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Evaluation of the Circulating IL-12 in Women with Breast Cancer in Erbil City

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Abstract

The current study investigated that malignant breast cancer (BC) was shown to be associated with a single nucleotide polymorphism (SNP) on chromosome 12 in the IL-12 subunit p40 gene (rs3212227, A/C) malignant BC. In an Erbil city case–control research involving 140 infected persons and 70 healthy women, the allelic and genotypic occurrences of SNPs were examined. Having the minor allele (A) raised the chance of getting malignant BC by a whopping 109% (95 percent confidence interval [CI]: 0.68 to 1.74). P < 0.05; OR = 0.92; 95 percent confidence interval [CI]: 0.57 to 1.47) was found for the primary allele (C). Genetic assessments in the current study have found that homozygosis in the CC and AA allele groups (P< 0.05; OR = 1.06 & 0.94, 95 percent confidence intervals: 0.54 to 2.06 & 0.46–1.91) was related with a developed hazard of malignant BC, while the CA allele group (P< 0.05, OR = 0.80, 95 percent confidence intervals: 0.37–1.70) was associated with a lower risk. There was no link between the heterozygous genotype and breast disease in the research. There may be a link between the A allele's increased production of IL12B p40 mRNA and an increased risk of BC, but further research is needed to confirm this.

Keywords: Breast cancer, Interleukin 12, Single Nucleotide Gene polymorphism.

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تقييم انترلوكين -12 الدائر في الدم لدى النساء المصابات بسرطان الثدي في مدينة اربيل

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الخلاصة

حققت الدراسة الحالية في أن سرطان الثدي الخبيث (BC) حيث شوهدت أنه مرتبط بتعدد أشكال نيوكليوتيد واحد (SNP) على الكروموسوم 12ل IL-12للوحدة الفرعية p40 في جين سرطان الثدي الخبيث (rs3212227, A/C) . تم فحص التكرارات الأليلية والوراثية للنيوكليوتيدات SNPs في دراسة الحالات والشواهد في مدينة أربيل، شملت 140 شخصًا مصابًا و 70 امرأة سليمة. أدى وجود الأليل الصغير (*A*) إلى زيادة فرصة الإصابة بالورم الخبيث BC بنسبة هائلة بلغت 109٪ (فاصل الثقة% CI:95 0.68 إلى 1.74, P <0.05 . (OR= 0.92 ؛ فاصل الثقة% 95: CI 0.57 إلى 1.47) للأليل الأولي *C* . وجدت التقييمات الجينية في الدراسة الحالية أن التماثل الزيجوت في مجموعات أليل CC و AA، OR = 1.06 & 0.94 ، فواصل الثقة 95 في المائة: 0.54 إلى 2.06 و 0.46-1.91) كان مرتبطًا بخطر متطور لورم خبيث BC، بينما أليل CA المجموعة(P <0.05 ، OR = 0.80، 95٪ فواصل الثقة: 0.37-1.70) ارتبطت بمخاطر أقل. لم يكن هناك ارتباط بين النمط الجيني متغاير الزيجوت وامراض الثدي في البحث. قد يكون هناك ارتباط بين زيادة إنتاج الأليل *A* لـ IL12B p40 mRNA وزيادة خطر الإصابة بـ BC ، ولكن هناك حاجة إلى مزيد من البحث لتأكيد ذلك.

كلمات مفتاحية: سرطان الثدي ، إنترلوكين 12 ، تعدد الأشكال الجيني أحادي النوكليوتيدات.

Introduction

More than 85% of the time, BC originates in cells of the glandular tissue's thin outer layer (epithelium) (15 %). At this point in the tumor's development, cancer has not spread to other areas of the body (Ginsburg et al., 2020).

According to the American Cancer Society's latest estimates, female BC has now surpassed female lung disease as the most commonly diagnosed cancer in women, accounting for an projected 2.3 million modern infections per year (11.7 %). Moreover, 90% of cancer deaths were caused by colorectal cancer, and eight percent by liver cancer. Lung cancer caused 88%

of cancer deaths overall. (Sung et al., 2021). Breast cancer is now more common than lung cancer in both developed and developing countries. In Eastern Africa, the incidence rate of BC is 19.3 cases per 100,000 women, while in a number of developed European countries; the rate is 89.7 cases per 100,000 women. They're high (above 80 per 100,000) in industrialized parts of the globe (outside of Japan) and low (less than 40 per 100,000) in most poor countries, according to a recent research (Zaidi & Adlane Dib, 2019).

