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Serum Level of ST2 with Gene's Distal Promoter Polymorphism in Iraqi Females with Breast Cancer

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Abstract

Breast cancer is the most important and frequently diagnosed cancer among women worldwide. IL-1RL1 is a member of the IL1R superfamily also known as ST2. Its receptor for IL-33 member of the IL-1 cytokine family. Expression of the *ST2* gene is regulated by a proximal and distal promoter that direct two distinct types ST2L and sST2 are produced by alternative splicing in human cells. The aim of the present study of the association of ST2 serum level and polymorphism with breast cancer in Iraqi women suffers from breast cancer.

Materials and methods: peripheral Blood samples were collected from 66 Iraqi patient women diagnosed with breast cancer distribution on two groups pre-treatment (PT) and under treatment with chemotherapy (UTC) patients, and 34 healthy women were matched with patients as a control. ELISA technique has been used to determine the serum level of ST2 receptor. The polymerase chain reaction (PCR) performed on 40 patients and 20 control, to determine the genetic variation in the distal promoter region of ST2 gene. The PCR product was sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea. Then analyzed using genious software.

Results: the serum level of ST2 for understudying groups recorded highly significant difference in mean ± SE for PT (12722.45 ± 464.42 pg/ml) and UTC (11884.23 ± 479.78 pg/ml) patients as compared to control (9259.24 ± 724.87 pg/ml) under ($p<0.01$). Sequencing revealed six different polymorphisms (-27609A/T, -27607A/G, -27601A/T, -27595A/G, -27208A/C, -27128C/T) identified in the distal promoter region only one polymorphism (-27128C/T) of which had significant difference under ($P<0.01$) according fisher's exact probability.

Conclusion: elevation of serum concentration of sST2 could consider as a clinical biomarker for diagnosis breast cancer female patients. There are some difference noticed in SNP of ST2 promoter gene with SNP (-27128C/T) are showed significant differences between patients and control, T allele of (-27128C/T) SNP may have role to prevent the risk of breast cancer in Iraqi females.

Key words: breast cancer in Iraq, IL-1RL1, ST2, IL-33 R, ST2 gene, distal promoter.

INTRODUCTION

Breast cancer is the most important and frequently diagnosed cancer among women worldwide and the leading cause of cancer-related deaths in developing countries (1). In Iraq Breast cancer considered the most common cancer. There were a 4529 case in 2013 considered 4422 females and 107 males, the percentage of total constitute around 18.84% with rate 12.9 for each 100000 population (2). The ST2 receptor is a type-1 transmembrane protein encoded by the *IL1RL1* gene (3). Located in chromosome 2q12 and contains 11 exons, a proximal, and a distal promoter (4). The *IL1RL1* gene expression is regulated by a distal and proximal promoter that govern two distinct types ST2L and sST2 are produced by alternative splicing in human cells (5). Both sST2 and ST2L isoforms can be transcribed from either promoter, with promoter usage governed by the cell type. while both mast cells and the human leukemic cell line UT-7 can transcribe ST2 isotypes using either the proximal or distal promoter, the distal promoter is predominantly used in this cell line for expression of both sST2 and ST2L (6). Conversely, almost all transcriptions are initiated from the proximal promoter in fibroblasts (7). Different polymorphisms in the promoter region have been associated with different diseases, example association between atopic dermatitis and the - 226999G/A SNP in the distal promoter region of the ST2 gene (8).

IL-33/ST2 pathway is facilitating the expression of pro-angiogenic VEGF in tumor cells and attenuating tumor necrosis and this crucially involved in the growth of mammary tumor (9). IL-1RL1 is a member of the IL1R/TLR superfamily (10). Also known as ST2, T1, DER4 or Fit-1 (11). IL-33 is a member of the IL-1 cytokine family. It has been identified as a specific ligand for the ST2L receptor (12). There are three isoforms of ST2 in humans, which are produced by differential splicing of a single transcript: soluble ST2 (sST2), a membrane-bound form (ST2L), and variant form (ST2V). sST2, sST2 is expressed in embryonic tissues, mammary tumors, and fibroblasts (10). ST2L is expressed in several immune cells, such as mast cells, monocytes, dendritic cells (13). ST2V is expressed mainly in gastrointestinal organs such as the stomach, large and small intestine, and spleen (14). sST2 inhibited the binding of IL-33 to ST2L-positive cells and that suppressed activation of NF-β and the production of Th2 cytokines in the IL-33 signaling were suppressed in the presence of sST2, sST2 acts as a negative regulator of Th2 cytokine production by the IL-33 signaling (15). sST2 as a decoy receptor of IL33, is also thought to have a critical role in several diseases, including systemic lupus erythematosus (16); rheumatoid arthritis (17); Hepatocellular (18). The IL-33/ST2 axis has been implicated in numerous disease states, including Alzheimer's disease (19). Inflammatory bowel diseases, rheumatoid arthritis and colorectal cancer (20, 21). Some evidence is supportive of a critical role for the IL-33/ST2 axis in the initiation and maintenance of wound healing responses (22). According to the all above the present study designed to evaluate the association of ST2 serum level and its polymorphism with breast cancer in Iraqi patient women.

