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RESEARCH ARTICLE

RETN gene polymorphisms and serum resistin levels in patients with breast cancer

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ABSTRACT

Background: Increased serum resisitin levels act as an effective pro-inflammatory mediator, which may play a key role in cancer progression and promotion and may be caused by *RETN* gene polymorphisms. **Aim of the work:** We investigated serum resistin levels in breast cancer patients as well as the relationship between the *RETN* single nucleotide polymorphisms (rs7408174 and rs3219175) and breast cancer susceptibility. **Subjects and Methods**: This case control study included 80 breast cancer patients and 80 healthy controls. Genotyping of *RETN* rs7408174 and rs3219175 gene polymorphism were determined by real time polymerase chain reaction (RT-PCR) TaqMan allelic discrimination assay. Serum resistin levels were quantified using Enzyme-linked immunosorbent assay (ELISA). **Results:** Breast cancer patients had higher serum resistin levels compared to healthy controls. Patients with rs3219175 AG and rs7408174 CT/CC genotypes had significantly higher serum levels of resistin. Carriers of the single nucleotide polymorphism (SNP) rs3219175 heterozygous (AG) and homozygous (AA) genotypes had a significantly higher breast cancer risk than carriers of the wild (GG) genotype (Odd ratio (OR): 2.437; 95% CI: 1.262-4.707 and 3.091; 0.723-13.222, respectively). Patients carrying rs3219175 AG/AG+AA and rs7408174 CT/CC genotypes had a higher risk of developing advanced tumour size (T3+T4), lymph node (LN) infiltration, and advanced TNM stage (III+IV). **Conclusion:** There is a strong link between *RETN* SNP rs3219175 and high serum resistin levels and the risk of breast cancer. Furthermore, the *RETN* SNPs rs3219175 and rs7408174 are linked to poor clinicopathological status.

Keywords: Breast cancer, Resistin, *RETN* gene polymorphism

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INTRODUCTION

Breast cancer (BC) is considered one of the most diagnosed cancers among women with increasing mortality rates. Each year, an estimated 2.3 million new cases of BC are diagnosed worldwide (Barzaman et al.,2020). Several risk factors of developing breast cancer have been identified: non-modifiable risk factors such as age, genetic mutations, family history, reproductive and gynaecological factors, and modifiable risk factors such as obesity, smoking, lack of physical activity and hormonal therapy (Łukasiewicz et al., 2021).

Mammography screening and genetic testing have limited sensitivity and specificity for determining the level of breast cancer risk. However, genotyping of single nucleotide polymorphisms (SNPs) has shown to predict BC risk and help in disease prognosis and management (Kleibl et al., 2016).

Several genetic mutations, including BRCA1 and BRCA2 gene mutations, have been associated with a high risk of breast cancer (Abu-Helalah et al., 2020). Moreover, advances in DNA sequencing techniques, such as next generation sequencing, have assisted in the identification of other breast cancer susceptibility genes, such as TP53, CDH1, PALB2, and PTEN, as well as several rare gene variants that have been linked to an increased risk of breast cancer (Subaşıoğlu et al., 2023).

Resistin is a cysteine-rich protein released by macrophages and adipose tissue. It has been reported to act as a potent pro-inflammatory mediator and to promote cancer progression (Sudan et al., 2020). Resistin has been linked to cancers such as prostate, breast, and colorectal (Yang et al., 2016 , Zhang et al., 2019).

The *RETN* gene (Resistin coding gene) is located on chromosome 19, and various single nucleotide polymorphisms (SNPs) in the *RETN* promoter and 3'-untranslated region have been identified (Elkhattabi et al., 2019). Several studies have found a link between *RETN* genetic variants and the risk of multiple diseases such as metabolic syndrome, type 2 diabetes, and colorectal cancer (Zhou et al., 2017, Hashemi et al., 2018). *RETN* SNPs have also been linked to disease progression in patients with lung cancer (Hu et al., 2017). The *RETN* gene has been reported to be overexpressed in polycystic ovary syndrome and breast cancer tissue (Vallega et al., 2016, Nambiar et al., 2016).

