ORIGINAL ARTICLE

Granzyme-B gene Polymorphisms and Susceptibility of Breast Cancer Patients in Egypt

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Key words:
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ABSTRACT

***Background**: Breast cancer (BC) is the most common type of female cancer in Egypt. Granzyme B (GrB) is primarily found in cytotoxic granules and has been considered the most abundant Granzyme. However, recent research has revealed various other crucial roles for GrB. **Objectives**: In this study, we aimed to examine Granzyme-B (GrB) expression as well as to investigate whether a common genetic variation in the gene encoding GrB, among two single nucleotide polymorphisms (rs8192917, rs2236338), is associated with breast cancer risk in Egyptian women. **Methodology:** This study included 195 participants, with 103 cases diagnosed with breast cancer and 92 serving as controls. Flow cytometry was used to assess anti-Granzyme B expression, while genotyping of the Granzyme B gene variants (rs8192917, rs2236338) was conducted. **Results**: We observed a substantial decrease ($p < 0.001$) in the percentage of Granzyme B in breast cancer patients compared to healthy women, with an odds ratio of 10.110 (95% confidence interval: 4.966-20.582). In rs8192917, The GG genotype and G allele could be considered risk factors for breast cancer (OR= 1.22, 95% CI: 1.02-1.22, P<0.001) and G allele (OR = 5, 95% CI: 2- 12.53, P=00). Additionally, in rs2236338, GC and GG genotypes , and the C allele was; OR =3.91 (95% CI: 1.07-14.3, OR = 0.233 (95% CI: 0.069-0.86), and 4.09 (95% CI: 1.15-14.57), respectively could all be risk factors for breast cancer. **Conclusion**: The variant allele (rs8192917 and Granzyme-B (GrB) expression) could be considered as a predictive factor for breast cancer development in Egyptian females.*

INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed cancer globally, with over 2 million new cases reported in 2020. It represents the leading cause of cancer-related death among women, accounting for more than 680,000 deaths[1].

In Egypt, breast cancer is the most common prevalent among women, making up 38.8% of cases. In 2020, nearly 22,700 new cases were reported, with projections estimating about 46,000 in 2050. The mortality rate for breast cancer is approximately 11%, making it the second leading cause of cancer-related deaths after liver cancer[2].

Breast cancer is categorized into subtypes such as luminal A, which is ER-positive, PR-positive, and HER-2 negative, and luminal B, which is ER-positive, PR-positive, and HER-2 positive [3]. In contrast, basal-like breast cancers express ER, PR, and HER-2, while triple-negative breast cancer (TNBC) lacks expression of all three markers (ER, PR, HER-2) [3,4]. TNBC presents a clinical challenge due to its aggressive growth, recurrence, poor differentiation, and large tumor size [4]. Current breast cancer treatments are tailored to the tumor's subtype and stage and include radiation, chemotherapy, hormone receptor modulators, immunotherapy, and endocrine therapy[3,4].

Breast cancer tumorigenesis involves multiple stages including, proliferation, apoptosis, autophagy, invasion, migration, metastasis, and drug resistance [5,6]. Breast cancer is classified into various subtypes based on pathology[4], including non-invasive types, like Paget's disease, invasive breast carcinoma of special type (e.g. apocrine carcinoma or micropapillary carcinoma), and invasive breast carcinoma of no special type (e.g. lobular carcinoma or ductal carcinoma), as well as inflammatory breast cancer (IBC). Invasive breast cancer (IBC) is the most common type and is often associated with poor prognosis[3]. Effective management focuses on early diagnosis and prompt treatment[7]. Breast cancer treatment is multimodal, involving surgery, radiotherapy, chemotherapy, and

hormonal therapy. Axillary lymph-node dissection and external beam radiotherapy are key components in treating most cases of invasive breast cancer, with tumor removal playing a critical role depending on the stage [8].

Granzymes, especially granzyme B (GrB), are key enzymes in cytotoxic T-cells and natural killer cells that induce apoptosis via perforin-mediated pathways. Recent studies have revealed additional functions of GrB beyond its role in cytotoxic granules. GrB expression in both normal epithelial and cancer cells also impacts processes like extracellular matrix remodeling, epithelial-to-mesenchymal transition, and fibrosis [9,10].