MATERIALS AND METHODS

Blood samples were collected from 66 Iraqi patient women diagnosed with breast cancer from the Oncology Teaching Hospital of the Medical City, Baghdad. Samples included two groups pre-treatment (PT) and under treatment with chemotherapy (UTC) patients, during the period from September 2017 to December 2017, and 34 healthy women were matched with patients in age and gender. Volume of 5ml of peripheral blood samples was drawn under sterilized condition by using disposable Syringe and divided into two parts, 2ml placed in to EDTA tube and 3ml placed in a Gel tube, then left for half an hour, then centrifuged for 15 minutes at 3000 RPM, Serum was transferred into 2ml Eppendorf tubes and stored at -20 C for further analysis. Serum level of ST2 was measured by using an ELISA technique (Human ST2/IL-33R ELISA Kit, R&D Systems, USA).

DNA extraction and Polymorphism Genotyping

Total genomic DNA extracted from the whole blood was applied using genomic DNA extraction kits (Geneaid, Taiwan). Then, DNA concentration and purity were measured by nanodrop. DNA bands were visualized using UV light after electrophoresis in a 1% agarose gel in 75 volts for 1 hour. Extracted DNA samples were stored at -20C for furthered used. The polymerase chain reaction (PCR) performed in a 25µl reaction mixture, pre-mix 5µl (Bioneer, Korea), 2µl DNA, 2µl of each primer and 11µl of distilled water. The primer sequence of the ST2 distal promoter as shown in (Table 1). The program of PCR reaction as shown in (Table 1). The length of PCR product was 825bp. Sequencing is performed on PCR products of *ST2* gene, the PCR product was sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea. Then analyzed using genious software.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used for outcome of different factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means of parameters in this study (24). WINPEPI computer programs (version 11.63) was used to calculate the statistical significance of P-value that was calculated by Odd Ratio as well as Fisher s exact test. Hardy-Weinberg equilibrium was tested by chi-squared test that was done using OEGE - Online Encyclopedia for Genetic Epidemiology studies (25).

RESULTS AND DISCUSSION

Samples collected from patients with median age (<40, 40-50, >50) the number and percentages were 14 (21.21%), 34 (51.51%), 18 (27.27%), respectively. The results of the present study showed that, the most patients were in age between (40-50 year) represents the high frequency was group 51.51%. This study has been agreement with several studies which indicated that breast cancer was developing an increase in Iraqi females after the age 40 years (26, 27). During the period from 1991 to 2000 in Iraq the mean age was 45 years and no change in the age distribution in the 10year period (28). But this time breast cancer developed at an early age about 25 (personal communication).

1- ST2 concentration in serum.

The results of the current study, which included breast cancer patients divided into two groups PT and UTC and control, the serum level of IL-33R/ST2 in all understudying groups as shown in (figure 1). The results of the mean ± SE for PT and UTC groups were (12722.45 ± 464.42, 11884.23 ± 479.78 pg/ml), respectively, as compared to control (9259.24 ± 724.87 pg/ml) there was a highly significant difference under ($p<0.01$).

