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Impacts of RETN genetic polymorphism on breast cancer development in Beni-Suef females, Egypt

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Abstract

Breast cancer is the most common cancer among females with increasing incidence and death rates. Resistin is pro-inflammatory molecule which shares in diverse cellular signaling pathways. This study aimed to evaluate resistin and RETN rs3219175 gene polymorphism and their relevance to diagnostic susceptibility, prognostic value, and genetic risk among Egyptian female patients with breast cancer. Eighty female patients with breast cancer were recruited from the Oncology Department, Faculty of Medicine, Beni-Suef University. Breast cancer staging and grading were determined. Eighty age-matched normal females participated as controls. Quantitative determination of serum resistin was assayed by an enzyme-linked immunosorbent assay (ELISA). RETN rs3219175 gene polymorphism was determined by real time polymerase chain reaction (RT-PCR) TaqMan allelic discrimination assay. Serum resistin showed statistically significantly higher level among females with breast cancer when compared to controls (p <0.001). Resistin showed sensitivity of 80% and specificity of 67.5% at cut off value of 1.27 ng/mL for diagnosis of breast cancer (p =0.001). RETN rs3219175 gene polymorphism showed significantly higher frequency of AG, AA genotypes, and A allele among cases when compared to controls (p <0.001). No statistical difference was found in resistin level or RETN rs3219175 gene polymorphism regarding tumor characteristics including size, lymph nodes or distant metastasis. Resistin showed significantly higher level among carriers of AG followed by AA genotypes and among A allele (p <0.001). In conclusion, resistin could be proposed as a possible potential diagnostic marker and A allele of RETN rs3219175 gene might be suggested as a genetic risk allele among female patients with breast cancer.

Keywords: Breast cancer, resistin, RETN rs3219175, gene polymorphism.

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Introduction

Breast cancer is one of the most commonly diagnosed cancers with an estimated number of 2.3 million new cases worldwide each year. It is considered the fifth cause of cancer related deaths.[1] Breast cancer is the most common cancer among Egyptian females where most patients present at a late stage with subsequent poor outcome.[2] Two staging systems for breast cancer are provided by the American Joint Committee on Cancer (AJCC). The anatomic stage which is based on the extent of cancer including size of primary tumor (T), nodal status (N), and distant metastasis (M). And the prognostic stage, which includes anatomic TNM plus tumor grade, and status of the biomarkers estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).[3, 4] The addition of tumor grade, hormone receptor and oncogene expression including ER, PR, and HER2, and multigene panel to anatomic staging provides more accuracy. However, the anatomic staging is used in areas with unavailability of biomarker testing.[5] ER-, PR-, HER2-negative and triple negative biomarkers are more aggressive breast cancer subtypes with limited treatment options.[6]

Resistin is a pro-inflammatory cytokine which was initially discovered as a link between diabetes and obesity; being mediator of insulin resistance; hence the name resistin.[7] Resistin is a member of the resistin-like molecule (RELM) cytokine family which plays a regulatory role in many human chronic inflammatory diseases, metabolic disorders, infections, and cancers.[8] Resistin is a 12.5 kDa cysteine-rich protein that consists of 108 amino acids.[9] Resistin is expressed in various organs and tissues, including adrenal gland, pituitary gland, hypothalamus, adipose tissue, lung, spleen, intestine, placenta, pancreas, stomach, skeletal muscle, plasma, and skin epidermis.[10] Synthesis and release of resistin are regulated according to cell type and micro environmental stimuli.[9] It has been demonstrated that proinflammatory mediators such as lipopolysaccharide (LPS), tumor necrosis factor-alpha (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) can strongly induce the expression of resistin in monocytes/macrophages.[11] Resistin participates in various metabolic functions, modulation of satiety centers and somatotrophic cells, central nervous system regulation, production of cytokines, monocytes differentiation into macrophages, control of heart contractility, angiogenesis, smooth muscle activity, renal functioning, and remodeling.[12] Diverse studies propose the key role of resistin in proliferation, metastasis, angiogenesis, and regulation of metabolism in cancer cells.[7] Resistin promotes breast cancer cell survival through up regulation of B-cell lymphoma 2 regulator proteins, and through activation of toll-like receptor 4 which controls DNA transcription and cell survival.[12] Moreover, resistin impairs immune response, alters metabolic profile, induces stemness, and dysregulates microRNAs leading to cancer development and progression.[13] The gene encoding resistin (RETN) is localized on chromosome 19p13.2.[7] Several single nucleotide polymorphisms (SNPs) have been identified in the RETN promoter and 3'-untranslated regions.[14] This study aimed to evaluate the association of resistin and RETN rs3219175 gene polymorphism among female breast cancer patients with disease diagnosis, prognosis, and genetic risk.

