

Assessment of the Two-Glutathione S-Transferase Genotypes *GSTM1* and *GSTT1* Polymorphism Frequencies in Epithelial Ovarian Cancer in Nasiriya Province

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Abstract

The family of glutathione S-transferases (GSTs) is composed of multiple isozymes with significant evidence of functional polymorphic variation. Glutathione S-transferase *GSTM1* and *GSTT1* are members of the GST family, is responsible for metabolism of xenobiotics and carcinogens. Allelic variant of GST gene polymorphisms might impair detoxification function and increase the susceptibility to cancer. The null genetic polymorphism in the gene encoding GST Class μ , *GSTM1* and *GSTT1*, has been reported to be significantly elevated in cancer patient. This study aimed to investigate if these polymorphisms are useful markers for predicting ovarian cancer susceptibility. 30 cases of epithelial ovarian cancer and 30 controls was used to investigate the frequency of *GSTT1* and *GSTM1* genotypes by using multiplex conventional PCR protocol. The frequency of *GSTM1* null allele was significantly elevated (100%, $P = 0.004$) compared with *GSTT1* active allele (13% $P = 0.037$). *GSTT1* null showed no significant effect in prognosis epithelial ovarian cancer (17%, $P = 0.551$). We conclude that the GSTs might affect in both risk to ovarian cancer.

Keywords: *GSTM1*, *GSTT1* Polymorphism, Epithelial Ovarian Cancer, Nasiriya Province.

Introduction

Ovarian cancer is the main cause of death with gynecological malignancies^[1]. It is a disease of premenopausal and postmenopausal women; however, no age exempt from ovarian neoplasm. Here is no socio- economic bar for ovarian malignancies but epidemiologically it is more frequent in higher socio-economic and industrially developed countries^[2]. Risk factor of ovarian carcinoma include inflammation, excessive number of lifetime ovulation, increase in steroid. Hormone levels, infertility, age, asbestos, talk and reproductive factor such as nulli-parity^[3]. Oxidative stress is due to disturbance in the balance between the production of reactive oxygen species (ROS) and deficiency of antioxidant defense. In other words, oxidative stress results if excessive production of ROS overwhelms the antioxidant system or if there is a significant decrease or lack of antioxidant defense^[4]. Numerous genetic polymorphism that may be involved in deferential enzyme function or expression, cancer risk^[5]. Our study postulated that lack of detoxification,

which is determined genetically, might be risk factor of ovarian cancer. Glutathione S-transferase (GSTs) are a family of enzymes responsible for the metabolism of xenobiotics and carcinogens. *GSTM1*, one member of GST family was formerly termed *GST1* or *GST* class "mu"^[6]. *GSTM1* is critical in the detoxification of the oxidative stress product during ovulation^[7]. Because of detoxification properties of GST enzymes, it is logical to suspect the role of *GSTM1*-related gene in ovarian cancer patients^[8]. *Glutathione S-transferase theta 1 (GSTT1)* is located in long arm of chromosome 22 (22q11.23). Some studies reported that *GSTT1* protein was involved in the conjugation of ethylene oxide and halogenated metabolites^[10]. Glutathione S-transferase catalyze glutathione-mediated reduction of exogenous and endogenous electrophile with broad and overlapping substrate specificity^[11]. It has been hypnotized *GST* functional variant associated with less effect detoxification of potential carcinogenesis may confer an increased susceptibility to cancer. Especially in the presence of environmental stress. Such as smoking

and exposure UV radiation^[12]. Detection variant of null allele exist for *GSTT1* and *GSTMI* genes and these present biochemically as failure express protein^[13,14].

Reported studies about the effect of GST polymorphism on risk of ovarian cancer are still few, due to many limitations. *GSTT1* and *GSTMI* null genotypes was shown to be associated with proper survival and progression free interval in ovarian cancer cases ^[15]. This study has been done because of the lack of genetic studies related to these cases in south of Iraq.

