Breast Cancer Gene 1 (Brca 1) Mutation In Female Patients With Or Without Family History In Qalubia Governorate

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ABSTRACT: Breast cancer is the most common cancer in women and its impact on morbidity and mortality is significant and well documented. BRCA genes mutation account for most of the cases of familial breast cancer. Female BRCA1 mutation carriers have an 80% to 85% risk of developing breast cancer over their life-time. This study aims to detect 5382insC,185delAG and C61G mutations in BRCA1 gene in healthy females and breast cancer female patients in Qalubia Governorate and correlate them with the presence or absence of family history of breast &/ or ovarian cancer to allow identification of individuals at high risk**. Materials and methods:** 50 females divided into 20 healthy females and 30 breast cancer patients with or without family history of breast &/or ovarian cancers were included in the study.185delAG and 5382insC mutation were detected by multiplex mutagenically separated PCR (MS - PCR) and C61G mutation was detected using the RFLP method. **Results:** It was found that the incidence of BRCA1 gene mutation in the breast cancer group was higher than its incidence in the control group Also the incidence of BRCA1 gene mutation in the groups with family history was higher than in the groups without family history. In addition, multiple exons mutation frequency was higher than one exon mutation in the breast cancer group with family history.Moreover, 5382insC mutation was found to be the most frequent BRCA 1 gene mutation among the females of Qalubia governorate followed by C61G mutation and 185 delAG mutation. **Conclusion:** In conclusion, BRCA1 gene mutation and multiple BRCA1 exons mutations play an important role in the pathogenesis of familial breast cancer in Qalubia Governorate, Egypt.
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Key words: familial breast cancer, BRCA1 gene, 5382insC mutation, 185delAG mutation, C61G mutation.

INTRODUCTION:

Breast cancer is the most common cancer in women and its impact on morbidity and mortality is significant and well documented (Jemal et al., 2008).Epidemiological studies have revealed several risk factors associated with increased susceptibility to breast cancer including genetic, family history, reproductive history and environmental factors (Lacey et al.,2009). About 5% to 10% of breast cancer patients carry germ line mutations that predispose them to inherited disease (Malone et al., 1998). Several genes have been involved in the pathogenesis of hereditary breast/ovarian cancer, but mutations in the BRCA1 gene are by far the most recurrent (Baudi et al., 2001). Germ line mutations of the *BRCA1* gene account for 40% to 45% of hereditary breast cancers and 80% of the families whose members have a high incidence of both breast and ovarian cancers.

BRCA1 (breast cancer 1, early onset) was cloned in 1994 based on its linkage to early-onset breast and ovarian cancer and is one of the most important tumor suppressor genes associated with breast cancer (Zhang and Powell, 2005)..It is located on the long (q) arm of chromosome 17 at band 21and contains 24 exons and 5592 nucleotides encoding a large protein of 1863 amino acids (Malone et al.,1998; Antoniou et al., 2003 and Chen and Parmigiani,2007). This protein is called breast cancer type 1 susceptibility protein (Jaworowska,2009). It is present in many tissues including normal breast and ovarian epithelium. It is either altered, reduced, or absent in some breast and ovarian tumors (Miki et al., 1994). BRCA1 interacts with numerous proteins that are involved in many important biological processes/pathways which may contribute to its tumor suppressor activity. These processes affect cell cycle checkpoints, transcription, protein ubiquitination,

apoptosis, and DNA repair. BRCA1 deficiency causes abnormalities in the S-phase checkpoint, the G_2/M checkpoint, the spindle checkpoint and centrosome duplication since it is involved in all phases of the cell cycle. The genetic instability caused by BRCA1 deficiency also triggers cellular responses to DNA damage that blocks cell proliferation and induces apoptosis. Thus BRCA1 mutant cells cannot develop further into full-grown tumors unless this cellular defense is broken. On the other hand, the absence of BRCA1 allows further genetic alterations, including further tumor suppressor mutations and activation of oncogenes, which overcomes growth defects and ultimately results in breast cancer formation (Zhang and Powell, 2005 and Deng, 2006).

Researchers have identified hundreds of mutations in the BRCA1 gene, many of which are associated with an increased risk of cancer (Breastcancer.org, 2008). Female BRCA1 mutation carriers have an 80% to 85% risk of developing breast cancer over their life-time (Burga et al., 2009). In addition to breast cancer, mutations in the BRCA1 gene also increase the risk of ovarian, uterine, cervical, fallopian tube, pancreatic, colon and liver cancers (Thompson et al., 2002).