Subjects and Methods

Study participants

The present study involved 160 participants, 80 females with breast cancer and 80 age-matched normal females as controls. The patients were recruited from the Oncology Department, Faculty of Medicine, Beni-Suef University. This study excluded patients with history of other types of cancers, coronary artery disease, inflammatory diseases, and breast cancer under treatment.

Ethical considerations

The study protocol was reviewed and approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University (approval number FMBSUREC/06062021/Abd Elwahab). Informed consents were obtained from all participants before involved in this study.

Data collection

Study participants were subjected to 1- Full history taking including history of lactation, contraception use and menopause. 2- Family history taking of breast cancer. 3- Proper clinical examination for staging and grading. 4- Imaging for detection of tumor size, lymph node involvement and distant metastasis.

Breast cancer Staging and Grading

Breast cancer staging was based on the TNM system and grading depended on breast cancer cell differentiation, and were determined according to European Society of Medical Oncology (ESMO).[15]

Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) analyses

The analysis of ER, PR and HER2, by immunohistochemical testing, was determined according to the American Society of Clinical Oncology Guideline Recommendations (ASCO).[16]

Serum resistin analysis

Blood samples were collected from study subjects, serum samples isolated and stored at -20°C till used. Quantitative detection of serum resistin was assayed by enzyme-linked immunosorbent assay (ELISA) kits (Cat no DRSN00, Human Resistin ELISA Kit supplied by R&D Systems, Inc., USA), according to manufacturer's instructions.

Genotyping of RETN rs3219175 polymorphism

Whole blood samples were collected from study participants on ethylene diamine tetra acetic acid (EDTA) anticoagulant. Genomic DNA was isolated using DNA blood mini kits (cat no 51104, QIAamp Mini kit, Qiagen, USA), according to manufacturer's instructions. Isolated DNA samples were stored at -80°C until used. Genotyping of RETN rs3219175 was performed by real time polymerase chain reaction (RT-PCR) using TaqMan allelic discrimination assay kits (Applied Biosystem Inc, Foster City, CA, USA), according to manufacturer's protocol. TaqMan genotyping assay contained two primers for amplifying the sequence of interest and two TaqMan Minor groove binder (MGB) probes for detecting alleles. The context sequence probes [VIC/FAM] CTCCAGCCCTTACTGTCTGCTCAGG [A/G]GCTTCC TCTTGGCCCCGGATGTGGG were used. The total PCR reaction volume was 20ul, consisted of 10 ng of genomic DNA, 10µl universal TaqMan master mix II, 0.5 µl SNP assay mix, and adjusted to a final volume of 20µl using nuclease free water. The PCR was performed by Step One real-time PCR (Applied Biosystem Inc, Foster City,CA, USA); under the following conditions: initial step of 95°C for 10 min, followed by 40 cycles each of 95°C for 15 sec and 60°C for 1 min. Interpretation of data was made using an allelic discrimination plot as a scatter plot of allele 1 (Victoria, VIC dye) versus allele 2 (fluorescein amidites, FAM dye) using the life Technologies real time instrument software plot (Figure 1).

Statistical analysis

Data were collected, coded to facilitate data manipulation, and double entered into Microsoft Access and data analysis was performed using the Statistical Package of Social Science (SPSS) software version 22 in windows 7 (SPSS Inc., Chicago, IL, USA). Independent samples t test was used to compare quantitative measures between two independent groups. Chi square test was used to compare between two of more than two qualitative groups. One-way ANOVA test was used to compare quantitative measures between more than two independent groups of quantitative data. Description of quantitative variables was in the form of mean and standard deviation (SD). Description of qualitative variables was in the form of number (No.) and percent (%). The receiver operating characteristic (ROC) curve was used for sensitivity, specificity, and prediction of cut off values. A *p*-value <0.05 was considered significant.[17]

