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The Effect of Genetic Polymorphism of *Resistin* Gene among Iraqi Breast Cancer Women

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Abstract: Breast cancer is a heterogeneous disease defined by molecular types and subtypes. It constitutes the most commonly-diagnosed cancer and the leading cause of cancer death in women, worldwide, according to the International Agency for Research on Cancer (IARC) World Cancer Reports in 2020. The study aimed to detect the relationship between *Resistin* Gene polymorphism (rs1862513, rs3745367) with the age and BMI of the participants diagnosed with breast cancer. A total number of 105 samples; three groups of 35 fresh blood samples were collected as malignant, benign and apparently healthy control. Conventional PCR with specific primers followed by sanger sequencing was used to characterize the two SNPs. The results revealed that (rs1862513) GG allele carriers (GG) had a breast cancer risk of (1.78) in compare with (CG) allele carriers; (OR=0.337). Genotyping and allele frequency of *RETN* gene at SNP (rs3745367) in three groups (control, benign and malignant) showed that AA allele with (OR=1.44) may considered as a risk factor for incidence of breast cancer. The results showed that the incidence of breast cancer was significantly higher in middle age group (40-49) year with a percentage of 42.85%. It was concluded the relation of BMI and breast cancer remains conflicting, the study show a negative correlation of BMI with tumor size, stage and grade of the tumor.

Keywords: *RETN* gene, rs1862513, rs3745367, Breast Cancer, BMI.

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Introduction

Breast cancer is the most detectable malignancy in females; it is the second cause of death worldwide. The risk of developing breast cancer is modified by various factors including age, reproductive and gynecological factors, and physical activity, consumption of alcohol and tobacco, as well as family history (1, 2 and 3).

Traditional genetic methods approaches for assessing breast cancer risk are still limited. According to research, genotyping single nucleotide polymorphisms (SNPs) may help forecast an individual's risk of breast cancer and guide disease therapy. Certain SNPs have an effect on breast cancer susceptibility. Breast cancer risk is increased in individuals who carry *BRCA1* and *BRCA2* gene mutations as well as genetic polymorphisms such as high-mobility group box protein 1 (HMGB1) and fascin-1 (FSCN1) (4- 9).

Resistin is a small cysteine-rich adipokine encoded by the *RETN* gene is secreted by adipose tissue or constitutively secreted by macrophages. There is *in vitro* evidence of up-regulated *RETN* gene expression in samples of human breast cancer tissue and polycystic ovary syndrome (15). SNPs are found in the *RETN* promoter and 3'-untranslated regions. Genetic

variation at the *RETN* locus carries a risk of several diseases, including the breast cancer. Previous study, carried out in Mexico has reported that the (rs1862513) SNP in *RETN* increased breast cancer risk (8, 9). Common SNPs in the *RETN* gene, including promoter rs1862513 (C-180G/ C-420G) and rs3745367 (G + 299A), have been previously analyzed for their contribution to the progression of several diseases, including breast cancer and other diseases. The meta-analysis of the *RETN* rs3745367 polymorphism in colorectal cancer and breast cancer, found no statistically significant risk association in any genetic model (10-16). Resistin concentration is associated with obesity and insulin resistance. It also involved in tumor progression through the activation of inflammation and the expression of adhesion molecules, promoting the proliferation, metabolism and invasion of tumor cells (5). It has been reported that Resistin can also confer resistance to chemotherapy in breast cancer cells, maybe there is a relation between adipocytes respond to the stimulation of excessive fat and thus initiate downstream cytokine reaction in breast cancer progression (6, 7). However, Resistin gene has more attention recently because it has been linked to increased risk of progression, angiogenesis and metastasis in various cancer models. Its role has been also associated with chemoresistance and stemness induction in cancer (3).

Material and methods

A total of 105 fresh blood samples were collected from the Oncology teaching Hospital/Medical City /Baghdad. (From March 2021 to September 2021). The sample categorized into three groups. Malignant group (Ductal, Lobular), benign group (Fibroadenoma), and control group collected from the negatively tested samples. Each group contains 35 samples. The patients were divided into five groups according to age, these groups ranged from less than 20 year old to upper than 60 year.