In a study of polish population on females with family history of breast & /or ovarian cancer, three important mutations in BRCA1 gene were detected including 5382insC, 185delAG and C61G on exons 20, 2 and 5 respectively (Grzybowska et al., 2002). In addition, exons 2 and 5 of BRCA1 gene were found to have a significant role in protein function. Moreover, other significant studies showed that exons 2, 5 are most likely to harbor germ line BRCA1 mutations (Yassaee et al., 2002).Also 5382insC mutation accounted for 80% of mutations found in both BRCA1 and BRCA 2 genes in the polish population (Górski et al.,2000). The aim of this study is to detect 5382insC, 185delAG and C61G mutations in BRCA1 gene in female patients in Qalubia Governorate and correlate them with the presence or absence of family history of breast or ovarian cancer to allow identification of individuals at high risk.

SUBJECTS AND METHODS

This study was performed on 50 females who were attending general surgery department - Benha University Hospitals Qalubia governorate –Egypt after taking written informed consent from them. They were divided into two groups: 1- Control group: including 20 healthy females, 10 were without and 10 were with a family history of breast &/or ovarian cancers.2- Breast cancer group: including 30 female patients, 15 were without and 15 were with a family history of breast &/or ovarian cancers.

❖ ***Detection of Mutation in BRCA1 gene:***

1-185delAG mutation on exon 2 was detected by *multiplex mutagenically separated PCR (MS - PCR)*

2-5382insC mutation on exon 20 was also detected by *multiplex MS - PCR*

3-C61G mutation on exon 5 using *the RFLP method*.

I- *Sampling:*

3 ml of venous blood was collected on vaccutainer tube containing EDTA. Each sample was mixed and divided into 2eppendorf tubes then stored at -80 for further processing.

II - *Genomic DNA extraction:*

Genomic DNA was extracted from 400 ul of whole blood using ***Genomix Easy Quick Blood DNA Extraction Kits***-USA, according to standard protocols (Sambrock et al., 2001). 50 ul of DNA was eluted. The DNA yields were determined from the concentration of DNA in the elute measured by absorbance at 260 nm. On the other hand the DNA purity was determined by calculating the ratio of the absorbance at 260nm to the absorbance at 280 nm. Pure DNA had an A260/A280 ratio of 1.7 -2.0. If there is a contamination with protein or phenol, the A260/A280 is significantly less than 1.7. On the other hand if there is a contamination with RNA, the A260/A280 is significantly more than 2.0 (Haque et al., 2003). The extracted DNA was then stored at - 20°C until further processing.

III -*Detection of 185delAG and 5382insC BRCA1 gene mutations by multiplex MS -PCR:*

1-DNA Amplification:

Primers were designed for amplification of 185delAG and 5382insC BRCA1 gene mutations in exons 2 and 20 respectively to be suitable for PCR for these genes (Chan et al., 1999). Table (1) shows the sequence of these primers.

Table (1): Sequence of the primers used for detection of 185delAG and 5382insC BRCA1 gene mutations

BRCA 1 gene	Mutation detected by primers	Primers	Primer sequence 5' ------ 3'	Alleles amplified using these primers
Exon 2	185delAG mutation	Common forward	GGT TGG CAG CAA TAT GTG AA	Wild type allele
		wild type reverse	GCT GAC TTA CCA GAT GGG ACT CTC	
		Common forward	GGT TGG CAG CAA TAT GTG AA	Mutant allele
		mutant reverse	CCC AAA TTA ATA CAC TCT TGT CGT GAC TTA CCA GAT GGG ACA GTA	
Exon 20	5382insC mutation	Common reverse	GAC GGG AAT CCA AAT TAC ACA G	Wild type allele
		wild type forward	AAA GCG AGC AAG AGA ATC GCA	
		Common reverse	GAC GGG AAT CCA AAT TAC ACA G	Mutant allele
		Mutant forward	AAT CGA AGA AAC CAC CAA AGT CCT TAG CGA GCA AGA GAA TCA CC	

Amplification was done using **Dream Taq Green PCR Master Mix (2x)** supplied by **Fermentas**, Germany. The PCR mix for each exon contained 25 ul of Taq PCR master Mix 2x, 2.5 ul of each of the three primers, 5 ul of the template DNA and 12.5ul of nuclease free water to reach a final volume of 50 ul. ***G storm*** thermal cycler Uk was used for amplification according to the following program: initial denaturation at 95 ºC for 3 mins, 35 cycles of denaturation at 95 ºC for 1 min, annealing at 50 ºC for 1 min and extension at 72 ºC for 2 mins, followed by final extension at 72 ºC for 10 mins then hold at 4 ºC.

2- Agarose gel electrophoresis:

10 µl of each amplified DNA & 1000 bp ladder (molecular weight marker) were separated on 2% agarose gel containing 0.3 µg/ml of ethidium bromide.The bands were visualized using UV transilluminator (254nm), photographed & analyzed table (2).

Table (2): Expected results on gel electrophoresis for detection of 185delAG & 5382insC BRCA1 gene mutations.

