GENOTYPING OF GLUTATHIONE-S-TRANSFERASE T1 AND M1 IN IRAQI BEHÇET'S DISEASE

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ABSTRACT

The Behçet's disease (BD) one of the multisystem diseases which usually presents with oral aphthous and genital ulcers, the elevation of free radicals production and decreased efficiency of antioxidant defenses have major role in this disease. The present study was carrying out to assess the possible association between BD disease and glutathione S-transferase Mu1 (GSTM1), glutathione S-transferase T1 (GSTT1) gene polymorphisms using multiplex PCR. The results of present study show four polymorphism of genes, GSTM normal, GSTT normal, GSTT with GSTM normal and gene deletion. GSTM gene was normal in 48% and 10% for patients and control. While 90% of patients were deleted in significant differences (p<0.0037). The GSTT show normal state in 88% in control meanwhile 20% of patients were normal, deletion of GSTT was higher in patients (80%) than control (12%) in significant differences (<0.0001). The normal state of both genes was observed in 48% for control and 6.6% patients in highly significant differences (<0.0001), while null appearances were shown in the 12% and 76.6% for patients and control in significant differences (0.0001). From these results there was strongly associated of antioxidants enzyme gene GSTT, GSTM with BD in Iraqi population.

Keywords: multiplex PCR, Behçet's disease, GSTT, GSTM


INTRODUCTION

The Behçet's disease (BD) considered as one of the chronic multisystem inflammatory disease described in the first by Behçetin 1937 (1). There are some clinical features can be used to diagnosis this disease including ocular and skin lesions, oral aphthous ulcers, and genital ulcerations. Other features also observed Systemic associations in BD, consist of neurologic, articular, gastrointestinal, cardiopulmonary, and vascular involvement (2). Main histopathological finding is vacuities in arteries and veins of various sizes (3).
Although the etiology of BD is ambiguous, but several mechanisms are participated. This disease as well as the other immune disorders, the genetically predisposed of an abnormal T cell and activation of neutrophil which response to environmental/endogenous factors. These factors can be causes increment of oxidative components which resulted from neutrophil excessive production of reactive oxygen species (ROSs) which causes oxidative tissue damage and tissue lesions genesis in BD.

Oxidative stress become one of the most interested problems which contributed in several disease, it's an imbalance between production and scavenger of ROSs led to accumulation free radicals in cells and tissues, which latter caused defect in some biological functions of the cells although of this harmful effect it play important role in cell signaling and biological process regulation as well as apoptosis, hormone action, growth factor cytokine, ion transport and transcription in addition of its involved in immune responses, investigation found that some factors induced overproduction free radicals like UV, X-ray and bad life style and nutrition.

The Antioxidant defense system consist of endogen and exogen factors including antioxidant enzymes, vitamins and minerals. The glutathione S-transferase Mu1 (GSTM1), glutathione S-transferase theta T1 (GSTT1) are two classes of multi-functional GST enzymes which activated in the oxidative stress states regarding to some syndrome. GSTM1 and GSTT1 variants were had role in the detoxification of some components produced by reactive oxidant tissue damage. Different isomers of GST in Human were recorded including GSTM1 is located at 1p13.3, GSTT1 is located at 22q11.2 which detection in present study, and GSTP1. Different studies have reported closely association of these enzymes with several kinds of oxidative stress-related diseases, including BD, hypertension, diabetes mellitus and lung cancer.

**MATERIALS AND METHODS**

**Sample collection**

The samples were collected from Merjan medical city from patients attendance to dermatology center, who diagnosed by specialist physician (Dr. Ali Akaza) by clinical and biomarker features, permission were taken from each patients that contributed in present study (39 patients enrolled in present study had age 36.15±14.23 consist of 73.5% male and 26.5% female, control group had age 30.20±11.36 consist of 52.9% male and 47.1% female. Five ml of vinous blood was collected from each study subjects to biomarker of disease diagnosis and DNA extraction.

**DNA extraction**

DNA was extracted from whole blood using (Genaid extraction kit) then concentration and purity were detection.

**PCR amplification and genes polymorphisms**

A multiplex PCR was used in present study by the following sequence GSTM1: f 5’-GAACCTCCCTGAAAAGCTAAAGC-3’, R 5’-GTTGGGCTCAATATAAGGTGG -3’. GSTT1: F 5’-TTCCCTACTGGTCTCACATCTC-3’, R 5’-TCCCAGGTCACCGGATCAT-3’(27). The PCR experiments implemented by Multiplex PCR using perdenaturation 5 min at 94°C, 35 cycles (60 sec at 94°C, 60 sec at 58°C, 72°C for 30 sec, finally 10 min at 72°C) the electrophoresis pattern of PCR products in agarose gel (1.5% agarose, 70 V, 20mA for 45 mints) was used.

