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The impact of IL-10 gene polymorphism on progressive Breast cancer

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Abstract

Breast cancer is the most frequently diagnosed malignancy and the second leading cause of mortality among women, the cytokine plays a critical role in cancer initiation, progression and elimination as well as the SNPs in IL10 gene -1082 linked with differential levels of IL-10 expression that impact on different type of disease . So the present study deal with correlation of IL-10 polymorphism and progressive of breast cancer .

Case-control study was performed to 70 female patients suffering from breast cancer with group of 70 healthy individual was used as control, the patients at age 35-79 which classified into stages and grade according to TNM classification . Blood sample was collected from all patients and control and used for DNA extracted for using SSP-PCR in detection the IL-10-1082A\G polymorphism.

The result shown that the age range (40-49) were highest than other age range. This result explain a significant association of family history , period of lactation , stage and grades of cancer with breast cancer . The result demonstrate that AA genotype and A allele is risk factor of severity in breast cancer patients, while GG genotype and G allele is protective factor for severity. Also the AA genotype had a tow- folded or more increase in T1-2 than GG genotype and 47.5 of patients have +ve results to spread of cancer into lymph node and founded that the cancer in about 43.4% of patients were metastasis into other organs . However high frequency 54.8 % of female patients in grade 3

Conclusion: The polymorphism in IL-10 at position (1082A\G) has association with progression of breast cancer at AA genotype, and IL-10 consider as predictive factor for severity of thalassemia

Key words: Brest cancer , TNM , IL-10 polymorphism

INTRODUCTION

Iraqi National Cancer Research Center (INCRC) considered breast cancer as the commonest type of malignancy in female, affecting one in eight women during their lifetime and the second most common malignancy worldwide and reported about 1,150,000 new cases/year with an incidence of 22 per 100,000 female populations (1). Breast cancer is a complex and multifactorial disease resulting in abnormal cell growth that leads to malignant tumor formation. (2 ; 3)

Smyth*et al*.,(2004) confirmed the role of cytokines in cancer immunity and carcinogenesis (4). Several study illustrated the association of immune-regulatory cytokines like pro-inflammatory cytokine (IL-6 , TNF-a , INF-y) and anti-inflammatory cytokine (IL-10) with several diseases like hepatocellular carcinoma , thalassemia and breast cancer (5 , 6 and 7) .

Human Interleukin- 10 produced by monocytes, macrophages, Th2 cells and regulatory T cells and act as anti-inflammatory cytokine that regulates the immune response by decreased the production of pro-inflammatory cytokines (8) . It has an important coordinated role in breast carcinogenesis (9). IL-10 might promote tumor development, by acting to suppress anti-tumor immune responses (10).

Polymorphic gene sequences in the promoter region of IL-10 gene may influence the gene expression (11) and consequently play a certain role in severity , susceptibility and clinical outcome to several disease by changing cytokine productionsuch as colorectal cancer (12), lung cancer (13) , gastric cancer (14) ,oral cancer(15) . So this study aim to focused on correlation between *IL-10* polymorphisms rs1800-896 (-1082 A>G) with breast cancer in Iraqi patients.

PATIENTS AND METHODS

Study population. During period from 1 October 2017 till june 2018, 70 women patients with breast cancer admitted to the Al-Sadder teaching Hospital and oncology unite were prospectively considered. The exclusion criteria were: (a) patient with other type of cancer (b) radiotherapy, or chemotherapy during the previous 3 week; and (c) other active medical conditions (benign breast tumor , heart, hepatic, renal failure, uncontrolled diabetes and infections). In addition to 70 healthy women without history family of breast cancer admitted to check up breast health were enrolled as control group

The study protocol was approved by the Ethics Committee of ministry of health in Iraq. Informed consent was obtained using a questionnaire completed by each participating subject .

The diagnosis of breast cancer was established by histopathologicalexamination by pathologist .Cancers were staged according to the International Union Against Cancer (IUCC) TNM system (16) .

70 blood samples were collected from all female patients and put in EDTA tubes to determination IL10,1082A/G polymorphism. The patients were classified according to . By taken swab from infection area and also after tonsillectomy , the sample surface is sterilized and opened with a sterile scalpel then taken swab from the fibrosis found in the tissue.

Genotyping. Genomic DNA was extracted from EDTA anticoagulant peripheral blood leukocytes using Accupower®Genomic DNA extraction kit (Bioneer. Korea). and then stored at -20 C till use.