Serum level of ST2 was elevated with a high significant in patients as compared with control. These results agree with studies indicated the elevation of serum concentration of soluble (sST2) may be a valuable indicator of poor prognosis in breast cancer (29, 30). High level of sST2 in serum association with increase motility of breast cancer cells also indicated that the sST2 secreted by breast cancer cells itself and found that sST2 secreted from metastatic tumor cells higher than primary tumor cells, and increased sST2 protein expression and secretion response to activation of ErbB2 tyrosine kinase receptor which increase tumor cells proliferation and motility and suggest that sST2 contribute to breast cancer metastasis (31). Another researcher pointed that IL-33/ST2 pathway facilitating expression of pro-angiogenic VEGF in tumor cells and attenuating tumor necrosis and that critically involved in the growth of breast tumor (9). sST2 may imply a therapeutic target for breast cancer as indicated in (32). According to the above ST2 serum level may consider a good diagnostic tool and to follow the progression of disease.

Table 1: sequence of the primers utilized in this study.

Primers	Sequences (5`→3`)	Product size	References
ST2	**Forward**: 5`-ATCCTTAGGCC TCTTCTCATCT-3` **Revers**: 5`-GCTGCATTGCTTTTTAT-3`	825bp	23

Table 2: PCR amplification program for *ST2* gene.

Steps	Temperature (ºC)	Time	No. of cycles
Initial denaturation	95	3 minutes	1
Denaturation	95	30 second	30
Annealing	54	45 second	
Extension	72	1 minutes	
Final extension	72	5 minutes	1

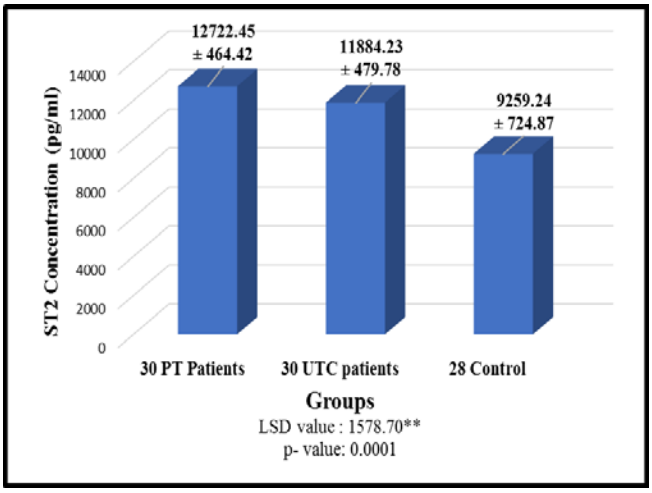

Figure 2: Comparisons between different groups in ST2 concentration (pg/ml).

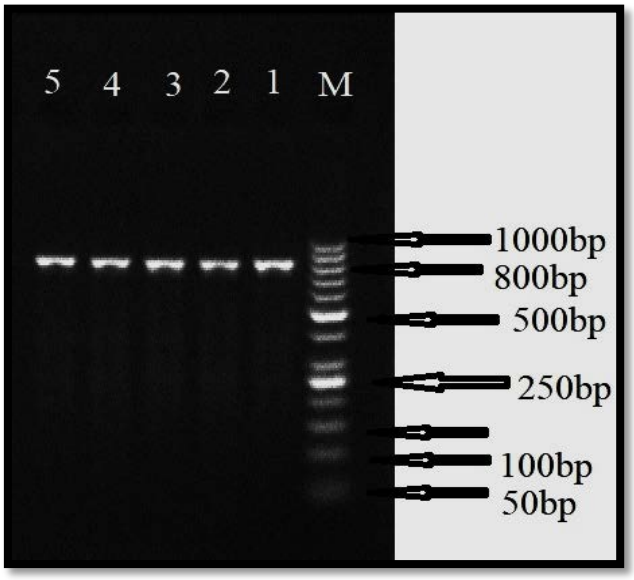

Figure (2): Gel electrophoresis for *ST2* gene (825 bp) with DNA ladder (M) on agarose gel (2%) in (100v, 1 hour), PCR product size was 825pb.

Table 3: polymorphisms for distal promoter region of *ST2* gene.

SNP	Substitution	Reference	Allele	Patients (40) No. (%)	Control (20) No. (%)
-27609	A/T	T	A	20 (50%)	12 (60%)
-27607	A/G	A	G	40 (100%)	20 (100%)
-27601	A/T	T	A	13 (32.5%)	5 (25%)
-27595	A/G	G	A	8 (20%)	3 (15%)
-27208	A/C	A	C	5 (12.5%)	6 (30%)
-27128	C/T	C	T	0	3 (15%)

SNP: single nucleotide polymorphism.