Materials and Method

Subjects: This case-control study was conducted between February 2016 and December 2017. Overall, thirty cases who recruited at Al-Habbobi teaching hospital were histopathological confirmed as epithelial ovarian cancer after surgery. Thirty healthy volunteers were chosen as control. Age ranged from 22 to 39 years in epithelial ovarian group and from 21 to 49 years in control group. Peripheral blood (5 mL) was drawn and collected into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The collected blood samples were frozen until DNA extraction and then PCR analysis was proceeded. A verbal consent had been taken from the patient and the control volunteer before engagement with the questionnaire.

Genotyping: Genomic DNA was isolated from whole blood as described previously^[16]. Eight microliter of DNA of each sample was subjected to qualitative assay by 0.8% agarose gel electrophoresis (70V/150Ma for 20 min). The homozygous of *GSTT1* and *GSTMI* genes were detected by multiplex PCR reaction, using primer set sequences in the table 1. To check successful amplification, albumin gene was used as internal control.

The absence of amplified *GSTT1* or *GSTMI* product (in the existence of the albumin PCR product) indicate the null genotyping for each respectively. Multiplex PCR reaction was proceed in a reaction volume 20 μ l which contain 5 μ l of master mix (Kappa, Korea), 1.25 μ l of each primer (10 pmol), 3.5 μ l of genomic DNA. Amplification condition for this multiplex reaction was as follows : (94°C for 3 min, 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1 min, and final cycle of extension at 72°C for 5 min). 10 μ l of each PCR product was loaded into 1.8% agarose gel containing ethidium bromide for electrophoresis (70V, 150 mA for 30 min) and visualized at room temperature under UV light.

Epidemiological Analysis: Relative association between studied genotypes and epithelial ovarian cancer risk were evaluated to calculate odd ratio (OR) and 95% confidence intervals (CI).

Results

Genomic DNA, which was extracted from blood leukocytes, was subjected to agarose gel electrophoresis assay as previously described, was illustrated in figure 1. Thirty samples of ovarian cancer and thirty samples of healthy volunteers (as control) genotyped by multiplex PCR assay for detection of *GSTT1* and *GSTMI* genes. Figure 2 represent multiplex PCR products which was analyzed by 1.8% agarose gel electrophoresis. The *GSTT1* active genotype frequency was 43.3% which was significantly different from control ($P=0.037$, OR 2.75, 95%), whereas the *GSTMI* null genotype frequency was 100% for all ovarian cancer cases ($P= 0.004$, OR 0.02, 95%). *GSTT1* null genotype showed no significant effect in risk of epithelial cancer ($P = 0.551$, OR 1.3, 95), as illustrated in table 1.