GrB, a 33 kDa protein encoded by the GZMB gene, has three common single nucleotide polymorphisms (SNPs) leading to missense mutations: Q55R, P94A, and Y247H [11,12]. Initially, it was believed that the RAH haplotype formed by these SNPs impaired GrB's apoptotic function [11], but later studies refuted this. Although their exact impact remains unclear, these mutations are located away from the enzyme's catalytic site. Notably, a study found that breast cancer patients with GZMB RAH alleles had a higher risk of developing the disease compared to those with QPY alleles. [13]

This study aimed to assess the expression of Granzyme B (GrB) among breast cancer patients and normal controls using flow cytometry. Additionally, it aimed to examine whether a common genetic variation in the gene encoding GrB, consisting of two single nucleotide polymorphisms (rs8192917, rs2236338), is associated with the risk of breast cancer in Egyptian women.

METHODOLOGY

Subjects and Sampling

Five milliliters of EDTA blood samples of peripheral venous blood were collected in sterile EDTA-tubes (KemikoVacutainer, Egypt). Each sample was labeled and numbered to the correspond with the other related investigations.

Samples were collected from 103 newly diagnosed, untreated breast cancer patients in Egypt. Control samples were obtained from 92 age-matched healthy female volunteers. Written consent was obtained from all participants. Patients were recruited from the Faculty of Medicine, Menoufia University, and hematology unit of Baheya Foundation for Early Detection and Treatment of Breast Cancer. Controls were recruited from the same population via invitations. All participants were recruited at a designated laboratory center, where investigations were conducted free of charge. The study was approved by the Ethical Committee of the Faculty of Medicine, Menoufia University (No: 2/2019 INTM2) from 2/2019 to 11/2020.

Exclusion criteria were; any cancer except breast cancer, any treatment (chemotherapy, radiotherapy and hormonal therapy), HCV, HBV and HIV viral infection, concomitant autoimmune disease, and use of immunosuppressive drugs.

All selected patients underwent physical examinations and a routine history assessment for diagnosis, including pathology for breast biopsy and immunohistochemical analysis. One hundred and three breast cancer patients, ranging in age from 32 to 82 years and in clinical stage from I to III be randomly selected (figure 1).

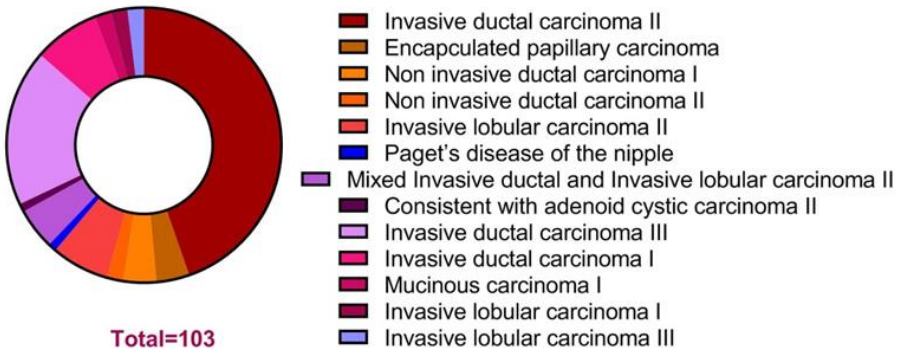

Fig. 1: Breast cancer types of the studied patients.

Immunophenotyping of peripheral blood leucocytes:

Anti-human Granzyme B (GrB) monoclonal antibodies were purchased from (Miltenyi Biotec GmbH, Germany). White blood cell phenotypes were analysed 'exvivo', following an immunofluorescence procedure recommended by Beckman coulter (Navios EX, France). Briefly, wash up was done to 50 µl aliquots of EDTA whole peripheral blood samples by adding 2 ml buffer (phosphate-buffered saline (PBS), pH 7.2) and centrifugation at 300xg for 10 minutes was performed [14].

Genotyping:-

Peripheral Blood Leucocytes' Isolation

Approximately 2 mL of EDTA blood samples were mixed with erythrocyte lysing buffer (1:4 v/v) within three hours of collection and incubated for 20 minutes at 30°C. The samples were then centrifuged for 5 minutes at 1500 rpm, repeating the process until a white pellet of leukocytes appeared [15] .The isolated pellets were stored at -80°C until DNA extraction.