2- *ST2* Gene Amplification

The region of ST2/IL-33R (distal promoter) was amplified from extracted DNA from 40 samples of patients with breast cancer and 20 healthy control. By using specific primer, the PCR was performed under optimum condition, then PCR product was electrophoresis on agarose gel (2%) in (100v, 1 hour) the results was a single clear band with molecular size (825 bp) compare with DNA ladder figure (2).

3- Sequencing of Amplified *ST2* Gene

In order to investigate the genetic variation in a distal promoter region that regulate transcription of *ST2* gene. Sequencing was performed to determine any genetic variation in (40) Iraqi females with breast cancer compared with (20) the apparently healthy control. The complete nucleotide sequence is examined and the results as illustrated in (Table 3) showed six different

polymorphisms. In present study not identify any homozygous rare allele in the under-studying groups. Results of sequencing were recorded in NCBI (Accession: LC437682, Version: LC437682.1. https://www.ncbi.nlm.nih.gov/nuccore/LC437682.) (Accession: LC437683, Version: LC437683.1. https://www.ncbi.nlm.nih.gov/nuccore/LC437683.) Accession: LC437684, Version: LC437684.1. https://www.ncbi.nlm.nih.gov/nuccore/LC437684).

3-1 Single nucleotide polymorphism (-27609 A/T)

Polymorphism of the *ST2* gene (-27609A/T) SNP which was observed two genotypes (TT, AT) while the other genotype AA didn't observe in 40 breast cancer patients and 20 control as shown in (Table 4) the results were demonstrated the observed genotype was equal (50%) of both homozygous TT and heterozygous AT in patients. The frequency of T allele was (75%) while for A allele was (25%) in patients, this result was not agreement with expected Hardy- Weinberg equilibrium, and there was a significant difference between observed and expected frequencies (his deviate may relate to the small sample size overlaps marriages deviate from Hardy-Weinberg (Table 5). In control homozygous genotype TT frequency (40%) and heterozygous genotype AT was (60%). The frequency of T allele was (70%) while A allele was (30%), this result was an agreement with Hardy-Weinberg equilibrium (Table 5). The T allele considered a Common allele in the Iraqi females because it is recorded the highest percentage (73%) in patients and control groups, and allele provide a good possibility for effected with disease.

The statistical analysis as shown in (Table 4) found that; in patients the frequency of homozygous genotype TT record OR (1.50) with a CI range between (0.50-4.61) under (95%) and show an ET fraction of the diseases it (0.167) and it showed a non- significant difference (0.502) according to fisher's exact probability. The heterozygous genotype AT recorded OR (0.67) with a CI between (0.22-2.01) under (95%) and showed a PR fraction of diseases its (0.2) and it showed a non- significant difference (0.502) according to fisher's exact probability. The allele frequency of T showed OR (1.29) with a CI range between (0.54-3.00) under (95%) and ET fraction (0.167) it showed a non- significant difference (0.590), while allele A showed OR (0.78) with a CI range between (0.33-1.86) under (95%) and PR fraction (0.067) it showed non-significant differences (0.590) according to fisher's exact probability.

3-2 Single nucleotide polymorphism (-27607 A/G)

In the (-27607 A/G) SNP. The homozygous genotype GG is only genotype observed in the present study and showed a high percentage (100%) in patients and control, while the other genotypes doesn't observe in the studying groups. The allele frequency of G allele (100%) in (40) breast cancer patients and (20) control, while for A allele don't appearing. From the present result G allele considered a Common allele in the Iraqi females because it is the only allele recorded the highest percentage (100%) in patients and control groups. The only genotype noticed in Iraqi female population is GG (homozygous).

3-3 Single nucleotide polymorphism (-27601 A/T)

Polymorphism of the *ST2* gene (-27601A/T) SNP which was observed two genotypes (TT, AT) while the other genotype AA didn't observe in 40 breast cancer patients and 20 control as shown in (Table 6) which illustrated the homozygous genotype TT showed a higher frequency in control (75%) than patients (67.5%). The genotype AT showed a lower frequency (32.5%, 25%) in patients and control, respectively. The allele frequency of the T allele (83.75%) in patients compared with control (87.5%) while for A allele (16.25%) in patients compare with control (12.5%). This result was an agreement with expected Hardy- Weinberg equilibrium (Table 7). According to this result a Common genotype in the Iraqi female is TT because it is recorded the highest percentage (70%) in patients and control groups.

