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Screening for Genetic Polymorphisms of Glutathione- S-Transferase Genes and Risk Factors Among Breast Cancer Patients in Iraq

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Abstract :

The study included 40 Iraqi women diagnosed as breast cancer patients who attended the Nuclear Medicine Hospital in Baghdad and 60 apparently healthy women as control .Genomic DNA extraction was performed using proteinase K / SDS method. Multiplex PCR techniques were followed to amplify the genes using specific primers. for detection of gstm1, gstt1 and albumin gene as a control. Genetic analysis showed that the percentage of deletions in breast cancer patients was 75% (gstm1 27.5%, null genotype 32.5 % and 15% for gstt1) versus 10 % were in the control samples (5 % gstt1, 3.33% gstm1 and 1.67% null genotype). Data were subjected to statistical analysis using Chi square (X2 test) to evaluate the association between etiological risk factors and having a risk for breast cancer . A significant association was considered at level ($P < 0.05$) . Blood group O and A showed an interesting association with breast cancer risk. The percentage of deletion was 90.9% and 86.6% in O and A blood group, respectively. Age and family history had no significant risk association with breast cancer ($P > 0.05$) in this study.

Key words:

GST genes, Breast Cancer, Iraq.

Introduction:

Although breast cancer cases were steadily rising in Iraq after the 1991 war, no attempt was done to study this problem at the molecular level. Breast cancer constituted 31% among other malignancies of women in Iraq.

Xenobiotic metabolism genes are considered to be environmental response factors .Variability in the metabolism of a number of endogenous and exogenous agents as a result of inherited genetic polymorphisms in the involved enzymes may affect cancer risk. However, recognized risk factors for breast cancer cannot fully explain the observed variation in breast cancer incidence over time and across geographic locations (1,2).

Environmental carcinogens, such as polycyclic aromatic hydrocarbons, could be responsible for some of the unexplained variations (3).Genetically determined differences in the activity of metabolizing enzymes involved in these reactions might contribute to host susceptibility to cancer (4). Taking these genetic factors into account may improve our ability to determine if environmental chemicals contribute to breast cancer (5).

Because of the potential carcinogenic effects of some of the GST-M1 and GST-T1 enzyme substrates and the possibility that the gstt1 gene deletion is associated with enhanced endogenous mutagenic processes, the gstm1 and gstt1 gene polymorphisms could be important in human carcinogenesis. The relationship between these polymorphisms and breast cancer risk has been evaluated in several small case-control studies, with contradictory results (6).

We present here our results of examining possible association among Iraqi Breast Cancer patients targeting polymorphisms in the Xenobiotic genes namely, Glutathione-S-Transferases (GSTs).

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Materials and Methods :

Blood samples (3-5 ml) were collected in EDTA tubes from patients attended the Nuclear Medicine Hospital in Baghdad , DNA was extracted from lymphocytes by proteinase K/ SDS digestion as described by Miller et al.(7).The polymorphism of gstm1 and gstt1 were analyzed by a multiplex PCR procedure. The following primers were used :

GSTM1:
F-(5/ - GAA CTC CCT GAA AAG CTA AAG C)
R-(5/ - GTT GGG CTC AAA TAT ACG GTG G)
GSTT1:
F-(5/ - TTC CTT ACT GGT CCT CAC ATC TC)
R-(5/ - TCA CCG GAT CAT GGC CAG CA)
Albumin:

F-(5/ - GCC CTC TGC TAA CAA GTC CTA C)
R-(5/ - GCC CTA AAA AGA AAA TCG CCA ATC)

The amplification reactions were carried out in a volume of 50 µl containing (25ng) DNA;10 mM Tris–HCl; 50 mM KCl ; 1.5 mM MgCl2;200 µM (each) dATP, dCTP,dGTP and dTTP (Promega); each primer was at 20 pM and 2.5 unit of Taq polymerase (Promega).
The amplification was carried out as:

Initial denaturation at 95 oC for 3 min, 30 cycles in thermocycler (Techne,Cambridge Ltd., England) as follow : 94 oC for1 min; 59 oC for1 min ; 72 oC for1 min and 5 min final extension for last cycle. The PCR products were analyzed on 2%Agarose gel electrophoresis to detect the absence or presences of these genes. Albumin gene used as internal control.

Results and discussion:

1. Genotyping

The internal control amplified Albumin fragment was 350 bp in length, whereas presence of the gstm 1 and gstt1 genes were identified by 215 and 480 bp fragments, respectively. Although these assays did not distinguish between heterozygote and homozygote positive genotypes, they conclusively identify the null genotypes (Fig 1).