Isolation of Total Genomic DNA

Genomic DNA was isolated from peripheral blood leukocytes using the Aljanabi and Martinez extraction method [16] . Leukocyte pellets were lysed in a buffer (50 mM NaCl, 1 mM Na_2EDTA, 0.5% SDS, pH 8.3) for 2 hours at 45°C. Proteins and cellular debris were removed with 4 M NaCl, while nucleic acids were precipitated using cold isopropanol. The resulting pellets were reconstituted in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at -20°C until further use.

SNP Selection and genotyping

Single nucleotide polymorphisms (SNPs) were chosen based on data published in PubMed's SNP database. In this study, SNPs were selected because of their significant association with the diseases being investigated [13,17].

To investigate the genotyping and allele analysis of polymorphisms in the Granzyme B gene (rs8192917 and rs11539752), a tetra primers amplification-refractory mutation system (ARMS-PCR) was conducted using a Mastercycler gradient thermocycler (Eppendorf, Hamburg, Germany). For both rs8192917 and rs11539752, DNA samples were first denatured at 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C, annealing at 65°C, and extension at 72°C, each for 1 minute. The primer sequences are detailed in table 1.

Table 1: Primer sequences for rs8192917 and rs2236338 detection using tetra primers amplification-refractory mutation system (ARMS-PCR)

rs8192917	Forward (5'-3')	Reverse (5'-3')
Outer	ATCCCTCCACTTACACCCTGGCC	AGGGGACAGTCGGTCCCCAG
Inner	GTGCGGTGGCTTCCTGATTCC	CAGCTGTCAGCACGAAGTCGTGTC
rs2236338		
Outer	TTGTCTCCTGATGGTGCTCAGCTTC	GGCCTTTTCTAAAAGGGGGCTTGAG
Inner	GGGGGCTTAGTTTGCTTCCTGTAGTTACTG	CACTGGATAAAGAAAACCATGAAACCCG

For rs8192917, the product sizes for the C and G alleles were 408 and 499 base pairs, respectively, while the two outer primers (control) yielded an amplicon of 862 bp. For rs2236338, the G and C alleles produced product sizes of 255 and 375 base pairs, respectively, with the outer primers (control) generating an amplicon of 572 bp. All fragment sizes were selected within the range of 255 to 862 bp. The amplification reactions were conducted in a single tube using all four primers simultaneously. Oligonucleotide primers were designed using the primer design tool for tetra-primer ARMS-PCR (PRIMER1 online software, http://primer1.soton.ac.uk/primer1.html, accessed on January 17, 2021). The amplicons were then resolved on 2% agarose gels (Sigma, St. Louis, MO, USA) and visualized using a UV trans-illuminator [18] .

Statistical Analysis

The results were gathered, organized into tables, and analyzed statistically using SPSS version 25 (SPSS, Inc., Chicago, IL, USA). Differences in allele frequencies and genotype distribution between the breast cancer patients and control group were evaluated using Pearson's χ^2 test. Odds ratios and their confidence intervals were computed to assess the relationship between genotype and breast cancer. When the assumption of Chi-square (χ^2) was violated, Fischer exact test was performed. Statistical significance was considered when the P-value was < 0.05. Also, Multinominal logistic regression was performed between normal and cancer groups as outcomes with the incorporation of SNP1 (rs8192917), SNP2 (rs2236338), age groups whether ≤40 or >40, and anti Granzyme B as the predictor variables

RESULTS

Clinical and demographic data:-

A total of 103 BC patients (100% were females) aged from 32 to 82 years old were included in this study. Healthy adult volunteers (n = 92, 100% were females) were included as control group with similar mean age of the patients group. The patients were sorted according to breast cancer type. Estrogen Receptor, (ER); Progesterone Receptor, (PR); Human Epidermal Growth Factor Receptor-2 (HER-2) were examined in the patients.Clinical features and receptors status of the patients are listed in (table 2).

Table 2: Breast cancer types of the studied patients.

	Receptors status		
No. of cases	**ER**	**PR**	**HER2**
85	+ve	+ve	-ve
2	+ve	+ve	+ve
8	-ve	+ve	-ve
6	+ve	-ve	-ve
2	-ve	-ve	+ve

Estrogen Receptor, (ER); Progesterone Receptor, (PR); Human Epidermal Growth Factor Receptor-2 (HER-2).