In the year 2020, in 276,480 estimated BC new cases, 42,170 estimated deaths are expected in women in the USA representing the principal reason of death amongst US women also calculate the entire number of cancer deaths prevented because of the continuous reduction in cancer death proportions during the early 1990s mainly due to mammography screening programs (Siegel et al., 2020).

Additionally, male BC death rates were considerably higher in transitional nations than in transitioned nations (15.0 per 100,000 and 12.4 per 100,000). The worldwide cancer burden is predicted to climb to 28.4 million new cases in 2040 as a result of demographic transitions, a 47 percent increase from 2020, with a superior escalation in transitioning nations (64 percent to 95 percent) than transitioning countries (32 percent to 56 percent). However, increased risk influences connected with globalization and a booming economy might worsen this (Sung et al., 2021).

Furthermore, on the basis of the most recent Iraqi Cancer Registry data, BC is the most communal method of female cancer in Iraq, attributing for approximately one-third of all female cancer cases recorded. This shows that BC is the most widespread cancer type in Iraqi women as a whole, even exceeding bronchogenic cancer. Karbala residents were revealed to have a variety of malignancies throughout the (2008- 2015). As well, according to the data, the frequency and age-specific rate of several types of cancer differed between men and women. Breast cancer is more common among women in British Columbia than everywhere else (AL-

Janabi et al., 2017). Iraqi women are more likely than the general population to be diagnosed with BC (Al- Hashimi and Wang, 2014).

Notwithstanding, it is widely established that cytokines of the IL-12 family regulate both distinctive and adaptive immune rejoinders. The cytokines of the IL-12 family have been extensively researched in the context of infection and autoimmune disease. There has been a great deal of investigation into how these cytokines impact the immune response to cancer. Likewise, cytokines of the IL-12 family are typically generated by innate immune cells, although adaptive immune cells can also release them depending on the disease or immune environment (Mirlekar and Pylayeva-Gupta, 2021). Since IL-12 may both activate innate (NK cells) and adaptive (cytotoxic T lymphocytes) immunities insusceptibility, it appears to be most suited for tumor immunotherapy. Cancer risk has been shown to be modulated by SNPs in the IL-12 genes IL12A and IL12B, in diverse populations and cancer types (Nunez-Marrero et al., 2020).The current study was aimed to evaluate the circulating IL-12 in women with breast diseases and also, to demonstrate the association of IL-12 +1188 SNPs with BC in Erbil city.

Materials and Methods

Subjects

One hundred and forty female patients who referred for operation with breast disease and BC were registered in the existing investigation. None of the infected people had been dealt with chemical therapeutics or radio-therapy before sample collection. Breast disease was diagnosed and confirmed by medical oncologists according to the surgical and pathological reports based on a clinical review and research laboratory test results.

Moreover, this study was conducted on patients affiliated simultaneously to several general and private hospitals in Erbil city. 70 patients were having malignant BC and another 70 patients were having benign breast disease. The mean age for BC patients were 33.5 and benign breast

disease were 52.5 respectively. 70 healthy female subjects (seemingly), were considered as a control group and their age range was 21 -66 years (mean 43.5 years).

Assessment of Cytokine Serum Levels

Sera of patients and controls were analyzed using commercially available kits (SUNLONG BIOTECH CO., LTD) using ELISA based on similar concepts for the level of cytokine (IL-12).

Genotyping of Cytokine Polymorphisms

There are several approaches for assessing genotypes of polymorphic genetic loci in individual patients. For their determination, most loci have only a limited number of identified methods. The polymorphic loci investigated in this analysis were all SNPs in the loci IL-12 on chromosome 5q31–33 and 3p12–q13.2.

The polymerase chain reaction was carried out with 2X Prime Taq Premix (GeneAll® Exgene TM Blood SV micro (105-101/ 105-152), Korea). In addition, we were able to carry out two independent procedures for each polymorphism using two tubes per sample. Two different forward primers, one for each allele, were employed in each reaction, as well as an anti-sense primer to ensure accuracy. Each 0.20 mL reaction comprised 1 l of each primer, 1 l of genomic DNA (100 ng/mL), 10 l of 2X Prime Taq Premix (GeneAll® ExgeneTM Blood SV small (105-101/ 105-152), Korea) and 7 l ddH2O. An electrophoresis technique utilizing a 2% agarose gel was utilized to separate the amplified components.