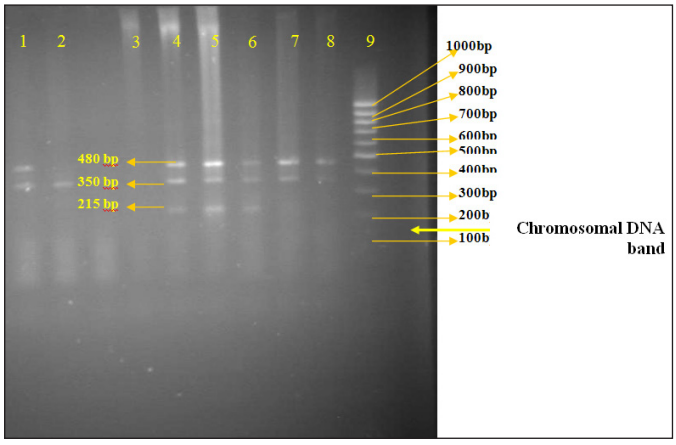

Fig .1 : Electrophoresis of PCR products on agarose gel 2% (70 volt / 1 hr) . All samples belong to patients.
480 bp = gstt1 band ,350 bp = albumin band (internal control),215 bp = gstm1 band.
Lane 1 : gstm1 deletion ,Lane2 : Null genotype,Lane3 : Negative control, Lane4 ,5,6 : Normal genotype, Lane7 , 8 : gstm1 deletion, Lane9 : DNA marker (100 -1000bp).

The results covered 40 cases of women diagnosed as breast cancer patients and 60 samples of control group(apparently healthy females) . The study has investigated possible correlation between gsts polymorphism and some etiological risk factors among breast cancer patients. Data of patients were distributed according to selected characteristics as major risk factors for breast cancer while control samples were subjected to some of these criteria except for the affected breast side. The screened factors were : age, , family history and blood groups. Other risk factors such as smoking and drinking alcohol were excluded based on the non habitual Iraqi population . In stead, the study focused on previous risk factors as well as the blood groups. Most genetic studies have ignored this last factor and almost non pointed to this kind of relationship. The descriptive parameters for both affected and healthy groups are summarized in table (1) .Thirty samples of patients were found to have deletion in one gene or both (Null genotype) .Only 10 samples were found normal . Among the 60 control group only six samples showed genetic deletion while the rest were of normal genotype.

Table 1 : Descriptive parameters of breast cancer patients according to genotype

Parameters	Samples number	Deletion	Normal
Age			
<50	25	19	6
≥ 50	15	11	4
Blood group			
A	15	13	2
B	8	3	5
AB	6	4	2
O	11	10	1

Fewer studies have examined the polymorphisms of glutathione S-transferase genes and its relation to breast cancer risk when compared with the other GST variants (8). Most literatures on the relationship of gst polymorphisms and breast cancer risk has been based on Caucasian women (9).

One of our primary reasons to study GSTs polymorphisms and their possible correlation to breast cancer is the fact that in highly populated and a war torn areas such Baghdad, the lack of an effective detoxification system might be a major risk .The GST family catalyzes reactive carcinogenic intermediates that are produced through the metabolism of environmental toxins by detoxification enzymes. The genes of the GST families are under the control of the aryl hydrocarbon receptor, which binds toxic chemicals such as dioxin, an environmental pollutant believed to be involved in breast cancer development (10).

In addition, GST is also involved in estrogen metabolism and its absence might expose estrogen-sensitive tissues to

a higher degree of stimulation (11). The influence of this polymorphism was already determined in other cancers such as lung cancer and ovarian cancer (10).Based on these facts; we have speculated for this study that gstm1 and gstt1 deletions contribute to the carcinogenesis of breast cancer. However, analysis of risk factors has indicated some kind of association.

2.Risk Factors

1. Age

From 40 patients, 25 were under 50 years of age, 19 of them had genetic deletion .The remaining six were of normal gsts genotype. Among the 15 patients who were above 50 years old, 11 had deletion; the others were of normal genotype. The 60 control samples included 40 women under 50 years of age . Six samples had genetic deletion and 34 were of normal genotype. The remaining 20 apparently healthy women above 50 years of age were all of normal gsts genotype.(Table 2).

Table 2: Association between age and gsts genotype of breast cancer patients and control group.

Age	Total	gstm1 deletion	gstt1 deletion	Null enoty pe	Normal
Patients					
<50	25	8	2	9	6
≥50	15	3	4	4	4
Total	40	11 (27.5 %)	6 (15 %)	13 (32.5 %)	10 (25%)
Control					
<50	40	2	3	1	34
≥50	20	0	0	0	20
Total	60	2 (3.33 %)	3 (5%)	1 67%).	54 (90%)

The percentage of genetic deletion in patients samples was 75% distributed as follows : null genotype (32.5 %) , gstm1 deletion (27.5 %) , gstt1 deletion (15%) while 25 % were of normal genotype.