Genomic DNA was extracted using Genomic DNA Extraction Kit (Favorgen / Canada) and the extraction was carried out following the manufacture instruction. PCR reaction was performed using Veriti thermocycler (ABI, USA) by applying a specific primers Table (1), and the following reaction parameters. initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification including denaturation at 95°C, annealing at 55°C, extension at 72°C (each comprising 30 s), and the final extension at 72°C for 5 min. The PCR product was checked by electrophoresis on 1.5% agarose gels with ethidium bromide (10 ng/100 mL of agarose solution in Tris borate EDTA buffer). All the genotypes were independently verified by Sanger sequencing using the previously prepared PCR amplicon (Macrogen, Seoul, South Korea). The Statistical Analysis System-SAS software was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) (11).

Table (1): Specific primers for RETN single nucleotide polymorphisms.

Primers	Primer Sequence(5'-3')	Product size(bp)
F- rs3745367	ATCAATGAGAGGATCCAGGAG-	600
R- rs3745367	AAGATCCTAGGGGAGTAGAGG-	600
F- rs1862513	TTTGTCATGTTTGCATCAGC-	469
R- rs1862513	ATGGAGGGAGTAGGATCTGC-	469

Results and discussion

Results revealed that most of the patients were between (40-49) years old with a percentage of 42.85% and the lowest in first group with age less than 20 as the percentage was 0 %. The benign group also showed that most of them were between (40-49) years old with a percentage of 42.85% and the lowest were with age less than 20 as the percentage was 0 %.

The three groups (control, benign and malignant) were subdivided into five classes according to their Body Mass Index (BMI kg/m2); normal weight (18.5-24.9), overweight (25-29.9), obesity class1 (30-34.9), obesity class 2 (35-39.9), obesity class 3 (≥40). In benign group 5 (14.28%) were with normal weight, 10 (28.57%) overweight, 14 (40%) were obesity class, 4 (11.42%) were obesity class II, and only 2 (5.71%) were obesity class. In patients group 5 (14.28%) were with normal weight, 14 (40%) overweight, 6 (17.14%) were obesity class I, 8 (22.85%) were obesity class II, and only 2 (5.71%) were obesity class III. (Table 2). The exact relation of BMI and breast cancer remains conflicting even after numerous studies. In a meta-analysis, the clinical importance of obesity on breast cancer risk was not seen (13).

The genotype variation of (rs1862513) depends on Hardy-Weinberg equilibrium ($p>0.01$) was as follow; in patients with malignant tumor CC 9 (26%), CG 12 (34%), GG 14 (40%). The benign group was; CC 16 (46%), CG 10 (28%), and GG 9 (26%). While in control group; CC 26 (74%), CG 9 (26%), and GG 0 (0%). According to the results there was a statistically significant difference in GG genotype compared with CC with OR (1.78), and it could be considered as a risk factor for breast cancer Iraqi women. While no significant with the CC and CG allele frequency (Table 3). A previous study in India found that CG genotype carriers had a 1.59- fold of risk of breast cancer (14). This association could reflect a risk of the tumor process itself and tumor progression through low-grade chronic inflammation. The SNP rs1862513 has been associated with increased transcriptional rate of resistin in the presence of guanidine (19).

The genotype variation of (rs3745367) was as follow; in patients with malignant tumor GG 7 (20.00%), GA 18 (51.43%), AA 10 (28.57%). The benign group was; GG 7 (20.00%), GA 19 (54.29%), and AA 9 (25.71%). While in control group; GG 24 (68.57%), GA 9 (25.71%), and AA 2 (5.71%). According to the results there was statistically significant difference with AA genotype compared with GG with OR (1.44), and it could be considered as a risk factor for breast cancer Iraqi women (Table 4).

A Chinese study examined associations between *RETN* single nucleotide polymorphisms (SNPs; rs3745367, rs1862513) and breast cancer susceptibility. Between-group differences were not significant for the proportions of breast cancer patients

with the rs3745367 and rs1862513 polymorphisms, as compared with healthy controls (9). Another meta-analysis study revealed that this *RETN* polymorphism significantly increased the risk of cancer.

The rs1862513 variant (CG, GG) significantly increased the risk of colorectal and breast cancer, but no correlation between the rs3745367 polymorphism (GA, AA) and cancer risk (18,19,20).

Cancer is a complex disease, and it has been proposed that individual genetic variants may only have a modest independent effect on the disease. Adipokines, secreted by the adipose tissue, are convincing candidates for the relationship between obesity and cancer risk (21, 22, 23). The patients were divided into five groups according to age; these groups ranged from less than 20 year old to upper than 60 year, the results than 20 as the percentage was 2.8 %. The demographic and clinical profiles of breast cancer in Alwan and Shawkat study clearly illustrate the prevalence among premenopausal women; where the peak age frequency is noted in the middle aged (30-49) groups (13, 17). These results revealed that breast cancer risk exist at any age, but it was increase at the middle age of women's life, and that also confirms the need of screening and early detection of breast cancer.