The statistical analysis as shown in (Table 6) found that; in the patients the frequency of homozygous genotype TT recorded OR (0.69) with a CI value between (0.19-2.32) under (95%) and show the PR fraction of the diseases was (0.231) and it showed a non- significant difference (0.665) according to fisher's exact probability. The heterozygous genotype AT recorded OR (1.44) with CI between (0.43–5.27) under (95%) and showed an ET fraction of the diseases was (0.10) and it showed a non- significant difference (0.665) according to fisher's exact probability. The allele frequency of T showed OR (0.74) with a CI range between (0.22–2.20) under (95%) and PR fraction (0.231) and it showed a non- significant difference (0.692), while allele A showed OR (1.36) with a CI range between (0.45–4.54) under (95%) and ET fraction (0.043) and it showed a non- significant difference (0.692) according to fisher's exact probability.

Table 4: Distribution of genotype and allele frequency of ST2 SNP (-27609 A/T) between patients and control.

-27609 A/T Genotypes	Patients NO (%)	Control NO (%)	OR	ET or PR	Fisher's exact probability	CI 95 %
TT	20 (50%)	8 (40%)	1.50	0.167	0.502 NS	0.50- 4.61
AT	20 (50%)	12 (60%)	0.67	0.2	0.502 NS	0.22- 2.01
AA	0	0	0	0	0	0
Total	40	20	---	---	---	---
Allele distribution						
T	60 (75%)	28 (70%)	1.29	0.167	0.590 NS	0.54- 3.00
A	20 (25%)	12 (30%)	0.78	0.067	0.590 NS	0.33- 1.86
NS: Non-Significant.						

Table 5: Expected frequencies of genotypes and allele of the -27609 A/T using Hardy- Weinberg equilibrium

Genotypes		TT	AT	AA	T	A	□2
Patients Genotypes	Observed no (%).	20 (50%)	20 (50%)	0	0.75	0.25	4.44*
	Expected no (%).	22.5 (56.25%)	15 (37.5%)	2.5 (6.25%)	No detect		
Control Genotypes	Observed no (%).	8 (40%)	12 (60%)	0	0.70	0.30	3.67
	Expected no (%).	9.8 (49%)	8.4 (42%)	1.8 (9%)	No detect		

* □2>3.84: results showed

frequencies.

Table 6: Distribution of genotype and allele frequency of ST2 SNP (-27601 A/T) between different groups.

-27601 A/T Genotypes	Patients NO (%)	Control NO (%)	OR	ET or PR	Fisher's exact probability	CI 95 %
TT	27 (67.5%)	15 (75%)	0.69	0.231	0.665 NS	0.19- 2.32
AT	13 (32.5%)	5 (25%)	1.44	0.10	0.665 NS	0.43- 5.27
AA	0	0	0	0	0	0
Total	40	20				
Allele distribution						
T	67 (83.75%)	35 (87.5%)	0.74	0.231	0.692 NS	0.22 - 2.20
A	13 (16.25%)	5 (12.5%)	1.36	0.043	0.692 NS	0.45 - 4.54
NS: Non-Significant.						

Table 7: Expected frequencies of genotypes and allele of the -27601 A/T using Hardy- Weinberg equilibrium.

Genotypes		TT	AT	AA	T	A	□2
Patients Genotypes	Observed no (%).	27 (67.5%)	13 (32.5%)	0	0.84	0.16	1.51
	Expected no (%).	28.06(70.15%)	10.89 (27.2%)	1.06 (2.65%)	No detect		
Control Genotypes	Observed no (%).	15 (75%)	5 (25%)	0	0.88	0.13	0.41
	Expected no (%).	15.31 (76.55%)	4.38(21.9%)	0.31 (1.55%)	No detect		

* □2>3.84: results show

control.

Table 8: Distribution of genotype and allele frequency of ST2 SNP (-27595 A/G) between different groups.