The demographic data of the studied subjects are shown in table (3) There was a significant difference in age among breast cancer patients and the control group (P<0.001).

Table 3: Demographic data of the studied subjects

	Control	**Breast cancer (BC)**	χ^2	**P value**	**Odds ratio 95% (CI)**
Number (%)	92 (47.2%)	103 (52.8%)			
Age (years)	46.52±10.43	53.70±12.10	12.92	<0.001	4.05(1.89-8.67)

SD, Standard deviation; χ^2, *Chi*-Square test; CI, Confidence interval; P value significant at < 0.05.

Detection of Granzyme B by flow cytometry:

Percentage of Granzyme B was significantly lower in the peripheral blood of breast cancer patients than control group (figure 2).

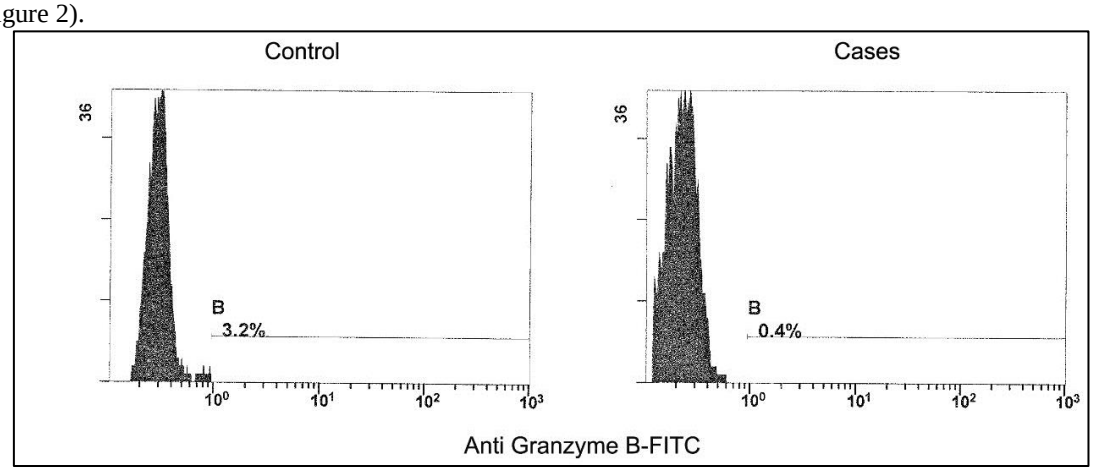

Fig. 2: Representative expression of Ggranzyme B using flow cytometry for control and breast cancer groups

We analyzed Granzyme B levels using flow cytometry with anti-Granzyme B (FITC) directly after blood sample collection. The results showed a significantly lower percentage of Granzyme B in breast cancer patients compared to healthy women (p<0.001), with an odds ratio of 10.11 (95% confidence interval: 4.97-20.58) (table 4)

Table 4: Immunophenotyping of Granzyme B (Anti human Granzyme B monoclonal

	Control N=92	**Breast cancer (BC) N =103**	χ^2	**P value**	**Odds ratio 95% (CI)**
Anti Granzyme B Abs.) (mean±SD)	3.35±2.52	0.42±0.40	40.69	<0.001	10.11 (4.97-20.58)

SD, Standard deviation; χ^2, *Chi*-Square test; CI, Confidence interval; P value significant at < 0.05.

Genotyping of Granzyme B:

The allelic frequency of rs8192917 was assessed using tetra primer ARMS-PCR, and the products were resolved on agarose gel. The results revealed a statistically significant difference between breast cancer patients and control group.

It was found that CC genotype was lower in breast cancer group than control group and GG genotype was higher in breast cancer group than control group (p<0.001) and odds ratio (95% confidence interval) in CC genotype between breast cancer and control groups 0.22 (0.06-0.77) and in GG genotype 1.12(1.02-1.22).

Breast cancer group had lower frequency for the C allele compared to control group (p <0.001) with χ^2 equal 14.05.

Moreover, there was a significant difference between the breast cancer and the control groups in rs8192917 genotypes (GG, CC) (p<0.001) as shown in table 5.