BRCA1 gene	Expected results on gel	Conclusion
Exon 2 (BRCA1 185delAG mutation)	One band of 533 bp size	Wild type allele
	One band of 638 bp size	Homozygous mutant allele
	two bands of 533 & 638 bp size	Heterozygous mutant allele
Exon 11 (BRCA1 5382insC mutation)	One band of 227 bp size	Wild type allele
	One band of 354 bp size	Homozygous mutant allele
	Two bands of 227& 354 bp size	Heterozygous mutant allele

IV- *Detection of C61G BRCA1 mutation in Exon 5 using the RFLP method:*

1-DNA Amplification:

Primers were designed for amplification of C61G BRCA1 gene mutation in exon 5 to be suitable for PCR for this gene (Grzybowska et al., 2002). The sequence of forward primer was CTC TTA AGG GCA GTT GTG AG and that of reverse primer was TTC CTA CTG TGG TTG CTT CC.

Amplification was done using (Dream Taq green PCR master Mix 2x) supplied by (Fermentas, Germany). The PCR mix contained 25 ul of Taq PCR master Mix 2x, 2.5 ul of each of the two primers, 5 ul of the template

DNA and 15 ul of nuclease free water to reach a final volume of 50 ul.*G storm* thermal cycler UK was used for amplification according to the following program : initial denaturation at 95 ºC for 3 mins,35 cycles of denaturation at 95 ºC for 1 min, annealing at 44ºC for 1 min and extension at 72 ºC for 2 mins, followed by final extension at 72 ºC for 10 mins then hold at 4 ºC.

2- Restriction endonuclease digestion:

The amplified products of exon 5 were digested by AvaII restriction endonuclease to detect C61G mutation, using Eco 471_AvaII kits (Fermentas) according to the following protocol: Preparation of a mixture of 10 ul of PCR reaction mixture, 18ul of nuclease free water, 2ul of 10xbuffer R and 4ul of Eco471. The product was mixed gently and spinned down for few seconds, incubated at 37 for 4 hours. Then thermal inactivation was done at 65 *ºC* for 20 min.This protocol was done in Thermo cycler (Hybaid, USA).

2-Agarose gel electrophoresis:

After AvaII digestion gel electrophoresis of the amplified products was done and one band was expected to be seen if no mutation and 3 bands if heterozygous mutation was present.

RESULTS:

Three germ line mutations in BRCA1 gene were analyzed including 185delAG, C61G & 5382insC mutations in exons 2, 5 & 20 respectively..Some clinical data of the subjects were taken and analyzed (tables 3, figures 1&2).Gel electrophoresis of the PCR products is shown in figures.3, 4 & 5.

Table (3): clinical data of the study group

groups / Clinical items	Controls		Breast cancer groups		X^2	*P*
	Without family history N =10	With family history N=10	Without family history N=15	With family history N=15		
	Number (%)					
Marital status Married	10/10 (100)	8/10 (80)	14/15 (93.3)	14/15 (93.3)	**2.8**	**>0.05**
Single	0/10 (0)	2/10 (20)	1/15 (6.7)	1/15 (6.7)		
Lactation +ve	10/10 (100)	6/10 (60)	12/15 (80)	12/15 (80)	**5**	**>0.05**
-ve	0/10 (0)	4/10 (40)	3/15 (20)	3/15 (20)		
Parity +ve	10/10 (100)	6/10 60	13/15 (87.7)	12/15 (80)	**5.74**	**>0.05**
-ve	0/10 (0)	4/10 (40)	2/15 (13.3)	3/15 (20)		
Hormonal Contraception +ve -ve	3/l0 (30) 70	3/10 (30)	3/15 (20)	5/15 (33.3)	**4.93**	**>0.05**
	7/10 (70)	710 (70)	12/15 (80)	10/15 (66.6)		
History of DM +ve	0/10 (0)	0/10 (0)	3/15 (20)	2/15 (13.3)	**4.07**	**>0.05**
-ve	10/10 (100)	10/10 (100)	12/15 (80)	13/15 (86.7)		
Clinical items	**Mean ± SD**				**F**	**p**
Age	34 ± 6.1	28 ±10.7	40.9 ±13.3	39.7 ± 6.9	**4.14**	**>0.05**
Onset of menarche	12.3 ±0.66	11.7 ±0.95	11.5 ±0.99	11.5 ±0.64	**2.52**	**>0.05**

p>0.05 : non significant

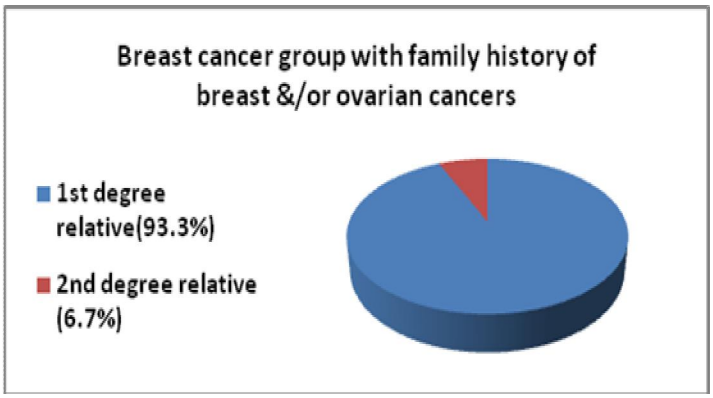

Figure (1): Percentage of the first & second degree of family relatives in breast cancer patients with family history of breast&/or ovarian cancer