Consequently, it can be hypothesized that breast cancer patients may have abnormal serum levels of resistin due to *RETN* gene polymorphisms that promote breast cancer development. Previous research has linked rs3219175 and other *RETN* gene polymorphisms to an increased breast cancer susceptibility and clinical staging (Wang et al.,2020, Sahan et al., 2022). Therefore, we aimed in this study to compare serum resistin levels in breast cancer patients to those in healthy subjects, and then investigate the possible relationship of *RETN* SNPs (rs3219175 and rs7408174) with breast cancer susceptibility, and prognosis.

SUBJECTS AND METHODS

Study design and settings

In this case-control study, 80 female patients with newly diagnosed breast cancer were recruited from Tanta University Hospitals' Oncology Unit, Egypt, from June 2021 to January 2022. Breast cancer had been confirmed histopathologically in all patients. Patients with history of other neoplasms, diabetes mellitus, inflammatory diseases and cardiac diseases and breast cancer under treatment were excluded**.** The control group comprised 80 age-matched healthy female participants, with a normal mammogram on routine checkup and free from chronic or acute illness.

Statement of Ethics

This study was approved by the Ethics Committee of the Faculty of Medicine, Tanta University and in accordance with the Declaration of Helsinki for experiments in humans (approval code 36174/12/22). All included subjects have provided written informed consent for the use of biological specimens for research purposes.

Data collection

The following sociodemographic and clinicopathological information were retrieved from medical records in all patients: age, BMI, tumour size, lymph node (LN) infiltration, distant metastasis, TNM staging, histological grading of the tumour and immunohistochemical study of progesterone receptor (PR), estrogen receptor (ER), and HER-2 status.

Blood samples

Five milliliters of venous blood was drawn from patients and health controls under complete aseptic conditions and subdivided into: 3 ml of blood into EDTA tube for molecular investigation, and 2 ml blood into plain tube then centrifuged at 3000g for 15 min, for serum resistin levels assay by ELISA. Separated serum and EDTA whole blood were stored at -80°C until used.

Genotyping of *RETN* gene single nucleotide polymorphisms (rs7408174 and rs3219175) by quantitative real-time PCR (RT-PCR) TaqMan allelic discrimination assay:

DNA extraction was performed following the manufacturer's instructions. DNA was isolated from the peripheral frozen whole blood samples by using DNA extraction kits (QIAamp DNA Blood Mini Kits Cat No./ID: 51104), then the extraction was stored in sterile 1 ml tubes at -20° C until the next step. Assessment of allelic discrimination for *RETN* SNPs was carried out using Applied Bio System, Step I Version). PCR was performed in a total volume of 25 µL, containing 3 µL genomic DNA, 12.5 µL TaqMan Universal PCR Master Mix (Thermo Fisher,

Applied Biosystems, Foster City, CA), 0.5µL probes and 9 µL nuclease free water. The sequence of 2 *RETN* SNP probes was described as follows:

- rs7408174,TTTTACCACAAAAAGGCCCGTTG TA[C/T]TGGAAACAAAGAA;
- rs3219175,CTCCAGCCCTTACTGTCTGCTCAG G[A/G]GCTTCCTCTTGGC.

The protocol included an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.
Data were further analysed with applied biosystem, step I version Software analysis modules.

Measurement of serum levels of resistin by ELISA: using Human Resistin (RETN) ELISA Kit from SunRed (Catalog no: 201-12-0339).

Data processing

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. Chi-square test was applied to investigate the association between the categorical variables. Alternatively, Monte Carlo correction test was applied when more than 20% of the cells have expected count less than 5. For continuous data, they were tested for normality by the Kolmogorov- Smirnov test Quantitative data were expressed as range (minimum and maximum), mean, standard deviation and median Student t-test was used to compare two groups for normally distributed quantitative variables. Odd ratio (OR) Used to calculate the ratio of the odds and 95% Confidence Interval of an event occurring in one risk group to the odds of it occurring in the non-risk group. Significance of the obtained results was judged at the 5% level.

RESULTS

Patient characteristics: Eighty breast cancer patients were included in our study, the majority (66.3%) of whom were under the age of 55, and 80 healthy individuals who were age matched as a control group. Between the 2 groups, there was no statistically significant difference in age (p=0.077; Table 1).

The body mass index of 62.5 % of breast cancer patients was more than 25 kg/m2. BMI differences between patients and controls were statistically significant (p=0.026; Table 1). Breast cancer patients had higher serum resistin levels, with a mean of 254.9 pg/ml as opposed to 115.5 pg/ml in the control group (p= <0.001; Table 1).