Single nucleotide polymorphisms (SNPs) related to the IL-10 (-1082) were determined using PCR with sequence-specific primers (PCR–SSP) in two reactions employing one common forward F:AGCAACACTCCTCGTCGCAACand two reverse primers R1: CCTATCCCTACTTCCCCC and R2:CCTATCCCTACTTCCCCT (17) , with an amplicon size of 179 bp. The reaction mix was done in 25 µl volumes include 5µl of template DNA ,GoTaq®Promega Green Master Mix 2X 12.5 µl ,Primers(foreword 2 µl and reverse 2 µl)and Nuclease Free water 3.5 µl (Applied PCR system, USA).

PCR conditions are initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 30s,annealing at 60°C for 1 min and 1 min of extension at 72°C, with a final extension of 7 min at 72°C., visualized by electrophoresis on a 1% agarose gel stained with 5% ethidium bromide. The gel was then photographed (Cleaver Gel Documentation System) by digital camera on UV light and scored for the presence or absence of an allele specific band

Statistical analysis

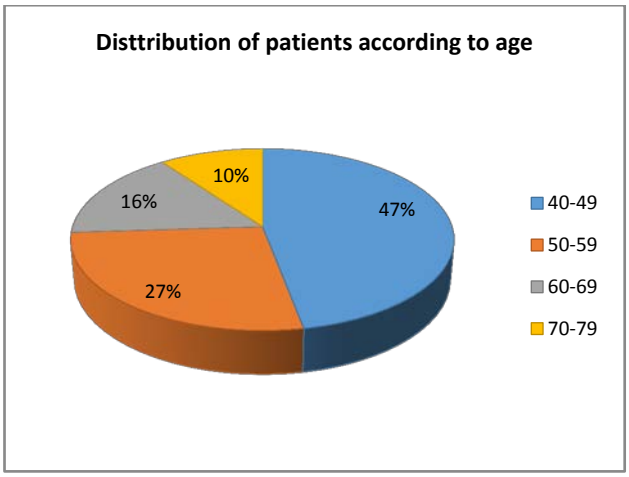
Statistical analyses were done using Graf pad prism 5 computer software . To measure the strength of association between 2 categorical variables, such as the presence of certain genotype and

diseasestatus , also differences of genotype and allele frequencies between breast cancer patients and control groups , the odds ratio (OR) and 95% condense intervals (CIs) was assessed by a special $\chi 2$ formula.The difference was considered significant, if $p < 0.05$.

RESULTS

1-Demography study

The present study show that maximum cases were observed in the age group (40-49) that record 47% while 27 % were observed in age range between (50-59) followed by 16% in (60-69) finally 10% (70-79) Fig (1) . A result was demonstrated prevalence of patients with family history more than patients without family history Fig(2) . Also results explain a significant difference P ≤ 0.05 in breast feeding and long period of lactation



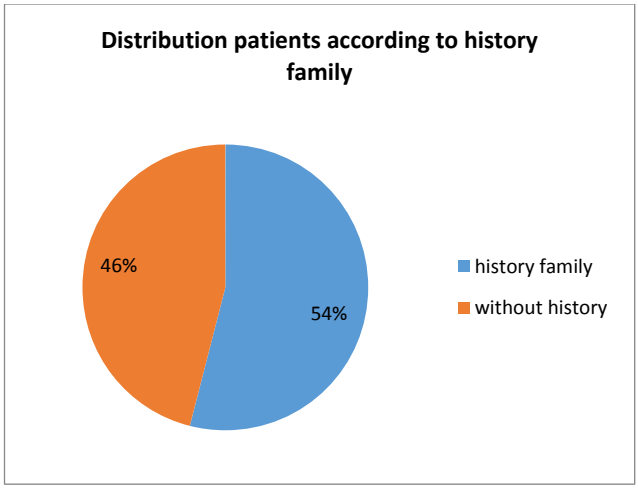


Figure : distribution of patients according to age (1) and family history (2)

Table(1) The breast feeding and the period in breast cancer women

Breast feeding	
yes	38(54 %)
No	32 (46 %)
Period of breast feeding	38 patients
1-6 month	5(13%)
7-12	7(18%)
1-2 year	10(26%)
More than 2 year	16(43%)

(X2 = 7.024 ,df = 1 , P value ≤0.05)

2-disterbution of patients according to Staging and Grading

As show in table (2) the distribution of patients according to size of tumor (T) , spread the cancer to lymph node (+ve) or not spread (-ve) as well as if cancer became metastasis (M1) or not (M0) .There is a significant difference among different stages (P≤0.05)

The results appear that 14 cases (20%) are grade I, 25 cases (33.7%) are grade II, 31 cases (44.3%) are grade III. There is a significant difference among different grades (P ≤0.05) as show in table (2)

Table (2) : TNM classification and grading in breast cancer patients

Gategory	**Breast cancer No (%)**
Tumor stage	
T 1-2	32 (45.7 %)
T 3-4	38 (54.3 %)
Lymph node	
N +ve	40 (57.2 %)
N -ve	30 (42.8 %)
Metastatic	
M0	24 (34.3%)
M1	46 (65.7%)
Grade	
1	14 (20 %)
11	25 (35.7 %)
111	31 (44.3 %)

X^2 = 16.060 ,df = 3 , P value≤0.01)

3- **Distribution of IL-10 Gene "-1082 A/G" polymorphism in**breast cancer patients

The genotypes frequency were AA, AG and GG 51.4%, 14.3% and 34.3 % in breast cancer patients and 22.9% , 24.2 % and 52.9 % in the healthy as show in Table (3) **.** This study explain that "A" allele have higher prevalent in the patients with percentage (69.3%) in compared to controls (31.4%).