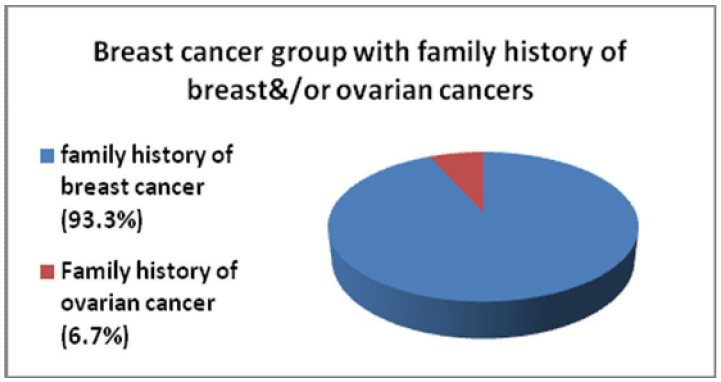

Figure (2): Percentage of the history of breast and ovarian cancers in breast cancer patients with family history of breast&/or ovarian cancer

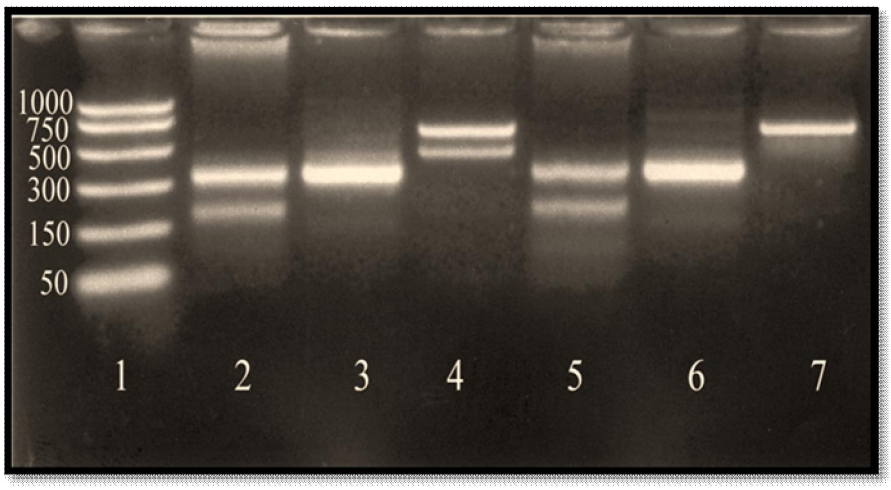

Figure (3): gel electrophoresis of amplified products of BRCA1 gene of exon 2 and exon 20.Lane 1 shows DNA ladder 1000bp.Lane 4 and7 show exon 2 amplification products ;with lane 4 showing heterozygous mutation and lane 7 showing homozygous mutation. Lanes 2, 3.5 and 6 show exon 20 amplification products; with lanes 2, 5 showing heterozygous mutation and lanes 3, 6 showing homozygous mutation.

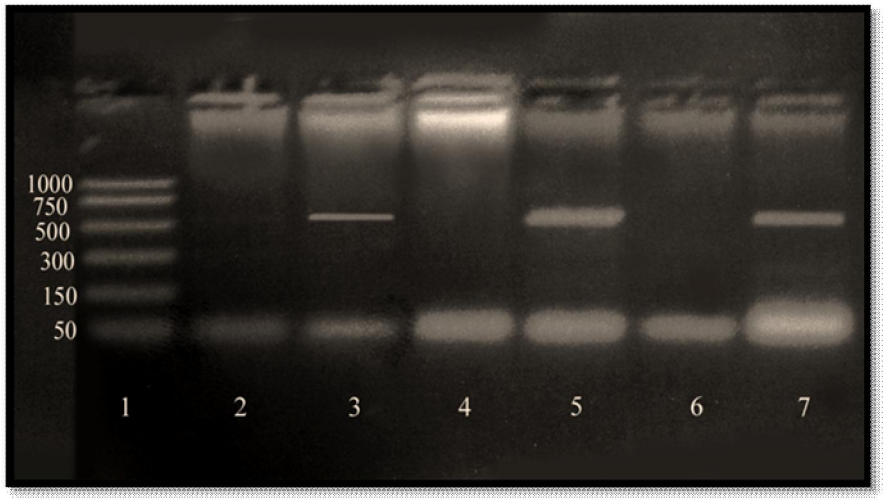

Figure (4): gel electrophoresis of the amplified products of BRCA1 gene of exon 5 before avaII digestion. Lane 1 shows DNA ladder 1000bp, Lanes 3, 5 and 7 show one band.

