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Genetic polymorphisms of *GSTM1* and *GSTT1* Genes and Endometrial Cancer in Basrah, south of Iraq

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Abstract

The genes glutathione S- transferase *MI* (*GSTM1*) and *GSTT1* involved in phase II metabolism catalyse glutathione – mediated reduction of exogenous and endogenous electrophiles. A case control study was designed to identify the association between polymorphisms at *GSTM1* and *GSTT1* genes and endometrial cancer risk. While there was a lack of association between *GSTM1* null genotype with the risk of endometrial cancer, the null genotype of *GSTT1* had a 5.7 fold increased risk toward endometrial cancer (OR=5.76; 95% CI = 2.07-15.97). Both *GSTM1*, *GSTT1* null genotype increased risk to about 3 fold. When stratified according to different grades of endometrial cancer the *GSTM1* was more representative in grade III (OR=2.6), the association becomes stronger when the *GSTT1* gene was also null (OR= 4.6).

Key words: endometrial cancer; genetic polymorphism; glutathione S-transferase.

1- Introduction

Cancer accounts for more than 20% of all the deaths in the world every year and is one of the most important medico-biological problems of this world. The central event in cancer development is the loose of genomic integrity which itself probably initiates from the assortment of genomic DNA by exogenous or endogenous carcinogens (1). The Phase II Glutathione S-transferases(GSTs) *GSTM1*,*GSTT1* and *GSTP1* catalyse glutathione-mediated reduction of exogenous and endogenous electrophiles (2). *GSTs* a multigene family of phase II metabolic enzymes, are

active in the detoxification of a wide variety of potentially toxic and carcinogenic electrophiles by conjugating them to glutathione (3) . These genes are thought to engage in the intracellular transport of endogenous metabolites and steroid hormones (4 , 5) Glutathione S-transferase *MI*(*GSTM1*) a member of the GSTs super family is polymorphic in humans (6), and approximately 45-50% of Caucasian and Japanese populations have the null genotype and are devoid of *GSTM1* enzymatic activity (7) .

GSTT1 , the other member of GSTs family ,which metabolizes various potential carcinogens such as monohalomethanes, and others, which are present in tobacco smoke . The null genotype frequency of this gene has been assumed in some ethic groups. The frequency is highest among Asian population (46.52%).Among European, the frequency ranges from 11-22% (8).

2- Material and Methods

2.1. Study population :

The study population comprised 50 patients women with endometrial cancer, aged between 15-72 year were contacted after surgery in the Basrah Hospital for deliveries and children , and 50 healthy volunteers who served as controls for genetic characterization .

Blood samples were collected from all patients and controls. Genomic DNA was isolated from samples by standard manual method (10) .

2.2. Genetic analysis:

Genotyping of the *GSTM1* and *GSTT1* genes was carried out by a multiplex PCR reaction in (Thermocycler , Thermo USA). The genotypes were analyzed according to the protocol of (11). Genotypes were amplified by using 6 set of primers(11) :

GSTM1(F):5- GAA CTC CCT GAA AAG CTA AAG C-3 ; **GSTM1(R):**5- GTT GGG CTC

AAA TAT ACG GTG G-3 ; **GSTT1(F):** 5- TTC CTT ACT GGT CCT CAG ATC TC-3 ;

GSTT1(R):5-TCA CCG GAT CAT GGC CAG CA-3 ; **Albumin(F):**5- GCC CTC TGC TAA

3. Results

Table 1 presents ORs and 95% CI for endometrial cancer patients in relation to the *GSTM1* and *GSTT1* genotypes, indicating that endometrial cancer is more likely to occur with *GSTM1* null genotype OR=1.34; 95% CI=0.55-3.59. In contrast , *GSTT1* null

Many reports have indicated an association between *GSTs* polymorphisms and endometrial cancer (4,2,9).

The present study reports the result of *GSTM1* and *GSTT1* polymorphisms in endometrial cancer patients in Basrah, southern Iraq comparing with healthy controls .

GTC CTA CTA-3 ;and **Albumin(R):**5-GCC CTA AAA AGA AAA TCG CCA ATC-3.

The reaction mixture (25µl) contained 200µm dNTPs , 1.5mm Mgcl₂, 1µm primers 1µg DNA and 2 unites of thermostable Taq DNA polymerase. A total of 30 PCR cycles with denaturation at 94° C for 1 minute, annealing at 58°c for 1 minute and extension at 72°c for 1 minute were conducted. An initial DNA denaturation at 95°c and final extension at 72°c were carried out for 5 minutes each.

The PCR product was then subjected to electrophoresis on a 2% agarose gel. The presence of bands of 480 and 215 bp was indicated of the *GSTT1* and *GSTM1* genotypes respectively , whereas the absence indicated the null genotype for that gene . Albumin indicated by a 350 bp product was used as an internal control.