Primer design and Protocols:

Web-based primer design tool (Primer1) was used to produce IL-12 primers for ARMS PCR and may be accessed at http://primer1.soton.ac.uk/primer1.html. The distinctiveness of the primers was determined using another web-based tool (Primer-BLAST), according to Collins and Ke (2012). The primers used were selected based on the prevalence of minor alleles (MAF 10). Similarly, researchers utilized allele-specific PCR and customized primers to detect the SNPs. The PCR amplification procedure was performed once the DNA had been extracted. The

PCR primers used were: ATGCCAAAGTGCTGAGTCACTA-3' is the proper response (common primer), 5'-TCGAGGGCAGAATGAGCCTC-3' (C allele primer), and 5'-TTGTTTCAATGAGCATTT AGC ATC G -3' (A allele primer) for IL12 A +1188 (rs3212227 A/C), the gene has its relative forward primers, with only one reverse for each SNP A & C.

Statistical Analysis

The GraphPad Prism 8 platform and the Student's t test were used to appraise the alterations in cytokine serum levels via an analysis of variance (ANOVA) approach. The statistical significance threshold was set at P values equal to or less than 0.05. There was a one-way ANOVA on rankings and a multiple comparison test (Dunn's) performed to compare different groups of women with breast cancer. Contrasting cytokine gene studies are estimated using Baptista-Pike odds ratio methods. The Fisher's exact test compares breast cancer types (benign and malignant) with those in a control group by utilizing a two-tailed P value.

Moreover, a Fisher's exact probability test for significant differences compared the IL12 +1188 allele and genotype distributions in BC patients and controls by means of a dual-tailed Fisher's exact test (p). In addition, the etiological fraction (EF) and relative risk (RR) were computed to discover the connection between alleles and genotypes and disease (Gonzalez-Galarza et al., 2011). Risk was estimated using odds ratio statistic with 95% confidence interval together with assessment of etiologic as well as preventive fractions.

Results and Discussion

Serum Level of IL-12

Total Patients

IL-12 cytokine showed significant variations in benign breast disease and malignant BC patients. The serum level of IL-12 in benign and malignant patients was considerably ($p<0.05$)

decreased when compared with controls (82.88±8.07 vs. 173.5 ± 28.34 pg/ml and (24.41± 1.55 vs. 173.5 ± 28.34 pg/ml).

Table 1: Serum level of IL-12B 1188 AC in total Benign and Malignant BC patients and controls.

Cytokine Serum Mean Level ± S.D. (pg/ml)			
Cytokine (N=70)	Breast disease Patients		Control
	Benign (N=70)	Malignant (N=70)	
IL-12B 1188 AC	82.88±8.07b	75.97±15.20b	173.50±28.34a

The current study results investigated whether immune system impairment manifested by reduced IL-12 levels ensue in BC patients. The present study demonstrated evidence indicating that IL-12 were decreased in BC patient's serum, suggested that tumor derived IL-12 were associated with tumor progression. These findings are compatible with a study finding that indicated the reduced IL-12 production may contribute in the tumor formation and development (Jafarzadeh et al., 2015). Also, the outcomes of this research are compatible with the conclusions of Ali, (2016), who revealed that lower serum level of IL-12B +1188 AC may be associated with the AA genotype, in addition, The SNP IL-12p40 +1188A genotype has been linked to lower levels of IL-12p40, an IL-12 subunit.

The 3'UTR of the coding gene has the SNP IL-12p40 +1188A/C, which has an effect on the gene's function. Additionally, this region, which does not code for proteins, has the power to affect protein translation because of its impact on mRNA stability and transcriptional/posttranscriptional activity (Matoulkova et al., 2012). Because of this, the SNP might have an effect on gene silencing and IL-12 mRNA expression levels (Kaarvatn et al., 2012).

IL12B Gene SNP at +1188 Position (rs3212227): -

Gene polymorphism of the studied cytokines IL12 was done by ARMS-PCR. The IL12B gene SNP at position +1188 was presented with two alleles (A and C) and three genotypes (AA, AC and CC). There is a significant disparity existed between observed and projected genotype

frequencies in BC patients ($p < 0.05$), and these genotypes did not match HWE at all in controls (Table 2). Research on the IL-12 +1188 gene's polymorphism was carried out at the site (rs3212227). IL-12 +1188A/C polymorphism role in patients' susceptibility to BC was evaluated in comparison to healthy persons in this study (control group). Figure 1 shows the findings of allele-particular PCR for the SNP IL-12 +1188A/C in BC patients and controls, correspondingly.