Tumor characteristics: T1 + T2 tumour size was found in 62.5% of the 80 patients, while T3+ T4 tumour size was found in 37.5%. LN infiltration was found in 51% of the patients. 5% of the patients had distant metastases. Sixty-three percent of the patients were in stages I+II, while 33.8% were in stages III+IV. Grade I cases made up 13.8% of the total, grade II cases made up 55.0%, and grade III cases made up 31.3%. The HER2neu mutation was found in 42.5% of the patients. The ER receptor was positive in 55% of the patients and the PR receptor was positive in 52.5% (Table 2).

Distribution of *RETN* rs3219175 and *RETN rs*7408174 genotypes among patients and controls

Among breast cancer patients, 41 of 80 (51.3%) had *RETN* rs3219175 polymorphism in the form of AG genotype and 6 of 80 (7.5%) had *RETN* rs3219175 polymorphism in the form of AA genotype whereas in the control group, only 26 out of 80 (32.5%) had *RETN* rs3219175 polymorphism in the form of AG genotype and 3 out of 80 (3.8%) had *RETN* rs3219175 polymorphism in the form of AA genotype. RETN rs3219175 polymorphisms varied between cases and controls in a manner that was statistically significant. (p=0.005; Table 3).

However, *RETN* rs7408174 genotypes did not differ significantly between the 2 groups. Among breast cancer patients, 30 of 80 (37.5%) had *RETN* rs7408174 polymorphism in the form of CT genotype and 5 of 80 (6.3%) had *RETN* rs7408174 polymorphism in the form of CC genotype while in the control group, 27 out of 80 (33.8%) had *RETN* rs7408174 polymorphism in the form of CT genotype and 4 out of 80 (5,0%) had *RETN* rs7408174 polymorphism in the form of CC genotype (p=0.521; Table 3).

Table 1. Age, BMI, and serum resistin level among the studied groups

	Patients (n = 80)	Control (n = 80)	p
Age (years)			
≤55	53(66.3%)	63(78.8%)	0.077
>55	27(33.8%)	17(21.3%)	
Mean ± SD.	51.5 ± 10.2	48.4 ± 9.1	0.051
Median (Min. – Max.)	51.5 (31 – 72)	49 (28 – 68)	
BMI			
≤25	30 (37.5%)	44 (55%)	0.026*
>25	50 (62.5%)	36 (45%)	
Resistin (pg/ml)			
Mean ± SD.	254.9 ± 51.3	115.5 ± 17.9	<0.001*
Median (Min. – Max.)	243 (194 – 409)	114 (73 – 153)	

*: Statistically significant at p ≤ 0.05.

Table 2. Tumor characteristics of patients group.

	No. (%)
Tumor size	
T1+T2	50 (62.5%)
T3+T4	30 (37.5%)
LN infiltration	
N0	29 (36.3%)
N1+N2+N3	51 (63.8%)
Distant metastases	
M0	75 (93.8%)
M1	5 (6.3%)
TNM Stage	
I+II	53 (66.3%)
III+IV	27 (33.8%)
Grading	
I	11 (13.8%)
II	44 (55%)
III	25 (31.3%)
HER2neu	34 (42.5%)
ER status	44(55%)
PR status	42(52.5%)

Breast cancer risk was nearly twice as high for patients with the *RETN* rs3219175 genotypes AG, AA, or AG+AA compared to GG. (OR: 2.437; 95% CI: 1.262-4.707, 3.091; 0.723-13.222, and 2.505; 1.325-4.736, respectively; p0.005 for all comparisons; Table 3). Additionally, women with the A allele of the *RETN* rs3219175 had a higher risk of breast cancer than women with the G allele. (OR: 1.981; 95% CI: 1.192-3.294; p= 0.008). Patients with the CT, CC, or CT+ CC genotypes of the *RETN* rs7408174, however, did not have a notably increased risk of developing breast cancer. (OR: 1.210; 95% CI: 0.626 – 2.338, 1.361 ;0.344 – 5.387 and 1.229; 0.654 – 2.309 respectively; Table 3).

Serum resistin level among different genotypes: Serum resistin levels varied statistically significantly between RETN rs3219175 and RETN rs7408174 genotypes. (p= 0.007* and <0.001 respectively; Table 4).