For control samples, the percentage was: 90% normal , 5 % gstt1 deletion, 3.33 % gstm1 deletion and 1.67 % null genotype.

The statistical analysis (Chi square) has indicated that there was no significant differences between genetic deletion of these genes and age ($p > 0.05$.(The study did not find a statistically significant difference in the breast cancer group with respect to age at diagnosis. Similarly Milikan et al. (12) observed no correlation between deletion of gstm1 and age at diagnosis. Garcia-Closas et al. (13) also reported no association for gstm1 null genotype in pre - postmenopausal women. As well, Bailey et al.,(14) reported that gstm1 null genotypes were not associated with breast cancer risk among pre- or postmenopausal women in African-American or Caucasian women. In contrast, Helzsouer et al., (8) found that the gstm1 null genotype was associated with an increased risk of breast cancer development, manifested predominantly in postmenopausal women .Park et al.,(9,15) and Parl (16) observed a significant multiplicative correlation between gstm1 and gstt1 null genotypes and high-risk status of parity factor in all women and in premenopausal women ($p<0.01$), but not in postmenopausal women ($p> 0.05$). Our findings support a direct interaction between genetic deletion and breast cancer incidence (75% in the breast cancer group versus 10 % in the control group). However, the results are in disagreement with other recent findings (17, 18, 19).

2. Blood group

Although this factor was not considered as risk factor in many studies , few clinical investigations referred to some breast cancer patients of certain blood groups had recurrence after successful therapy. This study included 15 patients with blood group A , 8 of B group , 6 of AB and 11 of O blood group . Control samples included 23 of A group, 12 of B group, 18 of AB and 7 were of O blood group. The percentage of deletion within A group represented 86.6 % and within O group 90.9 % (Table 3). These percentages were higher than their counterparts within control samples .A significant differences were noted ($p< 0.05$) .Accordingly, A and O blood group may be considered as risk factors for breast cancer in Iraqi population subjected to further screening studies.

Breast cancer link with blood type signaled variable results in the literature. Holdsworth et al., (20) found sister pedigrees with breast cancer to have an increased rate of type A compared to type O ($p<0.01$). The authors proposed that, based on this, there is a small association between blood type A and breast cancer development, they suggested that in a consecutive series of patients an excess of 7-20% type A would be found. A study of rapidly progressive breast cancer in Tunisian women found a slightly increased risk of a positive diagnosis in blood type A (21). Some researchers have gone far as to say that "blood groups were shown to possess a predictive value independent of other known prognostic factors" when discussing breast cancer (20).Other researchers have actually suggested that a degree of the susceptibility to breast cancer, from a gene perspective, might be a

result of a breast cancer-susceptibility locus linked to the ABO locus located on band q34 of chromosome 9 (22).The authors conclude that blood type A women have a generalized tendency to worse outcomes and a more rapid progression with this cancer. Research indicates that blood type A women are over-represented among breast cancer patients, and that this trend occurs even among women thought to be at low risk for cancer. A. women with blood type A have been observed to have poor outcomes once they are diagnosed with breast cancer. In complete opposition to these blood type A tendencies, It was found that blood type O infers a slight degree of resistance against breast cancer, and even among patients, blood type O showed a significantly lower risk of death (21). In conclusion, it appears that type A and O patients seems to have an increased risk . It is also true that breast cancer may have an association with blood group, but different blood groups are associated with different manifestations of the disease. Other cancers show various risk or lack of risk associated with blood group.

Table 3: Association between blood group and gsts genotypes in patients with breast cancer and control group.

Blood group/patients	Total No.	*gstm*1 deletion	*gstt*1 deletion	Null genotype	Normal
A	15	6	2	5	2
B	8	1	1	1	5
AB	6	2	0	2	2
O	11	3	3	4	1
Total	40	12	6	12	10
Blood group/control	Total No.	*gstm*1 deletion	*gstt*1 deletion	Null genotype	Normal
A	23	1	2	1	19
B	12	0	0	0	12
AB	18	1	1	0	16
O	7	0	0	0	7
Total	60	2	3	1	54

3. Family history

The forty patients included two cases with family history of breast cancer . These two cases were of abnormal gst genotype (deletion) .The 38 cases that have no history included 10 normal genotype while 28 had genetic deletions .Among the control group, the number of samples that had family history were three ,all of normal genotype. The remaining 57 included six cases with deletion . The Statistical analysis showed that there was no significant differences ($p > 0.05$), and in other words there was no association between family history and breast cancer (Table 4).