Table 5. Genotyping of rs8192917

	Control N (%)	**Breast cancer N (%)**	χ^2	**P value**	**Odds ratio 95% (CI)**
Genotype CC GG	 12 (80.0) 80 (44.4)	 3 (20.0) 100 (55.6)	7.02	0.01	 0.22 (0.06-0.77) 5 (1.36-18.33)
Allele frequency C G	 24 (80.0) 160 (44.4)	 6 (20.0) 200 (55.6)	14.05	0.00	 0.2(0.08-0.5) 5.0(2.0-12.53)

SD, Standard deviation; χ^2, *Chi*-Square test; CI, Confidence interval; P value significant at < 0.05.

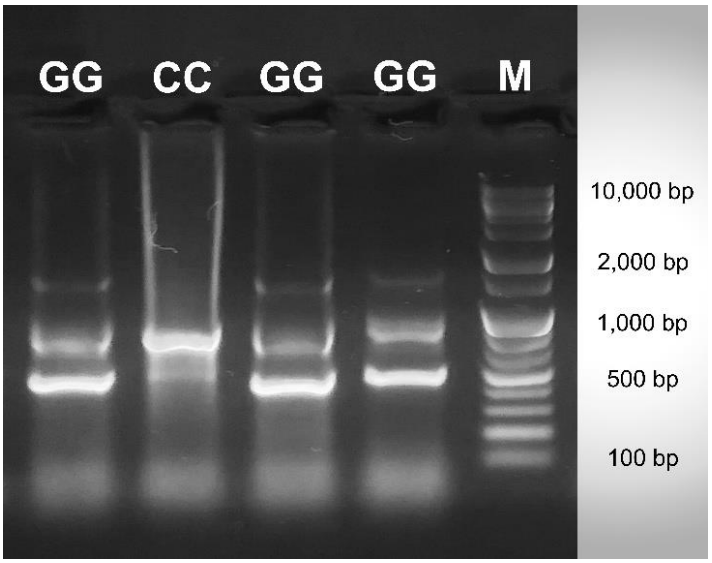

Fig. 3: Representative digital photograph of ARMS-PCR amplified products separated on 2.0% agarose gel electrophoresis showing the breast cancer (rs8192917) genotyping against Gene Ruler 100 bp DNA ladders (Willowfort, Birmingham Research and Development park, Birmingham)

The allelic frequency of rs2236338 was assessed using tetra primer ARMS-PCR, and the products were resolved on agarose gel. The current study revealed statistically significant difference between genotypes (CC, GG, GC) of breast cancer patients and control group (P<0.05).

It was found that CC genotype was non-significant in breast cancer group than control group (P>0.999), GG genotype was the most frequent genotyping in control group and breast group compared to other genotyping (P< 0.019) and it is considered protective as its odds ratio was 0.233(95% CI: 0.069-0.85) and GC genotype was considered risk predictive as it was higher in breast cancer group than control group (p=0.0325) and its odds ratio was (1.07-14.3).

The current study revealed a statistically significant difference between breast cancer and control groups regarding rs2236338 genotypes (Dominant comparison p<0.001 and recessive comparison, p<0/05), OR =4.29(1.18-15.55) in recessive comparison for (CC+GC) and =0.23(0.06-0.85) for GG, as shown in table (6).

There were not statistically significant difference between G and C alleles, OR=4.09(1.15-14.57) for C allele and =0.24(0.07-0.87) foe G allele.

Table 6: Genotyping of rs2236338

	Control N (%)	Breast cancer N (%)	χ^2 or Fischer exact test	P value	Odds ratio 95% (CI)
Genotype rs2236338					
CC	0 (0.0)	1 (100)		>0.999	infinity(NaN-infinity)
GC	3 (20)	12 (80)		0.0325	3.91 (1.07-14.3)
GG	89 (49.7)	90 (50.3)		0.019	0.233(0.069-0.86)
Dominant comparison					
GG+GC	92(47.4)	102(52.6)	1	<0.05	0(0-NAN)
CC	0(0)	1(100)			
Recessive comparison					
CC+GC	3(13.8)	13(81.3)	5.65	0.017	4.29(1.18-15.55)
GG	89(49.7)	90(50.3)			0.23(0.06-0.85)
Allele frequency					
C	3 (18.8)	13(81.3)	5.46	0.19	4.09(1.15-14.57)
G	181(48.5)	192(51.5)			0.24(0.07-0.87)

SD, Standard deviation; χ^2, *Chi*-Square test; CI, Confidence interval; P value significant at < 0.05.