***RETN* rs3219175 polymorphism and tumour characteristics:** Patients who had AG and AG+AA genotypes were more likely to have tumour size (T3+T4) (OR:15.625; 95% CI: 4.081 – 59.821, 13.500; 3.606 – 50.454 respectively),

Table 3. Odds ratio and 95% confidence interval of *RETN* genotypic frequencies in both groups

	Patients (n = 80)	Control (n = 80)	P	OR (95% C.I)
rs3219175				1.0 (reference)
GG	33 (41.3%)	51 (63.8%)	^{MC}p= 0.015*	2.437(1.262 – 4.707)
AG	41 (51.3%)	26 (32.5%)		3.091(0.723 – 13.222)
AA	6 (7.5%)	3 (3.8%)		2.505(1.325 – 4.736)
AG + AA	47(58.8%	29(36.3%)	0.005*	
Allele				
G	107(66.9%)	128(80%)	0.008*	1.0 (reference)
A	53(33.1%)	32(20%)		1.981(1.192 – 3.294)
rs7408174				
TT	45(56.3%)	49(61.3%)	^{MC}p= 0.807	1.0 (reference)
CT	30(37.5%)	27(33.8%)		1.210(0.626 – 2.338)
CC	5(6.3%)	4(5%)		1.361(0.344 – 5.387)
CT + CC	35(43.8%	31(38.8%)		1.229(0.654 – 2.309)
			0.521	
Allele				
T	120(75%)	125(78.1%)	0.509	1.0 (reference)
C	40(25%)	35(21.9%)		1.191(0.709 – 1.999)

*: Statistically significant at p ≤ 0.05

Table 4. Serum Resistin level (pg/ml) among different genotype in patients' group (n= 80)

	N	Resistin (pg/ml)		F	p
		Mean ± SD.			
rs3219175					
GG	33	236.42 ± 17.94		5.343*	0.007*
AG	41	272.29 ± 62.93			
AA	6	238.0 ± 53.30			
rs7408174					
TT	45	238.73 ± 16.68		11.106*	<0.001*
CT	30	266.07 ± 68.42			
CC	5	333.80 ± 58.44			

F: F for One way ANOVA test, *: Statistically significant at p ≤ 0.05.

LN infiltration (N1+N2+N3) (OR: 10.208; 95% CI: 3.333 – 31.268, 13.50; 3.606 – 50.454 respectively), TNM stage (III+IV) (OR: 11.579; 95% CI: 3.044 – 44.051, 10.435; 2.795 – 38.961 respectively), and associated with HER2neu mutation (OR: 3.088; 95% CI: 1.157 – 8.241, 3.030; 1.164 – 7.888 respectively) (Table 5).

***RETN* rs7408174 polymorphism and tumour characteristics:** Patients who had CT, CC, and CT+CC genotypes were more likely to have tumour size (T3+T4) (OR: 13.0; 95% CI: 4.130 – 40.922, 26.0 ; 2.470 – 273.674 and 14.182 ; 4.640 – 43.342 respectively), TNM stage (III+IV)(OR: 6.204 ; 95% CI : 2.109 – 18.252, 21.714 ; 2.102 – 224.271 and 7.238 ; 2.538 – 20.640 respectively) and pathological grade III disease (OR; 41.625 ; 95% CI: 10.096 – 171.624, 18.500 ; 1.817 – 188.389 and 35.844 ; 9.851 – 130.424 respectively) (Table 6). Patients with CT and CT+CC genotypes were more likely to have LN infiltration (N1+N2+N3) (OR: 3.826; 95% CI: 1.314 – 11.139 and 14.182; 4.640 – 43.342 respectively) (Table 6).

Table 5. Clinical classifications and *RETN* rs3219175 genotypic frequency in breast cancer patients: Odds ratios (ORs) and 95% confidence intervals (CIs).