Table (2): Comparison between difference groups in Age, BMI and Age at first child birth.

Group	Mean ± SD		
	Age (year)	BMI (kg/m^2)	Age at first child birth(year)
Control	33.02 ±11.32 c	24.94 ±5.13 b	**16.45 ±7.30**
Benign	42.40 ±12.24 b	30.57 ±5.20 a	**20.08 ±7.85**
Malignant	49.68 ±10.77 a	31.15 ±6.62 a	**19.20 ±9.40**
LSD value	5.434 **	2.701 **	**3.905 NS**
P-value	**0.0001**	**0.0001**	**0.163**

Table (3): Genotype and allele frequency of RETN gene at SNP (rs1862513) in three groups (control, benign and malignant).

Genotype RETN	Control N=35	Benign N=35	Malignant N=35	χ2	OR	CI
CC	26(74%)	16(46%)	9(26%)	13.84 **	Reference	--
CG	9(26%)	10(28%)	12(34%)	1.772 NS	0.337	**0.08-0.79**
GG	0(0%)	9(26%)	14(40%)	12.75 **	1.78	**1.02-3.67**
Allele						
C	0.87	0.60	0.43	--	--	--
G	0.13	0.40	0.57	--	--	--
** (P<0.01).						

Table (4): Genotype and allele frequency of RETN gene at SNP (rs3745367) in three groups (control, benign and malignant)

Genotype *RETN*	Control N=35	Benign N=35	Malignant N=35	χ^2	OR	CI
GG	24 (68.57%)	7 (20.00%)	7 (20.00%)	11.57 **	Reference	--
GA	9 (25.71%)	19 (54.29%)	18 (51.43%)	8.63 **	1.37	**0.72-2.36**
AA	2 (5.71%)	9 (25.71%)	10 (28.57%)	8.92 **	1.44	**0.89-1.92**
Allele						
G	0.81	0.47	0.46	--	--	--
A	0.19	0.53	0.54	--	--	--
**** (P<0.01).**						

Figure (1): Electrophoresis of *Resistin* gene (rs1862513 C>G) on agarose gel (2%) for 90mins at 70 Volt/cm in presence of DNA ladder marker.

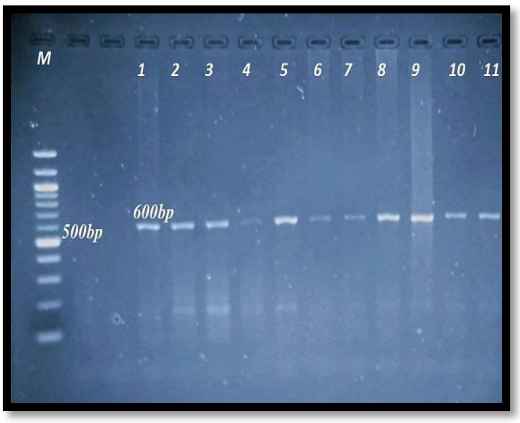

Figure (2): Electrophoresis of *Resistin* gene (rs 3745367 G>A) on agarose gel (2%) for 90mins at 70 Volt/cm in presence of DNA ladder marker.

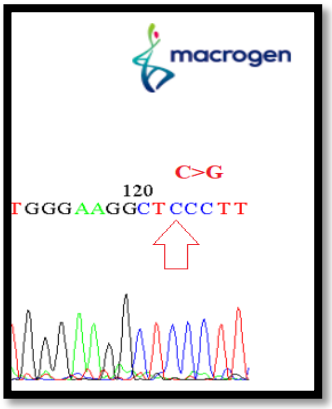

Figure (3): SNP location for rs 1862513 C>G

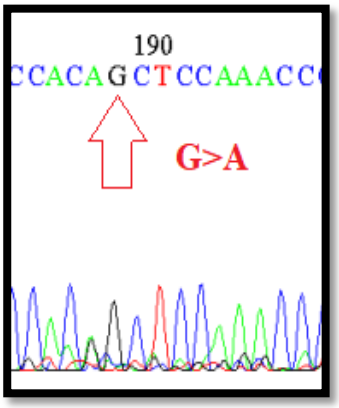

Figure (4): SNP location for rs 3745367 G>A

Conclusion

In conclusion, our investigation demonstrates an association between *RETN* gene variants and susceptibility for breast cancer and its progression among some Iraqi women carrying the *RETN* rs3219175 and rs7408174 polymorphisms.

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