = 40 100%	Fibroadenoma =10 16.66%
	Stages	Fat necrosis = 4 6.66%
	First=0	Dermatitis =5 8.33%
	Second=14 25.45%	Duct ectasia =4 6.66%
	Third=16 29%	Unknown = 17 28.33%
	Fourth=16 29%	
	Unknown=9 16.36%	
Family history		
No	%55 30	%75 45
First degree	16 29%	%8.33 5
Second degree	%9 5	%16.66 10
Third degree	%7 4	Nil 0%

The numbers of patients diagnosed as stage third and fourth were higher (29%) when compared with those stage two, unknown and first stage (25.4%, 16.3% and 0.0 respectively). According to the type of benign breast disease, the unknown cause showed higher percentage when compared with other types.

Results showed remarkable differences between studied group regarding the total white blood cell count. Granulocytes found to be increased significantly in BC, BBD and control group. The results of lymphocytes and monocytes were also revealed significant raising in both group of patients compared to control group (Table 2).

Table 3 reveal the variations in studied cytokine that might have a huge contributing in progression and development of breast cancer. All of IL-6, IL-8 and IFN- γ were remarkably increased in BC and BBD in comparison to control and even between BC and BBD the difference was significant.

Tumor marker CA15.3 was evaluated in patients and control group as its obvious from table (4a) the differences between studied groups were significant. Oxidative stress was

determined by measuring the ADA activity in serum of patients and control, the variations were notable between patients and control (Table 4b)

Complement proteins of the complement system were also determined in order to evaluate the body response to the immunological changes in the body. C3 and C4 were evaluated and there was a significant difference between patients and control, there was also remarkable differences between both group of patients (Table 5).

Table 6 showed the results of evaluation of serum immunoglobulins such as IgG, IgM and IgA in studied groups. The findings revealed that there was significant variation in the serum level of the mentioned immunoglobulins in patients and control.

Table 2: Mean± S.E of WBC count, and absolute leucocytes countin BC, BBD and control groups.

Group	Parameters in (Mean ± S.E)			
	WBC(x10^{3}cell/mm^3)	Granulocytes(cell/mm^3)	Lymphocytes(cell/mm^3)	Monocytes(cell/mm3)
BC	8.470^a ± 0.21	5854^a ± 243	3208^a ± 205	569^a ± 35
BBD	7.047^b ± 0.24	4532^b ± 196	2042^b ± 769	549^b ± 24
Control	6.265^c ± 0.15	3863^c ± 143	1838^c ± 58	372^c ± 16

Table 3: Mean± S. E concentration of IL- 6, IL- 8 and IFN- γ (pg/ ml) in BC, BBD and control groups.

Groups	Parameters in (Mean ± S.E)		
	IL- 6(pg/ ml)	IL- 8(pg/ ml)	IFN- γ(pg/ ml)
BC	315.8^a ±28.15	101.55^a ±3.59	453.65^a ±46.65
BBD	177.87^b ±14.79	82.77^b ±1.88	299.37^b ±13.34
Control	83.57^c ±5.73	68.37^c ±2.2	143.37^c ±9.13

Table 4a: Mean± S.E of CA15- 3 in BC, BBD and control groups.

Groups	Parameter in (Mean ± S.E)
	CA15.3 (U/ ml) Mean
BC	63.70^a ±4.51
BBD	34.70^b ±1.07
Control	25.12^c ±1.08

Table 5: Mean± S.E of C3 and C4 mg/dl in BC, BBD and control groups.

Groups	Parameters in (Mean ± S.E)	
	C3 mg/ dl	C4mg/ dl
BC	265.34^a ±6.43	44.62^a ±1.13
BBD	175.58^b ±4.63	31.75^b ±0.60
Control	158.25^c ±5.5	24.05^c ±1.26

Table 4b: Mean± S.E of ADA activity in BC, BBD and control groups.

Groups	Parameter in (Mean ± S.E)
	ADA activity (U/ml) Mean
BC	29.34^a ±1.93
BBD	21.5^b ±0.71
Control	14.96^c ±0.52

Table 6: Mean± S.E of IgG, IgM and IgA in BC, BBD and control groups.

Group	Parameters in (Mean ± S.E)		
	IgGmg/ dl	IgMmg/ dl	IgAmg/ dl
BC	1568.01^a ±51.13	364.60^a ±13.93	395.54^a ±24.54
BBD	1291.55^b ±38.48	274.23^b ±10.85	297.40^b ±9.55
Control	976.79^c ±53.54	220.11^c ±12.01	259.57^c ±15.88

DISCUSSION

Our study was in agreement with Montazeri*et al*. (2003) and Rennert (2009) which showed that the Iraqi BC patients had an age distribution that was nearly the same as that seen in Iran, Egypt and Jordan. In Sulaimanyiah- Iraq also agreed with our findings they found that currently diagnosed at advanced clinical stages with 60% of patients being under 50 years of age (Majid*et al*., 2010). A woman who began menstruating when she was younger than 12 years old has a 10- 20% increased risk of BC compared to one whose menstruation started when she was older than 14 years of age (CGHFBC, 2012). In addition, women who experience a delayed natural menopause (after the age of 55) are twice as likely to develop BC compared to women who experience menopause before the age of 45 (McPherson *et al*., 2000) which is in agreement with our study.