	Tumour Characteristics		**p**	**OR (95% C.I)**
	Tumour size			
	T1+T2 (n= 50)	**T3+T4 (n= 30)**		
rs3219175				
GG	30 (60.0%)	3 (10.0%)		1.0 (reference)
AG	16 (32.0%)	25 (83.3%)	<0.001*	15.625(4.081 – 59.821)
AA	4 (8.0%)	2 (6.7%)	0.128	5.0(0.630 – 39.669)
AG + AA	20(38%)	27(90%	<0.001*	13.500(3.606 – 50.454)
	LN infiltration			
	N0 (n= 29)	**N1+N2+N3 (n= 51)**		
rs3219175				
GG	21 (72.4%)	12 (23.5%)		1.0 (reference)
AG	6 (20.7%)	35 (68.6%)	<0.001*	10.208(3.333 – 31.268)
AA	2 (6.9%)	4 (7.8%)	0.182	3.500(0.556 – 22.029)
AG + AA	8(27.6%	39(76.5%)	<0.001*	13.50(3.606 – 50.454)
	Distant metastases			
	M0 (n= 75)	**M1 (n= 5)**		
rs3219175				
GG	33 (44.0%)	0 (0.0%)		1.0 (reference)
AG	37 (49.3%)	4 (80.0%)	0.998	–
AA	5 (6.7%)	1 (20.0%)	0.998	–
AG + AA	42(56%)	5(100%)	0.998	–
	TNM Stage			
	I+II (n= 53)	**III+IV (n= 27)**		
rs3219175				
GG	30(56.6%)	3(11.1%)		1.0 (reference)
AG	19(35.8%)	22(81.5%)	<0.001*	11.579(3.044 – 44.051)
AA	4(7.5%)	2(7.4%)	0.128	5.0(0.630 – 39.669)
AG + AA	23(43.4%)	24(88.9%)	<0.001*	10.435(2.795 – 38.961)
	Grading			
	I+II (n= 41)	III (n= 39)		
rs3219175				
GG	33 (80.5%)	0 (0.0%)		1.0 (reference)
AG	6 (14.6%)	35 (89.7%)	0.997	–
AA	2 (4.9%)	4 (10.3%)	0.998	–
AG + AA	8(19.5%)	39(100%)	0.998	–
	HER2neu			
	No (n= 46)	**Yes (n= 34)**		
rs3219175				
GG	24 (52.2%)	9 (26.5%)		1.0 (reference)
AG	19 (41.3%)	22 (64.7%)	0.024*	3.088(1.157 – 8.241)
AA	3 (6.5%)	3 (8.8%)	0.279	2.667(0.452 – 15.772)
AG + AA	22(47.8%)	25(73.5%)	0.023*	3.030(1.164 – 7.888)
	ER status			
	No (n= 36)	**Yes (n= 44)**		
rs3219175				
GG	9 (25.0%)	24 (54.5%)		1.0 (reference)
AG	23 (63.9%)	18 (40.9%)	0.015*	0.293(0.110 – 0.785)
AA	4 (11.1%)	2 (4.5%)	0.078	0.188(0.029 – 1.207)
AG + AA	27(75%)	20(45.5%)	0.009*	0.278(0.106 – 0.725)
	PR status			
	No (n= 38)	**yes (n= 42)**		
rs3219175				
GG	12 (31.6%)	21 (50.0%)		1.0 (reference)
AG	22 (57.9%)	19 (45.2%)	0.140	0.494(0.193 – 1.261)
AA	4 (10.5%)	2 (4.8%)	0.182	0.286(0.045 – 1.798)
AG + AA	26(68.4%)	21(50%)	0.097	0.462(0.185 – 1.150)

Table 6. Clinical classifications and *RETN* rs7408174 genotypic frequency in breast cancer patients: odds ratios and 95% confidence intervals.