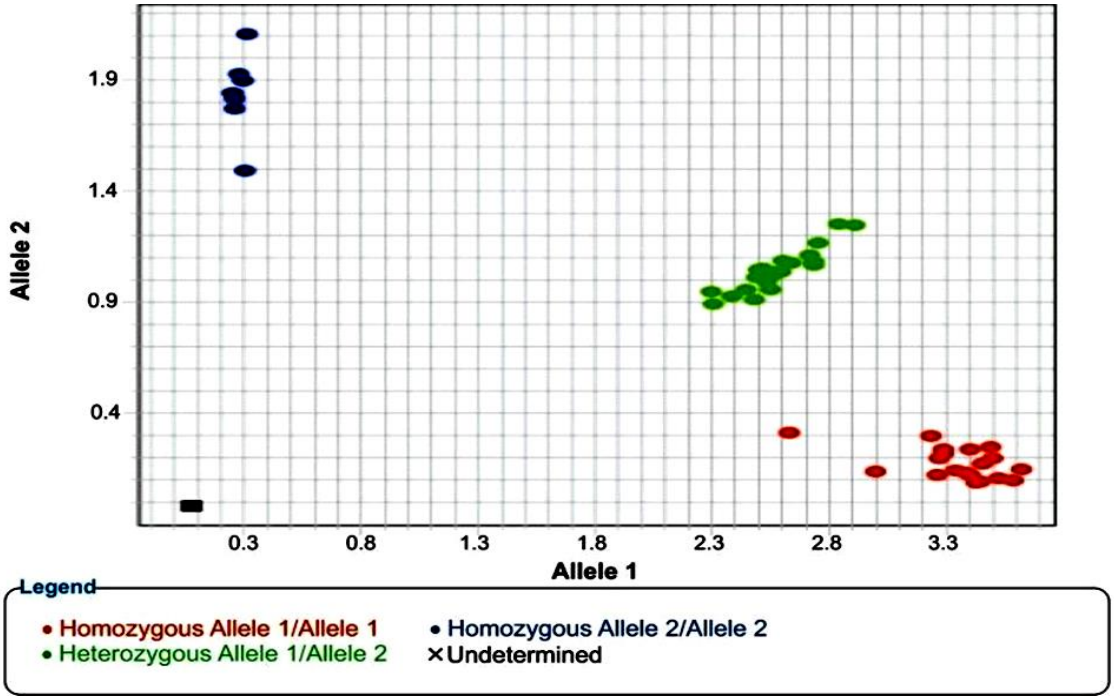

Figure 1. Allelic discrimination plot showing RETN rs3219175 gene.

Results

This study included 80 breast cancer female patients with mean age of 47.8±10 years and 80 age-matched female control volunteers with mean age of 44.9±11.9 (p =0.23). The use of contraceptives was significantly lower in breast cancer cases than in controls (p =0.006). However, other medical history data did not differ between cases and controls (p >0.05) (Table 1).

Table 1. Comparison of clinical history data between cases and controls.

Variables	Cases (N=80)	Controls (N=80)	p-value
	No. (%)	No. (%)	
Lactating			
Positive	74 (92.5%)	76 (95%)	NS
Use of contraceptives			
Positive	56 (70%)	76 (95%)	0.006
Menopause			
Positive	32 (40%)	30 (37.5%)	NS
Family history of breast cancer			
Positive	10 (12.5%)	4 (5%)	NS

*$P \leq 0.05$ is significant.

The status of ER, PR and HER2 was studied in breast cancer patients, showed negative ER in 30 (37.5%), negative PR in 44 (55%), and negative HER2 in 48 (60%) of the cases.

The mean resistin level was statistically significantly higher among breast cancer cases than controls (p <0.001) (Table 2 and Figure 2).

Table 2. Comparison of resistin level between cases and controls.

Variable	Cases (N=80)	Controls (N=80)	*p*-value
	Mean ±SD	Mean ±SD	
Resistin (ng/mL)	2.9±1.9	1.1±0.58	<0.001

*$P \leq 0.05$ is significant.