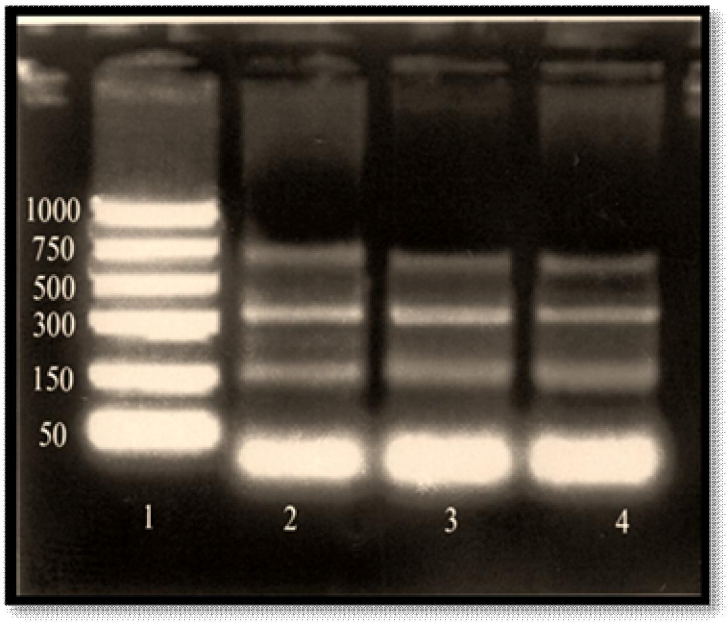

Figure (5): gel electrophoresis of amplified products of BRCA1 gene of exon 5 after avaII digestion. Lane 1 shows DNA ladder 1000bp.Lanes 2, 3 and 4 show three bands indicating heterozygous mutation.

The incidence of BRCA1 gene mutation in the breast cancer group (86.7%) was higher than its incidence in the control group (55%). Also the incidence of BRCA1 gene mutation in the groups with family history (73.35%) was higher than in the groups without family history (68.35%) (table 4). Besides that, the frequency of 2 or 3 exons mutation as compared to one exon mutation and normal gene is shown in table 4. Multiple exons mutation frequency was higher than single exon mutation frequency in the breast cancer group with family history (60% compared to 26.7%) and in the control and breast cancer groups with family history (45% compared to28.35%). On the other hand one exon mutation was more frequent than multiple exons mutation in the breast cancer group without family history (46.7% compared to 40%) and in the control group without family history (50% compared to 0%).

Table (4): Exons mutation frequencies in BRCA1 Gene among control and breast cancer groups

p	X^2	Breast cancer group		control		Groups / Exons Mutation frequency
		With family history N=15	Without family history N =15	With family history N=10	Without family history N =10	
		Number (%)				
>0.05	12.3	2/15 (13.3)	2/15 (13.3)	4/10 (40)	5/10 (50)	**Normal gene**
>0.05	23.3	13/15 (86.7)	13/15 (86.7)	6/10 (60)	5/10 (50)	**Mutant gene**
>0.05	12.3	4/15 (26.7)	7/15 (46.7)	3/10 (30)	5/10 (50)	**One exon mutation**
>0.05	12.3	9/15 (60)	6/15 (40)	3/10 (30)	0/10 (0)	**Two or three exons mutation**

The genotype frequency of 185delAG, 5382insC and C61G mutations was estimated in all studied groups (Table 5). The 5382insC mutation was the most frequent BRCA 1 mutation among the females of Qalubia governorate (56 %). It was followed by C61G mutation (40%) and 185 delAG mutation (22%).The same order of frequency of exons mutation occurred among the study groups with + ve family history, with 5382insC mutation being the most frequent (55%) followed by C61G mutation (50%) then 185 delAG mutation (26.665%). Also the most frequent mutations among the breast cancer group with + ve family history were 5382insC mutation and C61G mutation with the same frequency level each being (60%), while the least frequent mutation was 185 delAG mutation (33.33%). In addition, the absence of 185 delAG mutations in control women who had no family history of breast cancer was observed.

The allelic frequency of 185delAG, 5382insC and C61G mutations was also evaluated in all studied groups (Table 6). The evaluation revealed that the 5382insC homozygous mutation was more frequent than the heterozygous mutation in the studied groups but the heterozygous mutation was more frequent in the control group without family history..Also 185delAG homozygous mutation was more frequent than the heterozygous mutation in the studied groups except for the control group with absence of this type of mutation.