	Tumour Characteristics		**p**	**OR (95% C.I)**
	Tumour size			
	T1+T2 (n= 50)	**T3+T4 (n= 30)**		
rs7408174				
TT	39 (78.0%)	6 (20.0%)		1.0 (reference)
CT	10 (20.0%)	20 (66.7%)	<0.001*	13.0(4.130 – 40.922)
CC	1 (2.0%)	4 (13.3%)	0.007*	26.0(2.470 – 273.674)
CT + CC	11(22%)	24(80%)	<0.001*	14.182(4.640 – 43.342)
	LN infiltration			
	N0 (n= 29)	**N1+N2+N3 (n= 51)**		
rs7408174				
TT	22 (75.9%)	23 (45.1%)		1.0 (reference)
CT	6 (20.7%)	24 (47.1%)	0.014*	3.826(1.314 – 11.139)
CC	1 (3.4%)	4 (7.8%)	0.246	3.826(0.396 – 36.957)
CT + CC	7(24.1%)	28(54.9%)	<0.001*	14.182(4.640 – 43.342)
	Distant metastases			
	M0 (n= 75)	**M1 (n= 5)**		
rs7408174				
TT	45 (60.0%)	0 (0.0%)		1.0 (reference)
CT	26 (34.7%)	4 (80.0%)	0.997	–
CC	4 (5.3%)	1 (20.0%)	0.997	–
CT + CC	30(40%)	5(100%)	0.997	–
	TNM Stage			
	I+II (n= 53)	**III+IV (n= 27)**		
rs7408174				
TT	38(71.7%)	7(25.9%)		1.0 (reference)
CT	14(26.4%)	16(59.3%)	0.001*	6.204(2.109 – 18.252)
CC	1(1.9%)	4(14.8%)	0.010*	21.714(2.102 – 224.271)
CT + CC	15(28.3%)	20(74.1%)	<0.001*	7.238(2.538 – 20.640)
	Grading			
	I+II (n= 41)	III (n= 39)		
rs7408174				
TT	37 (90.2%)	8 (20.5%)		1.0 (reference)
CT	3 (7.3%)	27 (69.2%)	<0.001*	41.625(10.096 – 171.624)
CC	1 (2.4%)	4 (10.3%)	0.014*	18.500(1.817 – 188.389)
CT + CC	4(9.8%)	31(79.5%)	<0.001*	35.844(9.851 – 130.424)
	HER2neu			
	No (n= 46)	**yes (n= 34)**		
rs7408174				
TT	30 (65.2%)	15 (44.1%)		1.0 (reference)
CT	14 (30.4%)	16 (47.1%)	0.087	2.286(0.886 – 5.898)
CC	2 (4.3%)	3 (8.8%)	0.255	3.0(0.452 – 19.928)
CT + CC	16(34.8%)	19(55.9%)	0.062	2.375(0.957 – 5.895)
	ER status			
	No (n= 36)	**yes (n= 44)**		
rs7408174				
TT	18 (50.0%)	27 (61.4%)		1.0 (reference)
CT	16 (44.4%)	14 (31.8%)	0.257	
CC	2 (5.6%)	3 (6.8%)	1.000	1.0(0.152 – 6.593)
CT + CC	18(50%)	17(38.6%)	0.309	0.630(0.258 – 1.536)
	PR status			
	No (n= 38)	**yes (n= 42)**		
rs7408174				
TT	19 (50.0%)	26 (61.9%)		1.0 (reference)
CT	16 (42.1%)	14 (33.3%)	0.346	0.639(0.252 – 1.620)
CC	3 (7.9%)	2 (4.8%)	0.454	0.487(0.074 – 3.207)
CT + CC	19 (50.0%)	16(38.1%)	0.285	0.615(0.253 – 1.499)

DISCUSSION

Resistin displays biological, noxious, immune-mediated, proliferative, angiogenic, and metastatic properties via various cellular and molecular pathways (Alharithy.,2014). Diabetes mellitus, cardiac diseases, and cancers have all been linked to resistin and polymorphisms in the resistin-encoding gene *RETN* (Ozgor et al., 2019). In the present study, Breast cancer patients had significantly higher BMIs than the control group. This was supported by previous studies that hypothesized the excessive oestrogen production from adipose tissue, metabolic syndrome, and chronic inflammation may contribute to BC development (Kang et al., 2007, Picon-Ruiz et al., 2017). On the other hand, García -Estévez et al. (2021) reported that obesity seems to protect against breast cancer in premenopausal women.

According to our findings, serum resistin levels in BC patients exceeded those in healthy controls. This was consistent with previous studies (Dalamaga et al., 2013, Muñoz-Palomeque et al., 2018). According to Sahan et al. (2022), the overall mean of resistin concentration in malignant breast cancer patients was much higher than in benign breast cancer patients and the control group. Also, Vallega et al. (2016) reported that oestrogen receptor-negative breast cancers had notably elevated levels of resistin gene expression. A previous study reported that when breast cancer and BMI more than 25 kg/m2 were combined, the highest levels of resisitn were obtained (Crisóstomo et al., 2016). It was explained that increasing macrophage and adipose tissue which secrete more resistin in obese patients, could be responsible for this (Avgerinos et al., 2019). Inflammation may also play a role in the strong association between blood resistin levels and the risk of breast cancer (Obi et al., 2021). Past studies have reported the role of resistin as a proinflammatory cytokine, its direct correlation with other inflammatory markers like IL6 and C-reactive protein and that resistin expression has been upregulated during monocyte macrophage differentiation (Vallega et al., 2016, Li et al., 2018).