-27595 A/G Genotypes	Patients NO (%)	Control NO (%)	OR	ET or PR	Fisher's exact probability	CI 95 %
GG	32 (80%)	17 (85%)	0.71	0.25	0.609 NS	0.14- 2.98
AG	8 (20%)	3 (15%)	1.42	0.059	0.609 NS	0.34- 7.32
AA	0	0	0	0	0	0
Total	40	20				
Allele distribution						
G	72 (90%)	37 (92.5%)	0.73	0.25	0.626 NS	0.15- 2.85
A	8 (10%)	3 (7.5%)	1.37	0.027	0.626 NS	0.35- 6.70
NS: Non-Significant.						

Table 9: Expected frequencies of genotypes and allele of the -27595 A/G using Hardy- Weinberg equilibrium.

Genotypes		GG	AG	AA	G	A	□2
Patients Genotypes	Observed no (%).	32 (80%)	8 (20%)	0	0.9	0.1	0.49
	Expected no (%).	32.4 (81%)	7.2(18%)	0.4 (2%)			
Control Genotypes	Observed no (%).	17 (85%)	3 (15%)	0	0.93	0.08	0.13
	Expected no (%).	17.11 (86%)	2.78(14%)	0.11 (0.55%)			

* □2>3.84: results showed no significant differences under ($p < 0.05$) between observed and expected frequencies for patients and control.

3-4 Single nucleotide polymorphism (-27595 A/G)

Polymorphism of the *ST2* gene (-27595A/G) SNP which was observed two genotypes (GG, AG) while the other genotype AA didn't observe in 40 breast cancer patients and 20 control as shown in (Table 8) which illustrated the observe genotype was the homozygous genotype GG showed a high percentage (80%, 85%) in patients and control, respectively, while the genotype AG showed a lower percentage (20%, 15%) in patients and control, respectively. The allele frequency of the G allele (90%, 92.5%) in patients and control, respectively, while for A allele (10%, 7.5%) in patients and control, respectively. This result was an agreement with expected Hardy-Weinberg equilibrium (Table 9). From this result the homozygous genotype GG could consider a Common genotype in the Iraqi females because it is recorded the highest percentage (82%) in patients and control groups, despite the different alleles and its impact analysis but interpreted non-significant.

The statistical analysis as shown in (Table 8) found that; in the patients the frequency of homozygous genotype GG recorded OR (0.71) with a CI range between (0.14-2.98)

under (95%) and showed a PR fraction of diseases, it was (0.25) and it showed a non- significant difference (0.609) according to fisher's exact probability. The heterozygous genotype AG recorded OR (1.42) with a CI range between (00.34-7.32) under (95%) and showed an ET fraction of the diseases its (0.059) and it showed a non- significant difference (0.609) according to fisher's exact probability. The allele frequency of G showed OR (0.73) with a CI range between (0.15–2.85) under (95%) and PR fraction (0.25) and it showed a non- significant difference (0.626), while allele A showed OR (1.37) with a CI range between (0.35–6.70) under (95%) and ET fraction (0.027) and it showed a non- significant difference (0.626) according to fisher's exact probability.

3-5 Single nucleotide polymorphism (-27208 A/C)

Polymorphism of the *ST2* gene (-27208A/C) SNP which was observed two genotypes (AA, AC) while the other genotype CC didn't observe in 40 breast cancer patients and 20 control as shown in (Table 10) which illustrated the observe genotype was the homozygous genotype AA high frequency (87.5%) in patients compared to control (70%), the heterozygous genotype AC high frequency (30%) in control than in patients (12.5%). Allele frequency for A allele (93.75%) in patients compared with control (85%) while for the C allele (6.25%) in patients compared to control (15%). This result was an agreement with expected Hardy- Weinberg equilibrium (Table 11). According to this result a Common genotype in the Iraqi female is an AA because is recorded the highest percentage (82%) in patients and control groups. The most common allele in study groups was A allele record highly frequency (91%) compared to the lowest frequency (9%) of C allele. This may explain the absence of CC genotype in study groups compared to the sample size studied. At the same time the allele A make females more susceptible for disease.

The statistical analysis as shown in (Table 10) founded that, in the patients the frequency of genotype AA recorded OR (3.00) with a CI value between (0.74-12.10) under (95%) and showed an ET fraction of the diseases it was (0.583) and it has a non-significant difference (0.118) according to fisher's exact probability. The genotype AC recorded OR (0.33) with CI between (0.08-1.35) under (95%) and showed a PR fraction of the diseases its (0.2) and it showed a non-significant difference (0.118) according to fisher's exact probability. The allele frequency of A allele showed OR (2.65) with a CI range between (0.72-9.94) under (95%) and ET fraction (0.583) and it showed a non-significant difference (0.137), while allele C showed OR (0.38) with a CI range between (0.10-1.39) under (95%) and PR fraction (0.093) and it showed a non-significant difference (0.137) according to fisher's exact probability.