Kelsey et al., (25) reported no modification of gstm1 based on family history. The negative associations for the gstm1 polymorphism among women with a family history could be attributable to unknown genetic or environmental factors that interact with GST genes to increase the risk of breast cancer. However, Milikan et al.,(12) reported a slightly elevated risk among women with family history of breast cancer.

Table 4: Association between family history and genotype of gsts in breast cancer patients and control.

samples	family history	Total No	Normal genotype	Genetic deletion
patients	Yes	2	0	2
	None	38	10	28
control	Yes	3	3	0
	None	57	51	6

Since the type of breast cancer in this study is unknown, it may be due to environmental risk factors causing mutations or multiple alterations(26) .Women may have developed the cancer as results from mutations in the multiple cancer-associated genes, the first of these mutations could be either inherited from one parents

(familial cancer) or they could occur as a sporadic cancers (25). Most breast cancer cases (about two thirds) are known as sporadic, meaning that rare mutations have occurred. In these cases the initial rare cancer gene mutations occurred after conception; these cases have no connection to family history. Inherited breast cancer risk seen in families with only a few cases of breast cancer results from a second type of mutated genes, low penetrance genes. Low penetrance genes are much more common than high penetrance genes (26). Studies on low penetrance genes have focused on their variants or polymorphism shown to have varying levels of biological activity. These levels might link them with differing breast cancer risk (24) . A number of classes of genes with polymorphisms have been evaluated, including genes whose products play a role in reproductive hormone action, repair gene mutations, detoxify cancer-causing chemicals, or induce or prevent cancer themselves(27) .

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التحري عن التباين الوراثي لجين GSI وخطر الاصابة بسرطان الثدي عند المرضى العراقيين

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ألخلاصة:

هدفت هذه الدراسة إلى تقييم العلاقة بين التباين الوراثي لجينات (glutathione S- transferase genes (gstt1 & gstm1 وبعض العوامل المسببة للاصابه بسرطان الثدي بين المرضى في العراق .

شملت الدراسة 40 عينه لمريضات مصابات بسرطان الثدي من مراجعي مستشفى الطب النووي في بغداد(جميعهم نساء). وكذلك 60 عينه من النساء الأصحاء ظاهريا كعينات سيطرة.

تم تضمين الدراسة العوامل التالية : العمر(تحت عمر الخمسين،وفوق عمر الخمسين)، صنف الدم، التأريخ العائلي . تم استخلاص الحامض النووي منقوص الأوكسجين DNA من الدم باستخدام طريقة الـ proteinase K/ SDS لإجراء عملية التضاعف التسلسلي Multiplex PCR للحامض النووي.أجريت هذه ألعمليه للكشف عن وجود أو غياب الجينات واستخدم جين الألبومين كدليل سيطرة موجب . كان حجم حزمة الدليل (350 bp) بينما تم تحديد وجود gstm 1 وgstt1 عن طريق الحزم (215 bp و480 bp) على التوالي .على الرغم من إن هذا الاختبار لا يميز بين الكميتات المتماثلة والمتشابهة إلا انه يميز بينهما عن طريق الحذف الكلي للجين .

أظهر الاختبار بأن نسبة الحذوفات بين مريضات سرطان الثدي كانت (75%)توزعت كالآتي (Null genotype 27.5 % ، 32.5 % gstm1 و 15% gstt1) . بالمقابل أظهرت عينات السيطرة (10%) توزعت كالآتي : (gstm 3.33 1 ، 5 % gstt1 و 1.67% null genotype).

أخضعت جميع البيانات للاختبار الإحصائي باستخدام مربع كاي (X2) لتقييم العلاقة بين العوامل المسببة للاصابه ومدى قابلية الاصابه بسرطان الثدي . اعتبرت العلاقة مهمة إحصائيا عند مستوى ($P< 0.05$)

توصلت هذه الدراسة إلى مايأتي :

إن التباين الوراثي لجينات gstm1 و gstt1 كان عالي نسبيا (75%) بين مرضى سرطان الثدي في العراق وأعطى دلاله على قوة العلا قه.

أظهرت مجاميع الدم A و O علاقة مهمة مع الاصابه بسرطان الثدي وكانت نسبة الحذوفات (86.6%) بالنسبة للمجموعةA و (90.9%) للمجموعة O .

لم نلاحظ وجود علاقة مهمة إحصائيا ($P > 0.05$) بين العمر ، ألتأريخ العائلي و قابلية الاصابه بسرطان الثدي .