As a result, resistin may serve as a link between breast carcinogenesis and inflammation and it might be implied that therapeutic approaches that successfully inhibit resistin may be helpful in breast cancer management. A single SNP may only have a small independent effect in the pathogenesis of cancer, and multiple SNPs may give a more accurate picture of the risk (Alharithy., 2014). The current study investigated the relationship between the *RETN* loci rs7408174 and rs3219175 and breast cancer susceptibility, clinicopathological features, and prognosis in Egyptian women.

Compared to those who carry the wild (GG) genotype, carriers of the heterozygous (AG) and homozygous (AA) genotypes of SNP rs3219175 had a considerably greater chance of developing breast cancer. In addition, those who had the A allele of the *RETN* rs3219175 polymorphism had an increased probability of getting breast cancer than those who had the G allele. However, patients with the *RETN* rs7408174 polymorphism CT, CC, or CT+ CC genotype had a low probability of breast cancer development. The same findings regarding rs3219175 polymorphism was reported by Sahan et al. (2022).

Even though Wang et al. (2020) discovered no significant variations in the *RETN* rs7408174 polymorphisms between breast cancer patients and healthy controls, they did discover that those with the AG genotype of the *RETN* SNP rs3219175 and those with at least one A allele in the SNP rs3219175 had a greater chance of developing breast cancer than wild-type (GG) carriers. A previous study has reported a significant association of rs3219175 SNP of *RETN* with a high lung cancer risk (Hu et al., 2017). Unfortunately, studies examining *RETN* rs3219175 and rs7408174 polymorphisms in breast cancer are rare.

Research findings have shown that the *RETN* gene is frequently linked to genetic or epigenetic changes. Variant SNPs in the promoter region of DNA methylation may regulate the activity of genes, and thus influencing cancer development (Onuma et al., 2017). It is significant to note that the *RETN* SNP rs3219175 is situated in the promoter region and may control the gene's expression, which may account for its association with increased

breast cancer susceptibility (Wang et al., 2020). Additionally, our findings revealed that patients carrying rs3219175 AG genotype and rs7408174 CT/CC genotypes had significantly higher serum resistin levels. As far as we are aware, this is first study to investigate the relationship between the serum resistin level in breast cancer patients and the *RETN* SNPs rs3219175 and rs7408174. Wang et al. (2020) reported that carriers of rs7408174 CC genotype showed a higher resistin expression in BC tissue using immunohisto-chemistry and according to Sahan et al. (2022), patients with breast cancer who have the rs3219175 AG and AA genotype have much greater serum levels of resistin than the benign and control groups. These findings could indicate that rs3219175 and rs7408174 *RETN* SNPs may regulate resistin expression and influence breast cancer risk. Future research must focus on how these variants affect the expression of resistin in breast cancer cells. In terms of the relationship between *RETN* SNPs and clinicopathological characteristics in breast cancer, we discovered that patients with rs7408174 CT/CC genotypes exhibited higher rates of pathological grade III, advanced tumour size (T3+T4), LN infiltration and advanced TNM stage (III+IV). Furthermore, patients with rs3219175 AG/AG+AA genotypes had a higher risk of having advanced tumour size (T3+T4), late TNM stage (III+IV), LN infiltration and HER2neu positive breast cancer. Our results were supported by Wang et al. (2020) who revealed that *RETN* rs7408174 was linked to pathological grade III and late-stage disease.

CONCLUSION

The present study demonstrated a significant association between the risk of breast cancer and high serum resistin levels and the *RETN* SNP rs3219175. Furthermore, there was a significant relationship of the *RETN* SNPs rs3219175 and rs7408174 with poor clinicopathological status of breast cancer patients, suggesting that they may serve as targets for breast cancer treatment and genetic prognostic markers.

LIMITATION OF THE STUDY

The comparatively small sample size of subjects may represent a limitation in our study. Therefore, future studies with larger sample size in different ethnic populations are recommended to corroborate the current experimental results.

CONFLICT OF INTEREST

There are no conflicts of interest.

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Nil

ABBREVIATIONS

BC: Breast cancer
SNPs: single nucleotide polymorphisms
RETN gene: Resistin coding gene
PR: Progesterone receptor
ER: Estrogen receptor
SD: Standard deviation
t: Student t-test
$\chi 2$: Chi square test
p: p value for comparing between the studied groups.

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