3-6 Single nucleotide polymorphism (-27128 C/T)

Polymorphism of the *ST2* gene (-27128C/T) SNP which was observed two genotypes (CC, CT) while the other genotype TT didn't observe in 40 breast cancer patients and 20 control as shown in (Table 12) which illustrated the observe genotype was the homozygous genotype CC frequency (100%) in patients high than control (85.00%) with significant difference under (P<0.05). The genotype CT didn't observe in patients and observe with frequency (15%) in control. The allele frequency of the allele C (100%) in patients compared with control (92.5%) while for the allele C is not observed in patients compared to control (7.5%) with no significant difference. This result was an agreement with expected Hardy- Weinberg equilibrium (Table 13). According to this result a Common genotype in the Iraqi female is the homozygous genotype CC because is recorded the highest percentage (95%) in patients and control groups. The most common allele in study groups was C allele record highly frequent (95.5%) compared to the lowest frequency (2.5%) of T allele. This may explain the absence of TT genotype in study groups compared to the sample size studied, and allele C may lead to make females more susceptible to disease.

The statistical analysis as shown in (Table 12) founded that in the patients the frequency of genotype CC recorded OR (16.20) with a CI value between (0.83-316.91) under (95%) and show an ET fraction of the diseases it was (0.927) and it showed a significant difference (0.017) under (P<0.05) according to fisher's exact probability. The genotype CT recorded OR (0.06) with CI between (0.00 - 1.21) under (95%) and showed a PR fraction of the diseases it was (0.15) and it showed a significant difference (0.017) under (P<0.05) according to fisher's exact probability. The allele frequency of C showed OR (15.03) with a CI range between (0.77-292.16) under (95%) and ET fraction (0.928) and it showed a significant difference (0.018) under (P<0.05), while allele T showed OR (0.29) with a CI range between (0.01–6.36) under (95%) and PR fraction (0.032) and it showed a non-significant difference (0.714) according to fisher's exact probability.

Table 10: Distribution of genotype and allele frequency of ST2 SNP (-27208 A/C) between different groups.

-27208 A/C Genotypes	Patients NO (%)	Control NO (%)	OR	ET or PR	Fisher's exact probability	CI 95 %
AA	35 (87.5%)	14 (70%)	3.00	0.583	0.118 NS	0.74- 12.10
AC	5 (12.5%)	6 (30%)	0.33	0.2	0.118 NS	0.08- 1.35
CC	0	0	0	0	0	0
Total	40	20				
Allele distribution						
A	75 (93.75%)	34 (85%)	2.65	0.583	0.137 NS	0.72- 9.94
C	5 (6.25%)	6 (15%)	0.38	0.093	0.137 NS	0.10- 1.39
NS: Non-Significant.						

Table 11: Expected frequencies of genotypes and allele of the -27208 A/C using Hardy- Weinberg equilibrium.

Genotypes		AA	AC	CC	A	C	□2
Patients Genotypes	Observed no (%).	35 (87.5%)	5 (12.5%)	0	0.94	0.06	0.18
	Expected no (%).	35.16 (87.9%)	4.69(11.73%)	0.16 (0.4%)			
Control Genotypes	Observed no (%).	14 (70%)	6 (30%)	0	0.85	0.15	0.62
	Expected no (%).	14.45(72.25%)	5.1(25.5%)	0.45 (2.2%)			

*□2>3.84: results showed no significant differences under ($p < 0.05$) between observed and expected frequencies for patients and control.

Table 12: Distribution of genotype and allele frequency of ST2 SNP (-27128 C/T) between different groups.

-27609 A/T Genotypes	Patients NO (%)	Control NO (%)	OR	ET or PR	Fisher's exact probability	CI 95 %
CC	40 (100%)	17 (85%)	16.20	0.927	0.017*	0.83- 316.91
CT	0	3 (15%)	0.06	0.15	0.017*	0.00- 1.21
TT	0	0	0	0	0	0
Total	40	20				
Allele distribution						
C	80 (100%)	37 (92.50%)	15.03	0.928	0.018*	0.77- 292.16
T	0	3 (7.50%)	0.29	0.032	0.714 NS	0.01- 6.36
* (P<0.05). NS: non-significant						

Table 13: Expected frequencies of genotypes and allele of the -27128 C/T using Hardy- Weinberg equilibrium.