2.3. Statistical analysis :

The odds ratio(OR) and 95% confidence intervals(CI) were calculated as a measure of the association between genotypes and endometrial cancer and *P* = 0.005 values were considered significant(SPSS Software version 11).

genotype had a 5.7 fold increased risk towards endometrial cancer(OR=5.7;95% CI= 2.07-15.97) , while the *GSTM1,GSTT1* null genotype had increased the risk of the cancer to about 3 fold (OR=2.7;95% CI=0.34-21.159)

Tables 2 and 3 show the combined effects of *GSTM1* and *GSTT1* genotypes among different grades of endometrial cancer patients . The *GSTM1* null genotype was

more representative in grade III tumors (OR=2.69;95% CI=0.45-15.87) , and the association becomes stronger when the *GSTT1* gene was also null (OR=4.66)

Table 1: Distribution of polymorphisms of *GSTM1* and *GSTT1* among Case and controls

Genotype	Controls	cases	OR	95% CI
<i>GSTM1</i> (+)	40 (80%)	37 (74%)	1.00	-
<i>GSTM1</i> (-)	10 (20%)	13 (26%)	1.340	0.55-3.59*
<i>GSTT1</i> (+)	44 (88%)	28 (56%)	1.00	-
<i>GSTT1</i> (-)	6 (12%)	22 (44%)	5.761	2.07-15.97**

* P= 0.003

** P= 0.002

Table 2: Grade , + and - genotypes of *GSTM1* gene among endometrial cancer Cases

Grade	Total	<i>GSTM1</i> (+)	<i>GSTM1</i> (-)	OR	95% CI
I	12	10 (83.3%)	2 (16.6 %)	1.00	_____
II	18	14 (77.7%)	4 (22.2%)	1.24	0.36-4.26
III	20	13 (65%)	7 (35%)	2.69	0.45-15.87*

*P = 0.004

Table 3: Grade , + and - genotypes of *GSTT1* gene among endometrial cancer Cases

Grade	Total	<i>GSTT1</i> (+)	<i>GSTT1</i> (-)	OR	95% CI
I	12	8 (66.6%)	4 (33.3 %)	1.00	_____
II	18	11 (61.1%)	7 (38.8%)	1.27	0.27-5.87
III	20	6 (30%)	14 (70%)	4.66	1.00-21.65

4. Discussion

The ability to characterize polymorphic genes involved in metabolism of carcinogens has given a new approach for human cancer risk assessment (12, 13).

Individuals are exposed to a whole host of environmental carcinogens throughout their lives, it is clear that some individuals with genetically compromised detoxification pathways are at increased risk for a variety of cancers (14).

Although endometrial carcinoma is a common female malignancy, but little attention has been given to genetic factors. To our knowledge this is the first report of an association of *GSTM1* and *GSTT1* genes with endometrial cancer in Iraq.

In the present study ,the prevalence of genetic polymorphisms in the *GSTM1* and *GSTT1* genes with respect to their

association with the risk of endometrial cancer in Basrah (south of Iraq) has been investigated. The subjects with the null genotype for *GSTM1* had a slightly significant relationship to endometrial cancer with an OR of 1.34 (95% CI=0.55-3.59) , but the risk increased to around 6 fold with the *GSTT1* null genotype (OR of 5.76 ; 95% CI= 2.07-15.97).

The association was statically significant between *GSTM1* and *GSTT1* null genotype and grade of endometrial cancer with an OR of 2.6 and 4.6 (95% CI= 0.45-15.87; and 1.00-21.56) respectively. While the *GSTM1,GSTT1* null genotype together had increased the risk of this cancer to about 3 fold with an OR of 2.7.

The *GSTT1* and *GSTM1* null genotype were more common in endometrial cancer,

indicating that the deletion of these genes might be involved in the etiology of the cancer. These results correspond with the previous studies in the same context that tumors of different histology may have different etiologies (15, 16), and specifically that endometrioid is etiologically related (17,18). The result of this study is also in accordance with the finding of (19) that the null genotype of *GSTM1* and *GSTT1* genes are more common in endometriosis patients than in controls.

The increased frequency of the *GSTM1* null genotype was observed in a sample of 80 endometrial cancer patients compared with 60 patients control [OR=2.0] (4) While only the *GSTT1* null genotype was associated

with an increased risk of endometrial cancer [OR=1.55] in the study of Doherty *et al.*, 2005(9). GSTs are probably involved in the deactivation of estrogen-derived quinines (20) but it is not clear which of GSTs are involved (9). A recent report showed that *GSTP1* has this capability, because the GSTs have overlapping substrate specificity it is likely that other GSTs share this property (21).

In Conclusion, the *GSTM1* and *GSTT1* null genotype appear to be associated with increase the risk of endometrial cancer. Since the number of cases was small, thus needs to be verified by increasing the number in further studies.

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التعدد الوراثي للجينين *GSTM1* و *GSTT1* وعلاقتهام باخطورة الاصابة بسرطان بطانة الرحم في البصرة جنوب العراق

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الخلاصة

الجينين *GSTM1* و *GSTT1* من ضمن جينات الطور الثاني التي تعمل على ابيض المواد المسرطنة الداخلية والخارجية.صممت هذه الدراسة للتعرف على العلاقة بين التعدد الوراثي في الجينين *GSTM1* و *GSTT1* وزيادة الخطورة بالاصابة بسرطان بطانة الرحم في النساء . وجدت الدراسة انه لا توجد علاقة احصائية واضحة بين فقدان الجين *GSTM1* وخطورة الاصابة بسرطان بطانة الرحم بينما ازدادت هذه الخطورة بمقدار اكثر من خمس مرات عند فقدان الجين *GSTT1* . وعند توزيع عينات المرضى استنادا الى درجة المرض فقد وجد ان فقدان الجين *GSTM1* قد تمثل بشكل كبير في الدرجة الثالثة *Grade III* من المرض ($OR= 2.7$) . هذا الارتباط كان اكبر عندما كان الجين *GSTT1* مفقودا ايضا ($OR= 4.6$).