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Full Length Research Paper

Genetic polymorphisms of *GSTM1* and *GSTT1* genes and endometrial cancer in Basrah, South of Iraq

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The genes glutathione *S*- transferase M1 (*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 association between polymorphisms at *GSTM1* and *GSTT1* genes and endometrial cancer risk. Genotype frequencies in 50 patient's women with endometrial cancer were compared with 50 healthy volunteers who served as controls. While there was 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). The 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 become stronger when the *GSTT1* gene was also null (OR= 4.6).

Key words: Endometrial cancer, genetic polymorphism, glutathione *S*-transferase.

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 (Hanahan and Weinberg, 2000). 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 (Hanahan and Weinberg, 2000).

The phase II glutathione S-transferase (*GSTs*) *GSTM1, GSTT1* and *GSTP1* catalyse glutathione-mediated reduction of exogenous and endogenous electrophiles (Spurdle et al., 2001). *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 (Pemble et al., 1994; Hanahan and Weinberg, 2011). These genes are thought to engage in the intracellular transport of endogenous metabolites and steroid hormones (Esteller et al., 1997; Mangard et al., 1998). *GSTM1* a member of the *GSTs* super family is polymorphic in humans (Sobti et al., 2005), and approximately 45 to 50% of Caucasian and Japanese populations have the null genotype and are devoid of *GSTM1* enzymatic activity (Kelsey et al., 1997).

GSTT1, the other member of *GSTs* family, which metabolizes various potential carcinogens such as monohalomethanes, and other carcinogenetic molecules, which are presents in tobacco smoke. The null genotype frequency of this gene has been assumed in some ethnic groups. The frequency is highest among Asian population (16 - 52%) (Oke et al., 1998; Saadat et al., 2001). Among European, the frequency ranges from 11 to 22% (Steinhoff et al., 2000). Many reports have indicated an association between GSTs polymorphisms and endometrial cancer (Esteller et al., 1997; Spurdle et al., 2001 and Doherty et al., 2005). The present study reports the result of *GSTM1* and *GSTT1* polymorphisms in endometrial cancer patients in Basrah, southern Iraq comparing with healthy controls.

MATERIALS AND METHODS

Study population

The study population comprised 50 patients women with endometrial cancer, aged between 15 to72 years were contacted after surgery in the Basrah Hospital for deliveries and children, and 50 healthy volunteers who served as controls for genetic

Table 1. Genomic DNA was amplified by using 6 set of primers.

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 GTC CTA CTA-3
Albumin(R)	5-GCC CTA AAA AGA AAA TCG CCA ATC-3

characterization. Blood samples were collected from all patients and controls. Genomic DNA was isolated from samples by standard manual method (Sambrook et al., 1989).

Genetic analysis

Genotyping of the *GSTM1* and *GSTT1* genes was carried out by a multiplex polymerase chain reaction (PCR) reaction in (Thermocycler, Thermo USA). The genotypes were analyzed according to the protocol of (Arand et al., 1996). Genomic DNA was amplified by using 6 set of primers given in Table 1.

The reaction mixture 25 µl containing 25 ng DNA;10 mM Tris-HCl; 50 mM KCl; 1.5 mM $MgCl_2$; 200 mM dNTPs; 20 pM of each primer and 2.5 unit of Taq DNA polymerase. A total of 30 PCR cycles with denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min were conducted. An initial DNA denaturation at 95°C and final extension at 72°C were carried out for 5 min 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 use as an internal control.

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.

RESULTS

Table 2 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 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 3 and 4 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 become stronger when the *GSTT1* gene was also null (OR=4.66).

DISCUSSION

The ability to characterize polymorphic genes involved in metabolism of carcinogens has given a new approach for human cancer risk assessment (Shields et al., 1991; Anwar et al., 1996). 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 (Lallas et al., 2005). 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 this 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) have 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* and *GSTT1* null genotype 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 deletion of these genes might be involved in the etiology of the cancer. These results correspond with previous studies in the same context that tumors of different histology may have different etiologies (Kavale et al., 1988; and Obata et al., 1998), and specifically that endometrioid is etiologically related (Sainz et al., 1996; Paulson 1997). The result of this study also in accordance with the finding of Baranova et al. (1997) 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] (Esteller et al., 1997).The *GSTM1* null allele is an attractive candidate as an endometrioid ovarian cancer susceptibility allele since endometriosis is characterized by cyclical degeneration and chronic inflammation, conditions which will result in the production of reactive oxygen species. Consequently, impairment of GSTM1 function with an endometriotic lesion is likely to result in increased susceptibility to DNA damage and a propensity to malignant transformation (Baxter et al., 2001). 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). *GSTs* are probably involved in the deactivation of estrogen-derived quinines

Table 2. Distribution of polymorphisms of *GSTM1* and *GSTT1* among case and controls.

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

*P= 0.003, **P= 0.002.

Table 3. Grade, + and - genotypes of *GSTM1* gene among endometrial cases.

Grade	Total	*GSTM1* (+)	*GSTM1*(-)	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 4. Grade, + and - genotypes of *GSTT1* gene among endometrial cancer cases.

Grade	Total	*GSTT1* (+)	*GSTT1* (-)	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

(Butterworth et al., 1997) but it is not clear which of *GSTs* are involved (Doherty et al., 2005). A recent report showed that *GSTP1* has this capability, because the *GSTs* have overlapping substrate specifity it is likely that other *GSTs* share this property (Hachey et al., 2003).

In conclusion, the *GSTM1* and *GSTT1* null genotype appear to be associated with and may specifically increase the risk of endometrial cancer. The lack of function in one of these enzymes may render endometrial cancer more susceptible to DNA damage. Since the number of cases was small, thus needs to be verified by increasing the number in further studies.

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