Table(4) genotype and allele frequency in female breast cancer and healthy groups

		Patients N=70	Healthy N=70	OR (95% C.I)	P-value
Genotypes	AA	36 (51.4)	16 (22.9)	0.74(0.42-1.3)	0.05**
	AG	10 (14.3)	17 (24.2)	0.70(0.39-1.5)	0.47**
	GG	24 (34.3)	37 (52.9)	1	--
Allele type	A	97 (69.3)	44 (31.4)	0.86(0.5-1.1)	0.0001***
	G	43 (30.7)	96 (68.6)		

The study appear that AA genotype had a tow- folded or more increase in T1-2 than GG genotype and 47.5 of patients have +ve results to spread of cancer into lymph node . also the study founded that the cancer in about 43.4% of patients were metastasis into other organs . However high frequency 54.8 % of female patients in grade 3 as shown in table (5) .

Table(5): genotype frequency according to TNM and grade classification .

	AA N (%)	AG N (%)	GG N (%)	OR(95%)	P-value
Stage(no.of patients					
T1-T2 (32)	19 (59.4)	5 (15.6)	8 (25)	0.37(0.1-0.47)	0.005**
T3-T4 (38)	21 (55.3)	6 (15.8)	11 (28.9)	1.06(0.5-2.11)	0.18**
LN +ve(40)	19 (47.5)	9 (22.5)	12 (30)	0.95(0.1-0.55)	0.41**
LN -ve (30)	13 (43.3)	7 (23.3)	10 (33.4)	1.33(0.67-2.41)	0.50**
M 0 (24)	9 (37.5)	9 (37.5)	6 (25)	0.94(0.1-0.55)	0.55**
M 1 (46)	20 (43.5)	12 (26.1)	14 (30.4)	3.3(1.2-7.8)	0.006***
Grade					
1 (14)	5 (35.7)	5 (35.7)	4 (28.6)	1.72(0.5-1.01)	0.71**
11 (25)	10 (40)	6 (24)	9 (36)	1.6(1.009-2.49)	0.46**
111 (31)	17 (54.8)	6 (19.4)	8 (25.8)	0.86(0.57-1.10)	0.43**

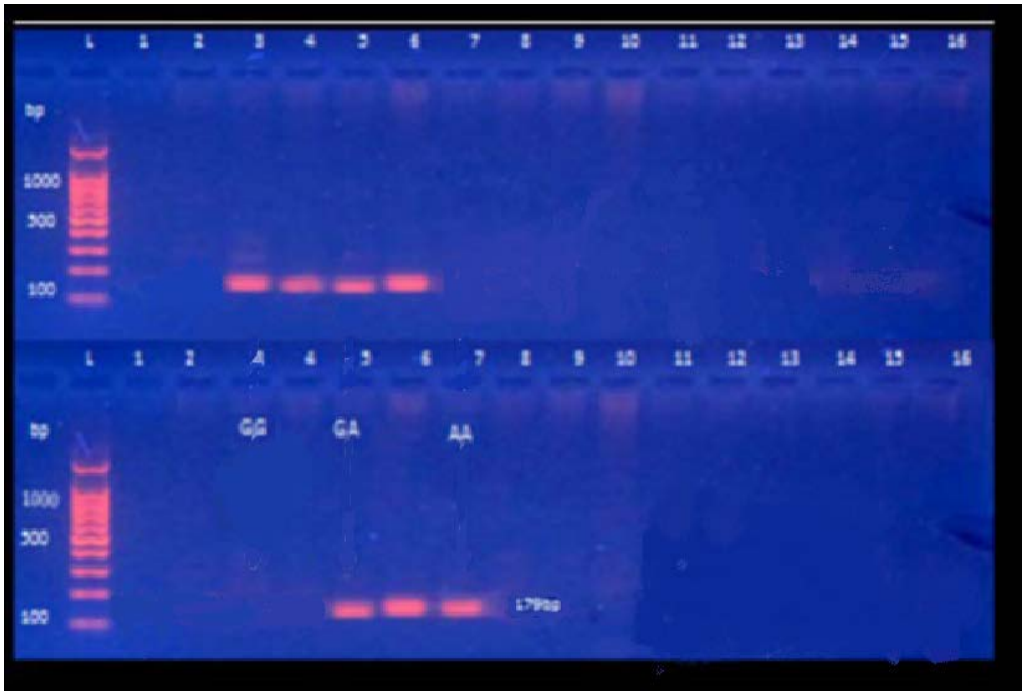


Figure (3): Gel electrophoresis and PCR product of amplified 179bp of IL-10 gene in breast cancer patients up part represent G allele and down part represent A allele .L : Ladder (100-1517bp) . lane (3,4) represent GG , lane (5,6) represent GA and lane (7) represent AA genotypes .