Genotypes		CC	CT	TT	C	T	□2
Patients Genotypes	Observed no (%).	40 (100%)	0	0	1	0	NAN
	Expected no (%).	40 (100%)	0	0			
Control Genotypes	Observed no (%).	17 (85%)	3 (15%)	0	0.93	0.8	0.13
	Expected no (%).	17.11(86%)	2.78(14%)	0.11 (0.55%)			

* □2>3.84: results showed no significant differences between observed and expected frequencies for patients and control.

Many studies support the role of IL-33/ST2 pathway in various fields of cancer (9, 32). In the present study, the effect of ST2 distal promoter region polymorphism (825bp) was studied on the susceptibility of breast cancer. Six polymorphisms -27609 A/T, -27607 A/G, -27601 A/T, -27595 A/G, -27208 A/C, -27128 C/T was identified in this region only one polymorphism (-27128 C/T) of which had significant difference under (P<0.01) according fisher's exact probability. The ST2 distal promoter region is highly polymorphic site, also a locus with great diversity among different racial and ethnic groups (23). In genetic study there is no previous study assessing the impact of ST2 distal promoter genetic polymorphism and breast cancer. This is the first study for the ST2 and breast cancer.

The result of the SNP (-27609A/T) the genotypes TT, AT record close frequencies in patients and control and the frequency of allele T was higher in patients than in control, according to fisher's exact probability T allele record as an etiological fraction with an odds ratio more than one (1.29) while A allele record as a preventive fraction with odd ratio less than one (0.78) in breast cancer patients. While the result of the SNP (-27607 A/G) showed the homozygous genotype (GG) is the only genotype noticed in Iraqi female population this indicate the common allele is G allele this different result may because different ethnic between Iraqi population and other population. The homozygous genotype TT of (-27601A/T) SNP is the most common genotype in present study showed groups which it records high frequency in patients and control, and allele T is the most common allele according to fisher exact record as a preventive fraction with odd ratios (0.74). While A allele record as etiological fraction with odd ratio (1.36) in Iraqi patients with breast cancer. In SNP (-27595A/G) homozygous genotype GG showed a high frequency in patients and control, and G allele represent the common allele in patients and control, according to fisher's test is recorded a preventive fraction with odd ratios (0.73) while A allele record etiological fraction with odd ratios (1.37) in breast cancer patients. The result of (-27208 A/C) showed a high frequency of homozygous genotype AA in patients and control and allele A record as etiological fraction according fisher's test with odd ratios (2.65) while the C allele record as a preventive fraction with odd ratios (0.38) in patients. No association was found between this SNPs and breast cancer disease. While the result of SNP (-27128C/T) showed significant difference between patients and control, the homozygous genotype CC showed high frequency in patient and control and represent the common genotype in Iraqi population, heterozygous genotype CT was observed in control group only, C allele record as etiological fraction with odd ratio (15.03) while T allele record as preventive fraction with odd ratio (0.29), according this result C allele from (-27128C/T) SNP may be have role to be the risk of breast cancer in Iraqi females individual with CC genotype may became susceptible for disease. There was no previous study about association between this SNPs and breast cancer however some studies have assessed the role of ST2 genetic polymorphism on the ST2/IL33 pathway activity and they established an association between atopic dermatitis and

asthma in children with the -26999 A allele in the distal promoter region (8, 33). And other study was reported two new polymorphisms in ST2 distal promoter polymorphism -27307 T/A and -27614 C/A on susceptibility and angiographic severity of coronary artery disease (23).

CONCLUSION

In concluding this study found significantly elevated serum level of ST2 in patients compared with control this elevation of serum concentration of sST2 could consider as a clinical biomarker in breast cancer female patients and may be associated with poor prognosis in breast cancer patients. According to the results SNP (-27128C/T) showed significant differences between patients and control, T allele from (-27128C/T) SNP may have role to prevent the risk of breast cancer in Iraqi females. Further studies about the ST2 gene should conducted in order to shed a light on the relationship between polymorphism and susceptibility for breast cancer.

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