Therefore, genotypic study of the IL12B SNP indicated significant differences between the patients and the controls. Those who were homozygous for the major allele (CC) had a 95 percent confidence interval of 0.54 to 2.06 times higher risk of breast cancer, as seen in Table 2. There was an arithmetically substantial alteration ($P < 0.05$) seen for all transporters of the foremost allele (AA), with 42.86 percent of breast cancer patients (30 /70) having the greater allele compared to 41.43 percent of women in the healthy group (29 /70). Those with a modest heterozygous genotype (CA) heterozygosis had a reduced risk of illness ($P = 0.05$; OR = 1; 95 percent CI: 0.48 to 2.01). A higher or major allele (C) was associated with a 52.86 percent reduced chance of developing the disease, since it was identified in half of the patients compared to controls, and was found 50% of the time in the patient group (vs. 55 percent in controls).

Table 2: Detected numbers and proportion occurrences and H-W steadiness of *IL-12 +1188* genotypes and alleles in Benign, Malicious BC patients and controls.

GROUPS			*IL12 +1188 GENOTYPE* OR ALLELE					H-W P ≤ 0.05
			CC	CA	AA	*C*	*A*	
BENIGN (N=70)	Observed	No.	30	19	21	79	61	Not Significant
		%	42.86	27.14	30	56.43	43.57	
	Expected	No.	22.29	34.42	13.29	Not Estimated		
		%	31.84	49.17	18.99			
Malignant (N=70)	Observed	No.	30	16	24	74	66	Significant
		%	42.86	22.86	34.29	52.86	47.14	
	Expected	No.	20.6	34.8	14.6	Not Estimated		
		%	29.43	49.71	20.86			
CONTROLS (N=70)	Observed	No.	29	19	22	77	63	Not Significant
		%	41.43	27.14	31.43	55	45	
	Expected	No.	21.17	34.66	14.17	Not Estimated		
		%	30.24	49.51	20.24			

Table 3: Statistical assessments of relations between IL-12 1188 genotypes or alleles, Benign and Malicious BC patients.

IL12 +1188 GENOTYPE OR ALLELE	STATISTICAL EVALUATIONS/ BENIGN				STATISTICAL EVALUATIONS/ MALIGNANT			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
CC	1.06	0.02	Not significance	0.54 to 2.06	1.06	0.02	Not significance	0.54 to 2.06
CA	1	ref	Not significance	0.48 to 2.10	0.80	0.06	Not significance	0.37 to 1.70
AA	0.94	0.02	Not significance	0.46 to 1.91	1.14	0.04	Not significance	0.56 to 2.29
C	1.06	0.03	Not significance	0.66 to 1.70	0.92	0.05	Not significance	0.57 to 1.47
A	0.94	0.03	Not significance	0.59 to 1.51	1.09	0.04	Not significance	0.68 to 1.74