Table (5): Genotype frequency of 185delAG, 5382insC and C61G mutations in exons 2. 20 and 5 respectively among the control and breast cancer groups

p	X^2	Total	Breast cancer group		control group		Groups / Genotype frequency
			With family history N=15	Without family history N =15	With family history N=10	Without family history N =10	
			Number (%)				
>0.05	4.16	11/50 (22)	5/15 (33.33)	4/15 (26.7)	2/10 (20)	0/10 (0)	**185delAG (Exon 2) Mutation**
>0.05	4.8	28/50 (56)	9/15 (60)	11/15 (73.3)	5/10 (50)	3/10 (30)	**5382insC (Exon20) Mutation**
>0.05	4.44	20/50 (40)	9/15 (60)	5/15 (33)	4/10 (40)	2/10 (20)	**C61G (Exon 5) Mutation**

Table (6): Allelic frequency of 185delAG, 5382insC and C61G mutations in exons 2. 20 and 5 respectively among the control and breast cancer groups.

p	X^2	Total	Breast cancer groups		control		Groups / Allelic frequency
			With family history N=15	Without family history N =15	With family history N=10	Without family history N =10	
			Number (%)				
>0.05	8.06	39/50	10/15 (66.7)	11/15 (73)	8/10 (80)	10/10 (100)	**185delAG (Exon 2):** • Wild
		2/50	2/15 (13)	0/15 (0)	0/10 0	0/10 (0)	• Heterozygous
		9/50	3/15 (20)	4/15 (26.7)	2/10 (20)	0/10 0	• Homozygous
>0.05	11.52	22/50	6/15 (40)	4/15 (26.7)	5/10 (50)	7/10 (70)	**5382insC (Exon 20):** • Wild
		7/50	2/15 (13)	2/15 (13)	0/10 (0)	3/10 (30)	• Heterozygous
		21/50	7/15 (46.7)	9/15 (60)	5/10 (50)	0/10 0	• Homozygous
>0.05	4.44	30/50	6/15 40	10/15 66.7	6/10 60	8/10 80	**C61G (Exon 5):** • Wild
		20/50	9/15 60	5/15 33	4/10 40	2/10 20	• Heterozygous

According to the current result, the age of females with BRCA1 gene mutations between 15 &30 years was 3/37(8.1%), above 30 to 40 was 23/37 (62.6%) and above 40 to 50 years was 11/37 (29.7%) Beside that the age of onset of breast cancer in the breast cancer patients with BRCA1 gene mutation was between 30 to 40 years in 17/26 of cases (65.4%) and above 40 to 50 years in 9/26 of cases (34.6%). The age of onset of breast cancer in the breast cancer patients with family history and BRCA1 gene mutation was between 30 to 40 years in 9/13 of cases (69.2%) and above 40 to 50 years in 4/13 of cases (30.8%).

DISCUSSION:

Breast cancer is the most common female malignancy and a major cause of death in middle-aged women (Lacey et al., 2009). Germ-line BRCA1 mutations confer a substantial lifetime risk of breast and ovarian cancer. The absolute risk of cancer by the age of 70 years conferred by a BRCA1 mutation is reported to be between 45% and 87% for breast cancer and between 36% and 66% for ovarian cancer (Thompson et al., 2002).Germ-line mutations of the BRCA1 gene are responsible for a substantial proportion of families with multiple cases of early-onset breast cancer (Haffty et al., 2009).

The magnitude of the risk of breast cancer in carriers of mutations in BRCA1 is critical for guiding decisions concerning cancer prevention options. Some women found to carry such mutations undergo prophylactic mastectomy and/or oophorectomy, because their cancer risk is extremely high. However, although it is very clear that mutations in these genes, segregating within these types of families, confer a substantial risk of both breast and ovarian cancer, the same may not apply to mutations detected in other settings, such as in families with less-extreme cancer histories or in incident cases, even those of early onset **(**Antoniou et al., 2003**)**.

The study was carried on 50 females to detect different mutations in BRCA1 gene in female patients in Qalubia Governorate and correlate them with the presence or absence of family history of breast or ovarian cancer to allow identification of individuals at high risk.

Multiplex mutagenically separated PCR was used to detect 5382insC mutation (exon20) and 185del AG mutation (exon2) in the BRCA1 gene as this method is easy, simple and rapid for detection of mutation (Chan et al., 1999). But for detection of C61G substitution mutation in exon 5 we used the RFLP method (Grzybowska et al., 2002).

In the current study, the percentage of the first degree family relative (mother, sister, or daughter) with breast and/or ovarian cancer (93.3%) was extremely higher than that of the second degree family relative (6.7%) in the breast cancer group with family history.

Couto and Hemminki (2007) reported that women with a first- and second-degree relative with breast cancer had a higher risk of breast cancer than women without such a family history. This risk was higher when the affected relative is a mother and/or a sister(s) compared to a grandmother and/or an aunt(s).