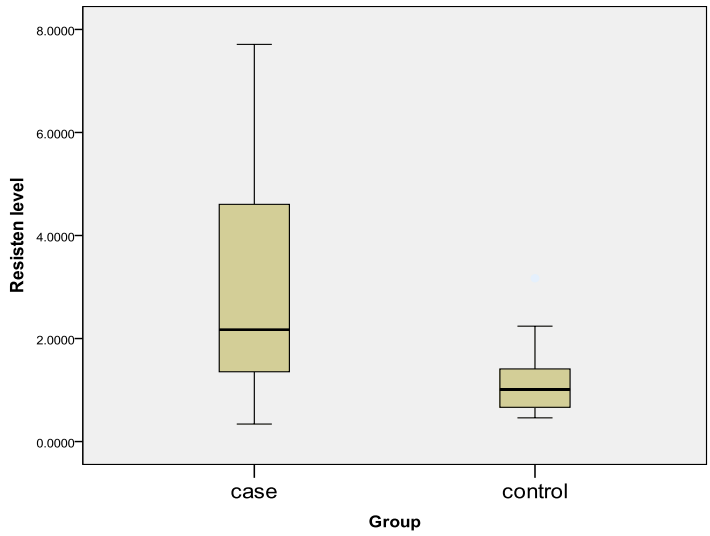

Figure 2. Comparison of resistin level between cases and controls.

Although there was a positive correlation between resistin level and age of participants, such correlation did not reach statistical significance (r =0.19, *p* =0.09). Among females with breast cancer, there was no statistically significant difference in resisitin level between different tumor characteristics including tumor size, lymph nodes affection, metastasis, staging and tumor grading (*p* = 0.7, *p* =0.4, *p* =0.7, *p* =0.9 and *p* =0.9, respectively). Also, no statistically significant difference was found in resistin level among ER, PR, and HER2 subtypes (*p* =0.3, *p* =0.8 and *p* =0.8, respectively).

Using the ROC curve analysis, resistin level illustrated sensitivity of 80% and specificity of 67.5% at a cut off value of 1.27ng/mL for diagnosis of breast cancer cases (*p*=0.001) (Table 3 and Figure 3).

Table 3. Sensitivity and specificity of resistin for diagnosis of breast cancer cases.

Variable	Sensitivity	Specificity	AUC	Cut off point	*p*-value
Resistin (ng/mL)	80%	67.5%	82.2%	1.27	0.001

AUC: area under curve. *$P \leq 0.05$ is significant.

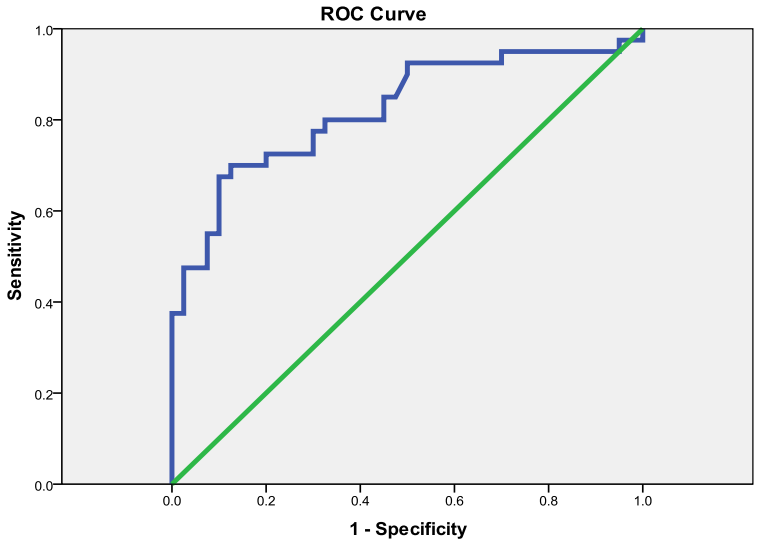

Figure 3. Receiver operating characteristic curve for serum resistin as diagnostic marker of breast cancer cases.

There was a statistically significant difference in RETN rs3219175 genotyping between cases and controls (p <0.001). Higher frequency of AG and AA genotypes was found among cases versus higher frequency of GG genotype among controls (Table 4).

Table 4. Comparison of different genotyping in study between cases and controls.

Genotyping	Cases (N=80) No. (%)	Controls (N=80) No. (%)	*p*-value
AG	56 (70%)	8 (10%)	<0.001
AA	18 (22.5%)	2 (2.5%)	
GG	6 (7.5%)	70 (87.5%)	

*$P \leq 0.05$ is significant.

On comparing RETN rs3219175 alleles, there was a statistically significant difference between breast cancer cases and controls, with a higher frequency of A allele among cases, and higher frequency of G allele among controls (p <0.001) (Table 5).

Table 5. Comparison of different alleles between cases and controls.

Alleles	Cases (N=80) No. (%)	Controls (N=80) No. (%)	*p*-value
A allele	92 (57.5%)	12 (7.5%)	<0.001
G allele	68 (42.5%)	148 (92.5%)	

*$P \leq 0.05$ is significant.