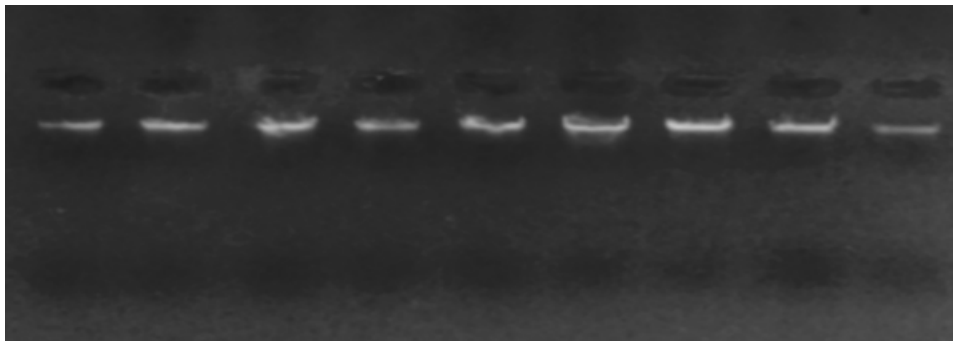


Figure 1. Extracted genomic DNA analyzed by 0.8% agarose gel electrophoresis

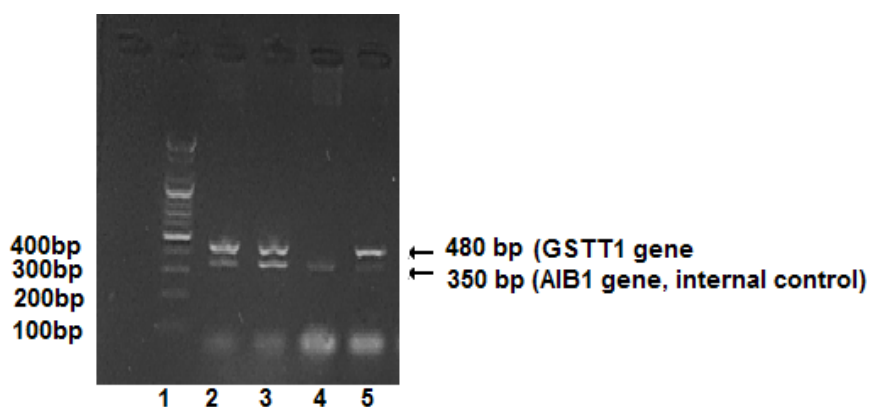


Figure 2. Detection of *GSTT1* and *GSTM1* genotypes by multiplex conventional PCR. Arrows indicate the position of 480 bp *GSTT1* product and 350bp *AIB1* product. lane 1 is a 100 bp marker. Lane 2. 3. 5 refer to *GSTT1* product, lane 4 was classified as *GSTT1* null. All lane exhibit *GSTM1* null at 215bp.

Table 1: Null frequency in ovarian cancer

	Total		<i>P</i> value ^a	OR (95% CI) ^b
Control	30			
<i>GSTT1</i> active	30	13 (43.3%)	0.037	2.75 (1.06-7.17)
<i>GSTT1</i> null	30	17 (56.6%)	0.551	1.3 (0.541-3.15)
<i>GSTM1</i> null	30	30 (100%)	0.004	0.02 (0.001-0.28)

a. Fisher exact test, b. Odd ratio with 95% confidence interval parentheses

Other risk factor had been assessed to be considered as non-significant risk factor

Discussion

This is the first study to investigate the association between *GSTT1* and *GSTM1* polymorphisms and the epithelial ovarian cancer in Nasiriya city. This study reported that *GTTI* active and *GSTM1* null have significant effects in risk of epithelial ovarian cancer. Except the genotype *GSTT1* null showed no significant effect of any examined cases. In the past several years, many studies have tried to illustrate the effects of genetic factors in susceptibility and development of ovarian cancer. However, the reported studies about frequencies mutation of previously mentioned genes were mostly not exhaustive^[17,18]. Recently, many studies reported that the polymorphism of GSTs could effect in susceptibility and developing ovarian cancer due to their important role in modification of the biological effects of carcinogens^[19,20].

Deletion of *GSTM1* and *GSTT1* genes results in “null” genotype characterized by a general deficit in enzymatic activity¹⁶. Individuals homozygous of

these deletions are thought to be at increased risk for malignancies as consequence of a decreased capacity to detoxify possible carcinogens^[21]. *GSTM1* gene deletion is a good marker for the early detection of many diseases, including, ovarian cancer, endometriosis^[7], cystic fibrosis^[22], bladder^[23], lung^[24] and stomach^[25] cancers. Null phenotype of *GSTT1* exhibits decrease in catalytic activity and has been associated with increased risk of cancer of the head, neck and oral cavity^[26]. Patients with *GSTM1* null or *GSTT1* null showed a better survival rate after chemotherapeutic treatment for the invasive ovarian cancer compared to other patient^[27].

Otherwise, this study found that the risk of ovarian cancer was increased in carrier of *GSTT1*-active genotype. This result seemed to be conflict, taking into consideration that several environmental carcinogens found in combustion products and tobacco smoke are among *GSTT1* substrates^[20]. However, there is proof that *GSTT1* might also be involved in bioactivation, rather than detoxification of several bifunctional alkylating

agents, present in environmental pollution and certain occupational hazards^[28]. As a conclusion, bioactive process can yield potent electrophiles that modify DNA and are potentially genotoxic^[29,30]. Thus, our result in conformity with the study of Sgambato et al. they showed that *GSTT1* null is associated with decreased risk of cancer^[31].

In summary, these results suggest that *GSTM1* null and *GSTT1* active alleles increase risk to ovarian cancer in Nasiriya population, but further large sample studies need to confirm the genetic role of GSTs in epithelial ovarian cancer.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Obstetrics and gynecology department and all experiments were carried out in accordance with approved guidelines.

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