A family represents a group of individuals sharing a common environment and genes. Hence, the higher incidence of breast cancer in the first and second degree relatives and the mechanisms leading to this higher risk of breast cancer could be environmental, genetic, or a combination of the two. The familial risk among first-degree relatives is so high that a substantial part of it must be caused by heritable factors. As environmental sharing between second-degree relatives is probably low, the breast cancer risk associated with having affected second-degree relatives is assumed to be due to heritable causes (Couto and Hemminki, 2007).So since first degree relatives share genes and environment more than second degree relatives, it may explain the higher incidence of breast cancer in the first degree relatives.

This study showed that the percentage of family history of breast cancer (93.3%) was much more than the percentage of family history of ovarian cancer (6.7%) in the breast cancer group with +ve family history. In support of this, other studies have indicated that the incidence of breast cancer and ovarian cancer in families is correlated with the location of the BRCA1 mutation. When mutations that result in a truncated BRCA1 protein occur in the first two-thirds of the gene, the risk of ovarian cancer relative to breast cancer in the family is significantly higher than when truncating mutations occur in the last one-third of the gene (Gayther et al., 1995). More recently, other researchers have found that mutations in a central region of BRCA1 were associated with a lower risk of breast cancer (Thompson and Easton, 2002). Also another study has reported that the risk of breast cancer increases with mutation position, from 5′ to 3′ (Risch et al., 2001). Researchers categorized BRCA1 mutations into three groups as: nucleotides 1–2400, 2401–4184, and 4185 onward. The relative risk of breast cancer for mutations in the central region as compared to that for mutations in the 5′ region was estimated to be 0.93, and that for mutations in the 3′ region was estimated to be 1.4; the corresponding risks for ovarian cancer were 1.8 and 1.1 respectively **(**Antoniou et al., 2003**)**.Note that in this study, the most frequent exon mutation was in exon 20 (5382insC mutation).this was followed by exon 5 and the least frequent mutation was in exon 2.

The incidence of BRCA1 gene mutation was higher in the breast cancer group than in the control group and in the groups with family history than in the groups without family history. In addition, multiple exons mutation frequency was higher than single exon mutation frequency in the control and breast cancer groups with family history. On the other hand one exon mutation was more frequent than multiple exons mutation in the control and breast cancer groups without family history. These results indicate that BRCA1 gene mutation plays an important role in the process of carcinogenesis in the breast cancer patients especially in those with family history and that multiple exons mutation plays a role in the carcinogenesis in the breast cancer patients with family history of breast cancer than in those without family history.

The higher frequency of BRCA1 gene mutation in the breast cancer patients is in agreement with the previous studies which reported that the prevalence of BRCA1 mutations in Korean women with breast cancer at a young age was high. They stated that certain variations of the BRCA1 gene lead to an increased risk for breast cancer and that researchers have identified hundreds of mutations in the BRCA1 gene, many of which are associated with an increased risk of cancer (Choi et al., 2004).

The higher frequency of the multiple BRCA1 gene mutations in the breast cancer patients and in the groups with family history is supported by a study which reported that most inherited breast cancer risk results from the interaction of several mutated genes. Families with this pattern of inheritance will contain only a few members with breast cancer. These mutated genes by themselves are associated with only a small increase in breast cancer risk, but when several of these genes are inherited together, they can lead to significant increase in breast cancer risk (Claus et al., 1996).Also, Warren and Devine (2003) reported that all breast cancer results from multiple gene mutations. The initial mutation can be inherited from one's parents.

According to the current study, the 5382insC mutation (exon 20) was the most frequent BRCA 1 mutation among the females of Qalubia governorate (56 %).It was followed by C61G mutation (exon 5) (40%) and 185 delAG mutation (exon 2) (22%). The same order of frequency of exons mutation occurred among the study groups with + ve family history, with 5382insC mutation being the most frequent (56%) followed by C61G mutation (52%) and 185 delAG mutation (28%). Also the most frequent mutations among the breast cancer group with + ve family history were 5382insC mutation and C61G mutation

with the same frequency level each being (60%), followed by 185 delAG mutation (33.33%).

It was reported that in the Upper Silesia population that the most frequent germ line mutation was 5382ins C mutation (73%),while mutations 185delAG and C61G were less frequent (Grzybowska et al.,2002). Also, it was found that there were 3 founder mutations in the BRCA1 gene in Polish families with breast-ovarian cancer including 5382insC, C61G, and 4153delA. They accounted for 51%, 20%, and 11% of all identified mutations respectively (Górski et al., 2000). Other ethnic groups with high frequencies of founder mutations include Ashkenazi Jews, Icelanders, French, Canadians, the Dutch, Norwegians, Swedes, and, possibly other populations of central and Eastern Europe (Streuwing et al., 1995; Szabo and King, 1997 and Dorum et al., 1999).