There was statistically significant difference between different RETN rs3219175genotypes as regards tumor stage among cases with higher frequency of AG and GG genotypes among those with stage III, and higher frequency of AA genotype among stage II (p =0.006). On the other hand, there was no significant difference between different genotypes as regards tumor size, lymph nodes involvement, distant metastasis, and grading (Table 6).

Table 6. Comparison of different genotyping between tumor characteristics among cases.

Variables	AG (N=56) No. (%)	AA (N=18) No. (%)	GG (N=6) No. (%)	*p*-value
Tumor stage				
Stage I	2 (3.6%)	2 (11.1%)	0 (0%)	0.006
Stage II	14 (25%)	16 (88.9%)	0 (0%)	
Stage III	30 (53.6%)	0 (0%)	6 (100%)	
Stage IV	10 (17.9%)	0 (0%)	0 (0%)	
Tumor size				
T1	6 (10.7%)	4 (22.2%)	0 0 (%)	NS
T2	22 (39.3%)	8 (44.4%)	0 (0%)	
T3	26 (46.4%)	6 (33.3%)	4 (66.7%)	
T4	2 (3.6%)	0 (0%)	2 (33.3%)	
Lymph nodes metastasis				
N0	14 (25%)	4 (22.2%)	0 (0%)	NS
N1	32 (57.1%)	12 (66.7%)	2 (33.3%)	
N2	8 (14.3%)	2 (11.1%)	2 (33.3%)	
N3	2 (3.6%)	0 (0%)	2 (33.4%)	
Distant metastasis				
M0	46 (82.1%)	18 (100%)	6 (100%)	NS
M1	10 (17.9%)	0 (0%)	0 (0%)	
Tumor grading				
G II	44 (78.6%)	16 (88.9%)	4 (66.7%)	NS
G III	12 (21.4%)	2 (11.1%)	2 (33.3%)	

P > 0.05 is not significant (NS).

On comparing the mean resistin level between different RETN rs3219175 gene variants, there was a statistically significant difference, with higher resistin level among AG genotype followed by AA genotype (p<0.001) (Table 7 and Figure 4). Moreover, resistin showed statistically significant higher mean level among A allele variant versus G allele variant (p<0.001) (Table 8 and Figure 5).

Table 7. Comparison of resistin level in different RETN rs3219175 genotypes.

Variable	AG Mean ±SD	AA Mean ±SD	GG Mean ±SD	*p*-value
Resistin (ng/mL)	3.1±1.9	1.9±0.96	1.1±0.78	<0.001

*$P \leq 0.05$ is significant.

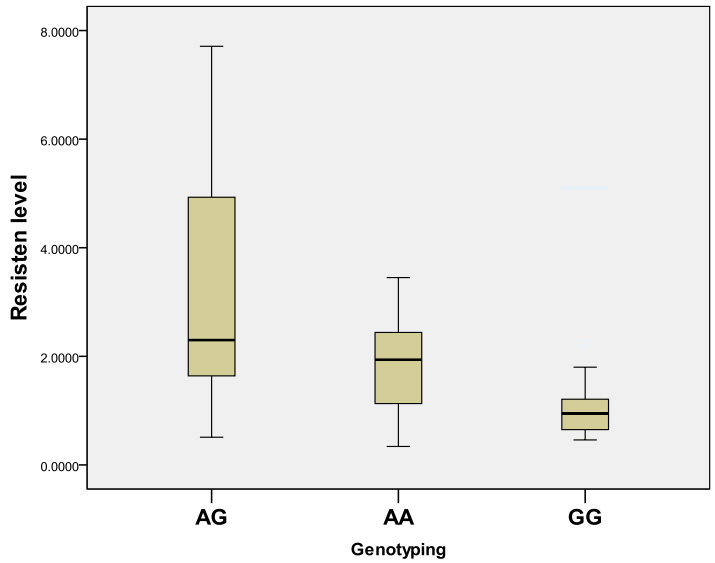

Figure 4. Comparison of resistin level in different RETN rs3219175 genotypes.

Table 8. Comparison of resistin level in different RETN rs3219175 alleles.

Variable	A allele	G allele	*p*-value
	Mean ±SD	Mean ±SD	
Resistin (ng/mL)	2.8±1.8	1.1±0.78	<0.001

*$P \leq 0.05$ is significant.