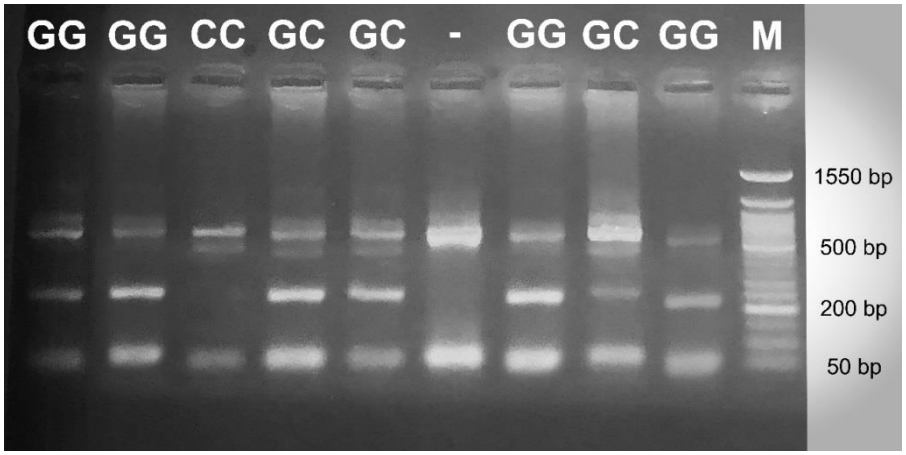

Fig. 4: Representative digital photograph of ARMS-PCR amplified products separated on 2.0% agarose gel electrophoresis showing the breast cancer (rs2236338) genotyping against Gene Ruler 50bp DNA ladder (Willowfort, Birmingham Research and Development park, Birmingham)

Hardy–Weinberg equilibrium (HWE) genotype frequencies of Granzyme B gene (rs8192917 and rs2236338) polymorphisms are demonstrated in table (7).

Granzyme B (rs8192917) genotype frequencies were different with HWE among controls and breast cancer group. Also, Granzyme B (rs2236338) genotype frequencies were different significantly from those expected by HWE among patients of breast cancer group and control group.

Table 7. Hardy–Weinberg equilibrium for Granzyme B gene (rs8192917 and rs2236338) genotypes among breast cancer patients and controls

	rs8192917					rs2236338				
	Genotype	Obs.	Exp.	χ^2	*P value*	Genotype	Obs.	Exp.	χ^2	*P value*
Control (N=92)	GG	80	69.56	92	<0.001	GG	89	89.02	0.025	>0.01
	GC	0	20.87			GC	3	2.95		
	CC	12	1.57			CC	0	0.024		
Breast cancer (N=103)	GG	100	97.09	103	<0.001	GG	90	89.48	0.67	>0.01
	GC	0	5.83			GC	12	13.05		
	CC	3	0.087			CC	1	0.48		

Obs., Observed; Exp., Expected; χ^2, *Chi*-Square test; P value significant at < 0.05.

CC genotype of Granzyme B (rs8192917) show high significant percentage of anti Granzyme B than GG genotype P=0.003. However, GG genotype of Granzyme B (rs2236338) show non-significant percentage of anti Granzyme B than GC genotype P>0.05, (table 8).

Table 8: The relation between AntiGranzyme B protein expression and studied SNPs.

	CC	GC	GG	*P value*
Anti Granzyme B				
rs8192917	3.95±0.77	-	1.63±0.16	0.003
rs2236338	-	1.29±0.60	1.86±0.17	0.145

Multinominal logistic regression helps to predict the effect of independent variables (predictors) on the dependent one (outcome). Estimating the probability of occurring breast cancer based on the given independent variables. In our model, the results of flow cytometry of Anti-Granzyme B were highly significant in our predicative model. Any increase by unit in Anti Granzyme B decrease the probability of cancer occurence compared to normal by 2.441. Also, the probability of occurrence of breast cancer increase in GC genotyping in SNP1 (rs8192917) compared to GG genotyping by 16.564. Moreover in SNP2 (rs2236338) the probability of occurrence of breast cancer increased with CC genotyping compared to GG genotyping by 16.880. On the other hand, patients of age groups ≤ 40 decrease the probability of occurrence of breast cancer by 0.316 compared with patients in age higher than 40. Odds ratio for Anti granzyme B, SNP1 (rs8192917), SNP2 (rs2236338) and Age groups were 0.087, 0.336, 8.631, and 0.669, respectively.