5382insC BRCA1 mutation was reported as the most frequent mutation (51%) detected in polish families with breast or ovarian cancer (Górski et al.,2000).Also it was reported that this mutation accounts for about 80% of the mutations found in the BRCA 1 and BRCA 2 genes in the polish populations (Grzybowska et al.,2000).This mutation is also common in Ashkenazi Jews, and it constitutes 25% of the mutations found in Jewish women with a high genetic risk of breast and ovarian cancers (Streuwing, 1997). The findings from small case series suggest that the 5382insC mutation also occurs frequently in families from Hungary and Latvia who have breast-ovarian cancer (Ramus et al., 1997 and Csokay et al., 1999). Also, the 5382insC mutation has been identified in other several countries, as Russia, Czech Republic and Lithuania, where it accounts, respectively for 94%, 33% and 50% of the BRCA1/2 gene mutations. In subjects with a family history of breast /ovarian cancer, the frequency in Russia is of 11%, in Greece of 8%, in Germany of 4%, in Italy of 3% and in Canada of 13%.On the contrary, a low frequency has been found in the Scandinavian countries, in Belgium and in Holland. It is thought that this mutation probably originated in the Baltic area 38 generations ago, with a gradual decrease going from east to west. A haplotype analysis indicated the likelihood of a single founder both in Europe and in North America for 5382insC mutations (Ferla et al., 2007).

The second most commonly observed mutation in the present study was the BRCA1 C61G mutation in exon 5. This is in accordance to a previous study which reported that the second most commonly observed mutation in the polish families with breast or ovarian cancers was the BRCA1 C61G missense mutation in exon 5 which accounted for 20% of families with mutations (Górski et al., 2000).

The least observed mutation in the present study was the BRCA1 185delAG mutation.This mutation was also found in 1% of Ashkenazi Jews and contributed to 16%–20% of breast cancer diagnosed before age 50. A second founder mutation in the BRCA1 gene, 5382insC, was found in 0.13% of this population. A combined analysis of several studies based on 22 different Ashkenazi populations had shown that breast cancer lifetime risk is similar in carriers of the 185delAG and 5382insC mutations (respectively of 64% and 67%), (Ferla et al., 2007).

In the current study, the 5382insC and 185delAG homozygous mutations were more frequent than the heterozygous mutation in the studied groups. On the contrary, the heterozygous5382insC mutation was more frequent in the control group without family history and the 185delAG mutation was absent in the control group without family history.This may be explained by that both alleles of the tumour suppressor gene BRCA1 must be affected before breast cancer to develop. This is due to the fact that if only one allele for the gene is damaged, the second can still produce the correct protein (Parkin et al., 2002). Also the low frequency of 185delAG mutation explains its absence in the control group without family history.

According to the current result, the age of females with BRCA1 gene mutations between 15 &30 years was 8.1%, above 30 to 40 was 62.6% and above 40 to 50 years was 29.7%.Also, the age of onset of breast cancer in the breast cancer patients with BRCA1 gene mutation was between 30 to 40 years in 65.4% of cases and above 40 to 50 years in 34.6% of cases. The age of onset of breast cancer in the breast cancer patients with family history and BRCA1 gene mutation was between 30 to 40 years in 69.2% of cases and above 40 to 50 years in 30.8% of cases.

Antoniou et al.(2003) reported that the breast cancer incidence in BRCA1-mutation carriers increased with age up to age 45–49 years but remained roughly constant thereafter. Women who carry BRCA1 mutations are particularly susceptible to the development of breast cancer before age 35–40.The relative risk of breast cancer in BRCA1-mutation carriers, relative to general population rates, declined with age from >30-fold at <40 years of age to 14-fold at >60 years of age. As a consequence of this, the incidence in BRCA1-mutation carriers raised to a plateau of ~3%–4% per annum in the 40–49-years age group and were roughly constant thereafter.

Ottini et al. (2000) reported that BRCA1 mutations were mostly found in breast cancer patients with disease diagnosis before the age of 50 years. Moreover, in cases with familial clustering of site-specific breast cancer, BRCA1 mostly accounted for tumours diagnosed before age 40 years.

Ford et al. (1995) estimated that the proportion of breast cancer in the general population due to BRCAl was 5.3% in women younger than the age of 40 years, 2.2% in women between the ages of 40 and 49 years, and 1.1% in women between the ages of 50 and 70 years. The proportion of breast cancer cases predicted to be attributable to BRCAl gene decreases markedly with age; approximately 33% of cases age 20-29 years compared with approximately 2% of cases age 70-79 years.

In conclusion, the 5382insC mutation was the most frequent BRCA 1 mutation among the females of Qalubia governorate followed by C61G mutation and 185 delAG mutation and multiple BRCA1 exons mutations play an important role in the pathogenesis of familial breast cancer in Egypt.

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