Older age at menarche typically is reported to be associated with reduced BC risk, while older age at menopause is associated with increased risk. Our results showed only one-fourth of the BC patients had irregular menstruation to the extent that irregular menstrual cycles reflect an ovulatory cycle, our findings support the hypothesis that the cumulative number of regular ovulatory cycles increases BC risk (Clavel-Chapelon, 2002). Rockhill*et al*. (1996) found little support for the hypothesis that a longer time until onset of regular menstrual cycling was associated with reduced risk of BC, which is in contrast with our study. Other reproductive events have also shown a linear association with risk for BC, specifically, women who gave birth for the first time before age 18 experience one- third the risk of women who have carried their first full- term pregnancy after age 20 (Yoo*et al*., 2002). Women who have their first full- term pregnancy at a relatively early age have a lower risk of BC than those who never have children or those who have their first child relatively late in life (Wohlfahrt and Melbye, 2001).

Premature termination of pregnancy appears to increase BC risk; the breast is exposed only to the high estrogen levels of early pregnancy and thus may be responsible for the increased risk seen in these women (Dall and Britt, 2017). Erlandsson*et al*. (2003) found no association between abortions and increased risk of BC. Patrick (2007) found strong relation between abortion and BC which is in contrast with our result.

Use of estrogen replacement therapy is another factor associated with increased hormone levels and it has been found to confer a modest (less than two-fold), elevation in risk when used for 10-15 years or longer (Brinton *et al*., 2008). The long- term (more than five years) use of postmenopausal estrogen therapy (ERT) or combined estrogen/ progestin hormone replacement therapy (HRT) may be associated with an increase in BC risk (Downing *et al*., 2007).

Similar to our findings, others found most women who get BC (approximately 80 percent) have no such family history of the disease (Porch *et al*., 2002). There was an increased risk of BC among Kurdish patients who had a positive family history compared to case controls. Although 19% of Kurdish BC patients gave a family history of BC and observed that BC risk increased with increasing number of first- degree family relatives with BC (Majid*et al*., 2010).

According of the WHO classification (WHO, 2011), the most common histological type determined microscopically was invasive ductal carcinoma. According to the AJCC system, the frequencies were 7.6%, 45.1%, 31.5% and 15.7% for stages I, II, III and IV respectively (Alwan, 2010). For patients with a designated stage, there was no significant relationship between tumor stage and age nor was the relationship significant when patients whose stage was unknown were included (Majid*et al*., 2010).

Some studies agreed with our results which they had shown that a high BMI was positively related with BC (Amadou*et al*., 2013).A study done in Basrah in 2005 showed significant association between increase body mass index and risk of BC (Dahooz and Hawaz, 2005). Obesity is another known risk factor for BC Obese women have an increased risk for postmenopausal but not premenopausal BC.

White blood cells count is a nonspecific marker that reflects systemic inflammation (Erlinger*et al*., 2004 and Shankar *et al*., 2006). An increase in WBC count within the clinically normal range was associated with increased risk of death, cardiovascular disease and cancers in several prospective studies (Grimm *et al*., 1985; Jee*et al*., 2005 and Kruse *et al*., 2011). Several studies reported that a greater WBC count may predict increased mortality for cancers (Friedman and Fireman, 1991; Jee*et al*., 2005; Shankar *et al*., 2006 and Thornton *et al*., 2008). The stromal tissues of tumors have a high WBC count, and the inflammatory cell number and their cytokines production seem to correlate with tumor severity and prognosis (Balkwill, 2004).

In the present study, serum IL- 6 levels were found to be significantly elevated in BC patients than that of control group. This result was certified by other studies. Jiang *et al*. (2000), Jablonska*et al*. (2001) and Benoy*et al*. (2002). Where they concluded that changes in values of certain cytokines could have a diagnostic and prognostic role in cancer disease, and the change in IL- 6 level mediated by tumor both directly and indirectly is an important parameter that affects the course of the disease. Elevated serum IL- 6 level would be a prognostic parameter in BC patients. Pre- treatment IL- 6 and IL- 8 levels are predictive mediators of response to therapy and prognosis of patients with recurrent breast cancer (Yokoe*et al*., 2000), which was also confirmed by Bachelot*et al*. (2003).