Table 9: Multinominal logistic regression between SNP1 (rs8192917), SNP2 (rs2236338), age groups, and Anti Granzyme B results

Diagnosis		B	Std. Error	Wald	Sig.	Odds ratio	95% Confidence Interval for Exp(B)	
							Lower Bound	Upper Bound
Cancer	Intercept	2.644	.389	46.129	.000			
	Anti Granzyme B	-2.441-	.416	34.403	.000	.087	.039	.197
	[SNP1 genotyping=GC]	16.564	.000					
	[SNP1 genotyping=CC]	-1.090-	1.143	.910	.340	.336	.036	3.156
	[SNP1 genotyping=GG]	0[b]	.	.	.	.	.	.
	[SNP2 genotyping=CC]	16.880	.000	.	.			
	[SNP2 genotyping=GC]	2.155	1.124	3.680	.055	8.631	.954	78.062
	[SNP2 genotyping=GG]	0[b]	.	.	.	.	.	.
	[AgeGroups=1]	-.316-	.740	.183	1	.669	.729	.171 3.109
	[AgeGroups=2]	0[b]	.	.	0	.	.	. .

a. The reference category is: Normal.
b. This parameter is set to zero because it is redundant.

Area under curve (AUC) value and parameters of validity of Multinominal logistic regression including positive predictive value (PPV) and negative predictive value (NPV) are displayed 80.18, and 94.52%. AUC was reported (AUC = 0.899, sensitivity = 82 %and specificity = 70%, p<0.0001) (figure 5).

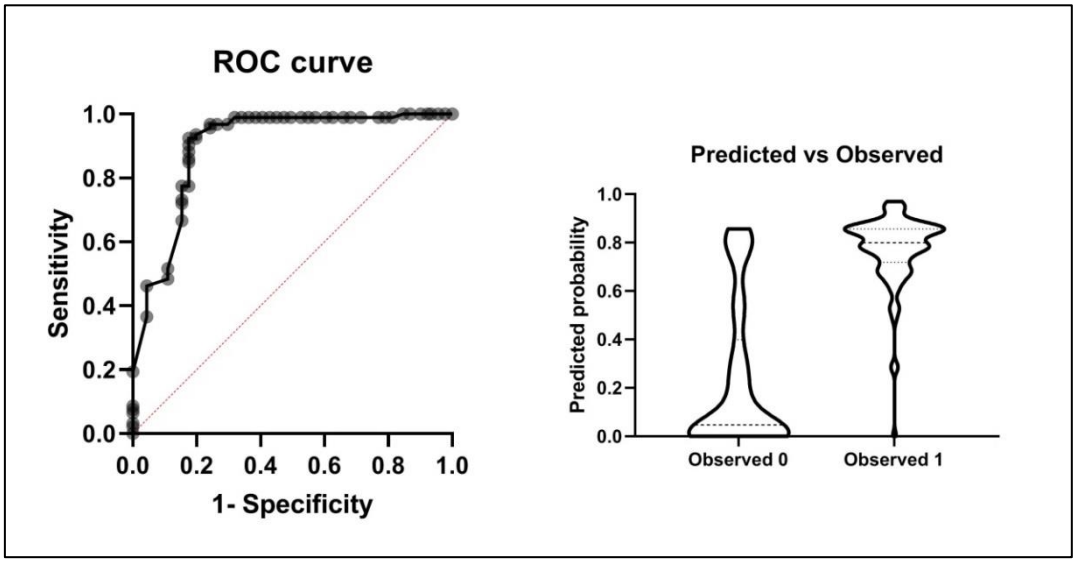

Fig. 5: ROC curve of multinominal logistic regression

DISCUSSION

The study examined the relationship between Granzyme-B (GrB) expression, as well as genetic polymorphisms (rs8192917 and rs2236338) that code for GrB, and the risk of developing breast cancer in Egyptian women. The direct lytic effect on tumor cells is mediated by the perforin/granzyme system, which induces apoptosis in target T cells by creating trans membrane pores and cleaving effector caspases, such as caspase-3[18]. Additionally, Granzyme B has been suggested to have caspase-independent mechanisms of cytotoxicity, resulting in DNA fragmentation and subsequent apoptosis[19]. Similar to other cytotoxic molecules, Granzyme B plays a role in various pathologies, including several viral infections, graft rejections, and graft-versus-host disease [20-22]. It is also believed to contribute to the inhibition of cancer growth and progression [23, 24, 25].