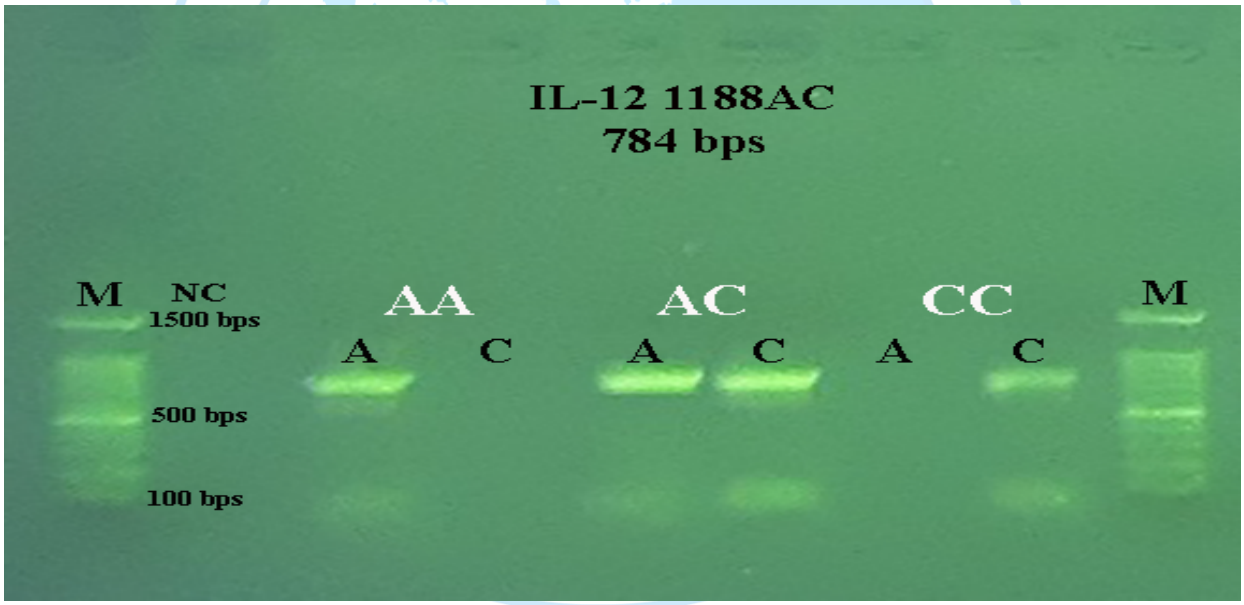

Figure 1: IL-12 1188 AC product of ARMS-PCR on agarose gel (2%) amplicon size (784bp): M: 100bp size DNA ladder. The 784 bp signifies the intensification of IL-12 1188A/C.

In this study, it was theorised that the IL-12 +1188A/C SNP affect the transcriptional levels of the cytokine may influence Th-1 immune responses to tumour cells, thus increasing susceptibility to MBC development. The presence of CC and AA genotype in the current study had significant association with BC development and act as etiological factor. The difference in distribution of the A and C allele in Benign breast disease, malignant BC patients and controls

were also significant. Correspondingly, the findings of the current study are in agreement with results of Kaarvatn et al., 2012, who demonstrated that patients with BC had considerably advanced allelic incidences of the main A allele of IL12B SNP (A>C, rs3212227) signifying that this allele deliberated augmented hazard for disease development expansion in Croatian Caucasian inhabitants.

Also, the results of the current study are confirmed by a study who established that individuals carrying homozygous wild type genotype AA are high IL-12 cytokine producers while those with a heterozygous state produce intermediate levels of the cytokine. Carrying the homozygous mutant genotype CC leads to reduced production of the cytokine (Oluocha, 2018). Interleukin-12 is heterodimeric and consists of two covalently linked P35 and P40 subunits (Lasek et al., 2014). The subunits are encoded by IL-12A and IL-12B genes which are positioned on chromosome 3p12q-q13.1 and 5q31-33 correspondingly (Sieburth et al., 1992). Studies have reported multiple polymorphisms on IL-12B gene including an IL-12B promoter region and a TaqI 3'UTR which leads to a transition from A to C at position +1188 (Seegers et al. 2002). Increased production of IL 12 by activated human monocytes has been linked to the TaqI polymorphism (+1188A/C) in TaqI. According to Kaarvatn et al. (2012), BC patients had substantially higher allelic occurrences of the main (C) allele of the IL12B SNP (rs3212227), showing that this allele was linked to an elevated risk of disease development in Erbil women. However, the allele A act as predisposition to the malignant BC.

Furthermore, immune checkpoint inhibitor, IL-12, helps prevent cancer from developing and progressing (Sangro et al., 2005). If a person's DNA sequence changes, the amount of IL-12 they produce influences their risk of cancer. For example, the IL12B 3'UTR A greater than C polymorphism, a significant functional SNP, affects Il-12 production and was found as a possible risk biomarker for several types of cancer. According to (Zhou et al., 2012), in a study done by (Abd-Alraoof, 2019), showed that the 3'UTR A/C (rs3212227) polymorphism IL-12 +1188 have been associated to a higher risk of cancer among Asians, particularly cervical and nasopharyngeal malignancies.

Conclusions

To sum up the information, this research has outlined the main factors associated with the appraisal of the circulating IL-12 in women with breast cancer in Erbil city. Moreover, based on the literature review it was concluded that malignant breast cancer was shown to be linked to an abnormal expression of the interleukin-12 subunit p40 gene. MBC risk increases if you have the minor allele (A). Furthermore, it has been illustrated that two of the three possible genotypes are found for the IL-12p40 +1188A/C SNP. The SNP IL-12p40 1188A/C increases the risk of BC illness among Erbil residents with the AA and CC genotypes. The blood levels of IL-12p40 were substantially lower in cancer patients compared to the healthy controls. Poor IL-12 p40 subunit production is allele dependently related with CA heterozygosis (CC > AA > AC), with the strongest inducer being CC and the lowest inducer being AA. A link exists between low levels of the IL-12p40 gene and the C allele frequency, as shown by this finding.

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