Interleukin- 8 was remarkably increased in patients with BC in comparison with the controls. Kozolowski*et al*. (2003) demonstrated that breast cancer is associated with elevated serum concentration of IL- 8 that is known stimulators of angiogenesis as well as cancer cells proliferation and growth (Koch *et al*., 1992 and Fu *et al*., 1998). However, results obtained by different authors clinically are somewhat inconsistent. Some authors (Zhang and Adachi, 1999; Yokoe*et al*., 2000; Tiainen*et al*., 2019) reported elevated IL- 8 serum concentration before treatment initiation as indicators of poor prognosis in BC patients, others (Petrini*et al*., 1992 and Barton and Murphy, 2001) did not reveal such dependence. Thus, clinical value of these interleukins serum concentration monitoring in BC patients remains to be fully elucidated.

Our study found high expression of IFN- γ in serum of BC and BBD patients. Several cytokines are known to promote the dissemination of BC tumors to target organs and one of them is interferon- gamma (IFN- γ). Recently, IFN- γ has also been implicated to have a prominent role in immune responses to tumors (Elpek*et al*., 2007). In contrary to this study, other study reported decreasing in serum IFN- γ breast cancer and BBD patients, and they suggested that tumor cells are able to escape from the control of this cytokine in the early tumor stages; this could be due to a decreased expression of IFN- γ (Ignacio *et al*., 2007 and Hrubisko*et al*., 2010).

Patients with metastatic disease were found to express higher level of CA 15- 3 in their serum than that of non-metastatic disease. Similar result was reported by others who found CA 15- 3 levels in the patients with primary BC patients were greater than normal control group (Miya*et al*., 1992 and Keyhani*et al*., 2005). The physiologic function of MUC1 is unclear, the glycoprotein appears to play a role in cell adhesion, immunity and the high levels present in cancer may be causally involved in metastasis (Duffy *et al*., 2001). Longitudinal studies of healthy women have demonstrated the statistical significance of this marker to monitor breast cancer patients (Agha-Hosseini*et al*., 2009).

The mean value for total ADA activity in the serum of BC and BBD were significantly higher than those of control group. This result was in agreement with other studies which reported an increased total ADA activity in the serum of BC patients (Walia*et al*., 1995; Canbolat*et al*., 1996 and Aghae*et al*., 2010).To discuss these findings, some authors suggest that high ADA activities play an important role in the salvage pathway activity of cancerous tissues and cells (Donofrio*et al*., 1978 and Dornard*et al*., 1982), while others propose that increased ADA activity may be a compensatory mechanism against toxic accumulation of its substrates (adenosine) due to accelerated purine and pyrimidine metabolism in the cancerous tissue cells (Namiot*et al*., 1996; Garcia-Gill *et al*., 2018).

In many pathological conditions such as cancer, some component of the complement system elicits typical primary or secondary changes (Whicher, 1978). An elevation in complement levels is associated with a wide variety of inflammatory disease and in cancer patients with progressing tumors. It has been reported that complement works synergistically with other system, especially with the immune system composed of immunoglobulins and T and B lymphocytes (Gminski*et al*., 1992; Chaplin, 2010). A study done by Vijyakumar*et al*., (1997) found elevated in the concentration of C3 and C4 in the sera of patients with breast cancer compared with benign and healthy group. They suggested that the complement activity increased significantly with the progression of the disease; also Li *et al*. (2005) found increases of C3 component in the sera of patients with breast cancer as well as benign breast diseases, which indicated activation of the complement system in response to a general disease process rather than proteins released by the tumor cells themselves.

In the present study serum Ig analyses depicted a significant increase in the level of serum IgG, IgA and IgM in breast and benign cancer than in that of the normal, which agree with the results reported by Ahmed *et al*. (2002); Roberts *et al*. (2006) and Mahdi (2011). Contrary to the present result, Lee *et al.* (2006) reported significantly decreased levels of serum IgG in patients with cancers of prostate and breast. Alsabti (2006) reported a positive correlation between the extent of metastatic breast cancer and the serum levels of various immunoglobulins particularly IgA. The association of breast cancer with rises in serum Ig levels particularly in IgA and IgG suggests a defense reaction against

increasing tumor load or the secretion of immunoglobulins against the tumor.

CONCLUSION

Age group (38- 47) was the most susceptible group for breast cancer. In breast cancer patients, some risk factors like (age at menarche, age at marriage, BMI, age at first pregnancy, OCP and family history) were more prevalent than BBD. In this study, the serum cytokines (IL- 6, IL- 8 and IFN-γ) were remarkably increased in breast cancer and BBD patients. CA15- 3 and ADA activity were significantly increased in breast cancer and BBD patients. Serum immunoglobulins (IgG, IgA and IgM) and complement components (C3 and C4) were increased in breast cancer and BBD patients when compared with the control group.

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