In the present study anti Granzyme B in the control group was higher than in breast cancer patients (p value <0.001) as measured by flow cytometry.

Granzyme B are significantly less expressed in breast cancer patients compared with the control group . Gaafar et al [13]. demonstrated that breast cancer patients had lower percentages of T cells, subsequently Granzyme B were significantly less in breast cancer patients when matched with normal donors.

The nonsynonymous SNP A-55G (rs 8192917) found in exon 2, leads to the amino acid substitution of Q-55R (numbering with reference to the human chymotrypsinogen A sequence) [11] .SNP (rs11539752) located in exon 3, is responsible for the amino acid substitution of P-94A and SNP (rs 2236338), located in exon five is responsible for the amino acid substitution of Y247H are the most studied polymorphisms in Granzyme B gene.

These polymorphisms have been previously linked to the occurrence of other cancers or immunological diseases [13]. The SNPs rs2236338 and rs8192917 are functionally active, leading to changes in amino acids. Jeong et al. previously demonstrated that these two SNPs form a haploblock in the Korean population [26]. Similarly to these results, by the analysis of the Egyptian population in breast cancer patients, we found that the CC genotype of rs8192917 was associated with decreased risk of breast cancer group significantly.

The C allele of rs8192917 has been linked to vitiligo, an autoimmune skin condition, in three independent cohorts [26-28]. Furthermore, it has been associated with subacute sclerosing panencephalitis [29] and postoperative keloids[30] in individual studies. Additionally, this SNP has been connected to transplantation outcomes following HLA-matched unrelated bone marrow transplants [31].

At the functional level, one study indicated that rs8192917 is linked to natural killer cell cytotoxicity [32] , though additional functional validation is required to clarify this finding. Moreover, another SNP in Granzyme B, independent of rs8192917, has been associated with joint destruction in rheumatoid arthritis, [32] highlighting the significance of Granzyme B genetic variants in immune-related pathogenic mechanisms. While these findings suggest that Granzyme B genetic variants may have broad disease-modifying effects, the underlying mechanisms remain unclear. Sun et al[12]. demonstrated that the minor RAH haplotype maintains the pro-apoptotic activity of Granzyme B. However, Granzyme B also plays various roles in processes such as extracellular matrix remodeling, epithelial-to-mesenchymal transition, inflammation, and fibrosis[10].

In this study, we found that CC genotype of Granzyme B (rs8192917) in breast cancer patients showed high significant percentages of anti Granzyme B than GG genotype.

This revealed that, CC genotype was associated with higher anti-Granzyme B levels.

Interestingly, Granzyme B gene polymorphism (rs2236338) in the current study shown the GC genotypes, were higher in breast cancer patients compared with control groups, similarly to Gaafar et al. [13] results.

Another study was done in Jordan and enrolled patients with colorectal cancer. Their study revealed a not significant association [17].

In the current study, the CC genotype (rs2236338) was the total frequent in breast cancer patients (100%) and lack in control group, followed by the GC (80%) and the least frequent was the GG (50.3%). Similarly, the distribution of genotypes, among patients with breast cancer and control group, based on Gaafer et al. [13] study revealed that GC genotype (50%) was higher than in the control group (42.5%) and C allele frequency (39.3%)in breast patients was higher than in control group (22.6) ,Similarily in this study C allele frequency in breast cancer patients (81.3) was higher than in control group (18.8%)

The polymorphism (rs8192917) has been linked to the occurrence of breast cancer in Egyptian patients. Conclusion, we found that CC genotype of Granzyme B (rs8192917) showed a higher percentage of anti Granzyme B than GG genotype. The prevalence of genotypes and alleles, as well as the location of Granzyme B polymorphisms, remains uncertain, and further research is needed to explore this aspect.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article. This manuscript has not been previously published and is not under consideration in another journal.

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