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for gene polymorphisms detection, the size product was GSTM1 215 bp and GSTT1312bp. The results were statically analyzed using odd ratio at CI 95% and (p value <0.05).

RESULTS

The results of present work show four polymorphism of genes which enrolled in present study GSTM normal, GSTT normal, GSTT with GSTM normal, and gene deletion. GSTM gene was normal in 48% and 10% for patients and control, While 90% of patients have deletion gene in significant differences (odd ratio 8.3077, CI 1.9926 - 34.6372, p<0.0037). the GSTT shows normal state in 88% of control meanwhile 20% of patients were normal, deletion of GSTT was higher in patients (80%) than control (12%) in significant differences (odd ratio 25.6667, CI 5.6733-116.118, P<0.0001). the normal state of both genes was observed in 48% for control and 6.6% patients in highly significant differences, while null appearances were shown in the 12% and 76.6% for patients and control in significant differences (odd ratio 46.00, CI 6.74 -313.93, P<0.0001) (table 1, figure 1).

Table 1: Distribution of GSTT and GSTM genotype in study subjects groups

<table>
<thead>
<tr>
<th>GENE</th>
<th>CONTROL 25</th>
<th>BD 30</th>
<th>Odd ratio</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM normal</td>
<td>12 (48%)</td>
<td>3 (10%)</td>
<td>8.3077</td>
<td>0.0037*</td>
</tr>
<tr>
<td>Deletion</td>
<td>13(52%)</td>
<td>27(90%)</td>
<td>1.9926 - 34.6372</td>
<td></td>
</tr>
<tr>
<td>GSTT normal</td>
<td>22 (88%)</td>
<td>6(20%)</td>
<td>25.6667</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Deletion</td>
<td>3(12%)</td>
<td>24 (80%)</td>
<td>5.6733-116.118</td>
<td></td>
</tr>
<tr>
<td>GSTM+GSTT</td>
<td>12 (48%)</td>
<td>2 (6.6%)</td>
<td>46.00</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Null</td>
<td>3 (12%)</td>
<td>23 (76.6%)</td>
<td>6.74 -313.93</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Electrophoresis pattern of GSTT and GSTM polymorphism in BD patients group

DISCUSSION

The present investigation was suggested to determination the role of antioxidant enzyme gene polymorphism in Iraqi BD patients, the oxidative stress became the most interesting problems which evolved in numerous disease in last years. The current results found that the deletion in GSTT and GSTM gene were more frequent in patients than control group in significant increments. As a result to transition in lifestyle and dietary habits, Iraqi population affected with many factors especially in induced and development disease in addition to genetic factors like BD.

The overproduction of free radicals by activated neutrophils in addition of defense in erythrocytes antioxidant may be associated to the pathogenesis of BD, also high level of malondialdehyde and superoxide dismutase levels, accompanied with lower activities of glutathione peroxidase was one of the investigations improvement about association the oxidative stress with BD. The present results observed that patients with BD had higher GSTM1 deletion than healthy individuals, also in GSTT and deletion both genes, the genetic deficiency of antioxidant enzyme which resulted from different factors causes imbalance in oxidative index led to accumulation oxidative damage like proteins disruptions and DNA mutations. Many processes happened during inflammatory like free radicals production from different source, cellular infiltrate is a main source of ROS, superoxide produced by stimulated monocyte, superoxide anion and hydrogen peroxide expressed by respiratory burst of infiltrating polymorphonuclear neutrophils in skin and nitric oxide synthase synthesis nitric oxide, thus if these free radicals accumulate in different tissue with present of genetic predisposition to incidence diseases it may be involved in disease development and severity as in BD.

Our study agreements with the study of Tursen et al., which found a higher percentages of the GSTM1-null genotype in Patients with BD compared with the healthy individuals. Other studies have suggested that the genotyping of GSTM1 and T1 may induced the capability to chemicals detoxifications like this contained in cigarette smoke. Which Some clinical manifestations of BD may be influenced by smoking, and this effect can be boosted in patients have GSTs genes polymorphisms.

CONCLUSION

The present study improved the higher association of GSTT and GSTM with BD in Iraqi population whose proved suffered from oxidative unbalanced in each individuals led to accumulated free radicals and resulting of oxidative damage in tissues, The present study is a first step from series investigations in relationship between BD and oxidative stress related genes.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