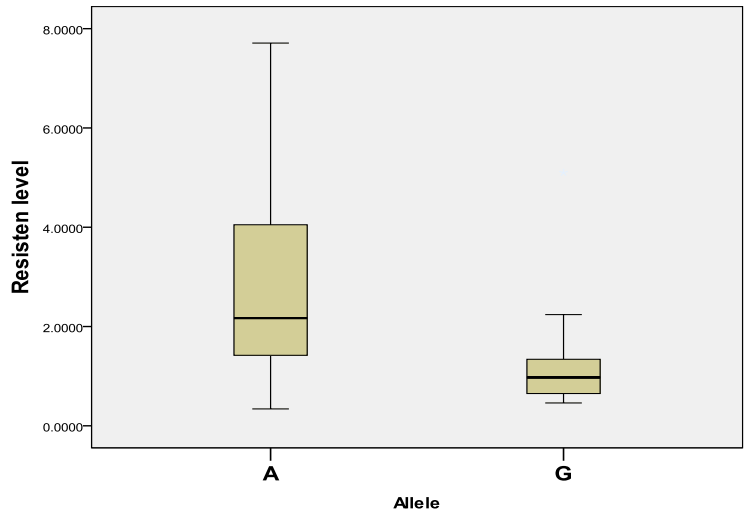

Figure 5. Comparison of RETN rs3219175 resistin level in different RETN rs3219175 alleles.

Discussion

Improvement of the accuracy of biomarkers is a major goal for breast cancer early detection which has its impact on recurrence and prognosis.[18] This study aimed to evaluate resistin and RETN rs3219175 gene polymorphism and their relevance to diagnostic susceptibility, prognostic value, and genetic risk among Egyptian female patients with breast cancer.

In the current study, serum resistin showed statistically significantly higher level among females with breast cancer when compared to controls. The sensitivity and specificity of resistin level in diagnosis of cases were 80% and 67.5%, respectively at cut off value of 1.27ng/mL (p= 0.001). However, no significant

difference was found in resistin level among the different studied tumor characteristics.

Our results came in accordance with an earlier study which reported increased resistin level in breast cancer patients compared to controls.[19]

Another study reported significant association of resistin with tumor and inflammatory markers, tumor size, cancer grade, tumor stage, lymph node invasion and decreased disease-free survival among females with breast cancer.[12] Resistin has been linked to increased risk of progression, angiogenesis, metastasis, and poor prognosis in various cancer models.[20] Resistin plays important role in several cellular signaling cascades involving tumor promoters leading to cell migration and invasion in breast cancer.[21]

In the present study, no difference was found in resistin level among ER, PR, and HER2 subtypes. This observation agreed with finding of a previous study regarding the status of ER/PR/HER2 in breast cancer patients.[12] However, in another study resistin was associated with ER-, PR-, HER2-negative and triple negative subtypes of breast cancers, possibly through inflammation as resistin stimulates expression of pro inflammatory cytokines such as IL-6.[22] Interleukin 6 plays a key role in contributing to breast cancer progression and was related to triple negative subtype of breast cancer.[23]

The current study demonstrated statistically significant increase of RETN rs3219175 AG and AA genotypes among cases when compared to controls. Also, the A allele showed statistically significant higher frequency among cases. On comparing resistin level with different RETN rs3219175 genotypes, resistin showed statistically significant higher level among AG followed by AA genotypes, and among carriers of A allele. In accordance with our study, a previous study reported an association between RETN rs3219175 gene SNP and susceptibility for breast cancer and its progression among Han Chinese women carrying the AG or the AG+AA gene variants and those with A allele.[24] Up regulation of RETN gene expression was found in *in-vitro* samples of human breast cancer tissue with SNP rs3219175.[22]

In conclusion, based on findings of this study, resistin can be proposed as a possible diagnostic marker in breast cancer. The study also suggested an association of RETN rs3219175 gene polymorphism among female patients with breast cancer. The A allele might be suggested as genetic risk allele for breast cancer.

Author Contributions

HMF and SAA contributed to the study conception and design. MER and TD contributed to clinical data collection and provided clinical support. HMF, SAA and NAAE contributed to material preparation, data collection and analysis. HMF and TD wrote the manuscript draft. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University (approval number FMBSUREC/06062021/Abd Elwahab).

Informed consent

We described the aim of this research to participants of this research before enrolment and informed consent was obtained from all participants.

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