DISCUSSION

The results revealed that the peak age frequency of (40-49 years) and (50-59 years).(18) recorded that peak age frequency in female patients between age group (40-49) and (50-59) by the Iraqi Cancer Registry . In Baghdad (19) who shows that about 68.2% of the patients were younger than 50 years. (20) they report that mean age of patients with breast cancer at diagnosis was 49 years .Also Alwan*et al*.,2017 (21) confirmed that about 70.8% of patients presented in the age range between 40-59 years and demonstrated a significant associations with respect to the breast cancer in the family. The higher incidence of breast cancer in this age may related to reproductive factors, or hormonal factors.

Breastfeeding is decreasing the risk of breast cancer among breastfeeding mothers. And the longer women breast feeding they are protected against breast cancer. The researchers find that the risk of breast cancer is decreased by 4.3% for every 12 months of breastfeeding and 7% for each birth . (22) that shows that there is a significant association in Turkish women with breast feeding and decrease risk of breast cancer. Also agree with a study done by (23) who suggest that breastfeeding for a year or more slightly reduces a woman's overall risk of breast cancer.

(24) who finds that family history of breast cancer indicates a strong association with risk of developing breast cancer. (25) demonstrated that the risk of developing breast cancer is twice as high in women who have an affected first-degree relative than women in the general population.Maruthi*et al*.(26) observed in their ' study that most breast cancer patients were in aggressive state with high tumor size and the cancer in most patients spread to lymph node and had a metastasis .Alwan ,2016 (27) explain that depending on TNM classification, about 9.8% of female patients presented with stage I disease, and 46%were diagnosed in stages III and IV and more than two-thirds of the patients (65.5%) had positive lymph node involvement . (28)observes that 43% of patients are diagnose in stages III . (29) who prove that (57%) of breast cancer cases are advance stage and grade. (30) confirmed that high frequency of patients were in high stage and grade which reflect the poor health education of the general population and their ignorance regarding the significance of clinical breast examination, breast self-examination and early medical consultation .

Several single nucleotide polymorphisms (SNPs) have been identified in the IL10promoter (31) . Three functional IL10SNPs have been characterized; these are an adenine (A) to guanine (G) substitution at nucleotide -1082 (rs1800896), a thymine (T) to cytosine (C) substitution at nucleotide -819 (rs1800871), and an A to C substitution at nucleotide -592 (rs1800872). These polymorphisms led to different *IL10* expression levels and determined inter-individual differences in IL-10 (32).Moorem(33) mention that Interleukin-10 inhibited T-cell proliferation , function and promoted proliferation and (26) observed a significant association of rs1800896 with BC in patients group, where AA genotype showed a two- fold increased risk towards BC and three fold risk towards metastasis. In-silico analyses strengthened observation revealing the alteration in transcription binding site in the IL10 promoter by the mutant allele G.(34) reported that IL10 AA genotype is correlated with a marked increase in breast cancer risk.IL-10-1082 AA genotype was associated with increased BC risk (35).Giordani et al. (36) confirmed a correlation between The -1082 polymorphism with cancer risk in Italy women patients

The seven-fold increased risk of BC seen in patients with low producing genotype (AA) and elevated frequency of low producing AA genotype in patients suggests that predisposing role of this polymorphism in South Indian women (37).In Turkish population observed that haplotypes A frequency is high in BC patients than controls and lower frequency in lymph node negative BC cases (38).. Similarly (39) appeared that *IL-10* "1082G/A heterozygous GA genotypes and A allele were significantly high in asthmatic patientsin contrast homozygous GG genotype and G allele that found at low frequency with asthmatic patients.Chand-Bhayal*et al*, who revealed that patients with AA genotype more susceptible to cancer than GG genotype (40) . This result demonstrate that AA genotype and A allele is risk factor of severity in breast patients,

greater IL-10 protein concentrations are seen in serum of breast cancer patients than in that of healthy individuals and this is associated with poor clinical outcomes , also confirmed that IL-10 producing capability makes individuals susceptible to more aggressive course of the disease (41) .

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