Identification of the Oxidative Stress–Related Genes in Rheumatoid Arthritis patients

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**Abstract:** Oxidative stress become the most important factor which have side effects in development disease, the present study aims to detection Glutathione S-transferases M1 and T1 in Romatiod Arthritis patients , whole blood used to extracted DNA from (30) patients and (30) healthy and genotype of GSST and GSTM gene was detected used multiplex PCR , the results show that deletion of GSTM was more frequency in RA patients than control it was 22%, 36.84% in control and patients respectively , the GSST gene deletion was more frequent than GSTM also it more frequent in patients than control , it was 52%, 73.68% control and patients respectively. The present study conclude that there was association between GSTs deletion and RA diseases.

**Key words:** Glutathione S-transferases, Romatiod Arthritis patients, multiplex PCR.

**Introduction**

Oxidative stress define as un balance between free radical production and consumption in the cell, this cause by different factors which included endogenous and exogenous inducers, this lead to cellular dysfunction (1,2,27).the common free radicals which derive from oxygen like superoxide free radical anion (O2·−), hydroxyl free radical (OH), lipid peroxyyl (LO), lipid alkoxyl (LOO)and lipid peroxide (LOOH) as well as non-radical derivatives such as hydrogen peroxide (H2O2) and singlet oxygen(^1O2) (2,3). These reactive oxygen species (ROS) are produced from two major sources in the biological system; cellular metabolism and environmental sources, the cellular source include mitochondrial electron transport chain, endoplasmic reticulum oxidation, NADPH oxidase, xanthine oxidase, prostaglandin synthesis, reduced riboflavin, nitric oxide synthetase, reperfusion injury, cytochrome P450, activated neutrophils and phagocytic cells while environmental source include like drugs, pesticides, transition metals, tobacco smoke, radiations and high temperature(4).

Glutathione S-transferases (GSTs) are metabolic isozymes in prokaryote and eukaryote, it's one the important enzymes of detoxification, its regulate the conversion of toxic compounds to hydrophilic metabolites (5,6) Also it protect cells against oxidative stress like neural cells (7). The GSTM1 gene is located at 1p13.3, GSTT1 gene is located at 22q11.2, and The GSTP1 gene is located at 11q13, The polymorphisms of GSTM1, GSTT1, and GSTP1 have been investigated with various diseases(8).

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disease primarily affecting synovial membranes of joints. The Pathogenesis happened in multistep process where cellular and humeral interactions mediated by lymphocytes (T and B cells) and non-hematopoietic cells like fibroblasts, connective tissue cells, and bone cells play a role. At site of inflammation, activation of T cells and macrophages leads to a large increase in oxygen consumption, thus it causes increased ROS production(9). Both, hematopoietic, and connective tissue cells are effected by oxidative stress process in arthritis. in RA patients enhancing of neutrophils and monocytes circulating causes by activity of NADPH- oxidase, the O2− production was increased
(2-8 fold) than in normal state (10), also decrease in neutrophil SOD activity and an increase in the levels of iron loose in the plasma lema of RA neutrophils and monocyte(11). In RA patients the production of O2- by neutrophils stimulating by N-formyl-methionylleucyl-phenylalanine (12).

In RA patients the effect of oxidative stress appeared as DNA oxidative damage and lipid peroxidation which present in the inflammatory synovium(13), in the early study Maurice et al (14) improved that the chronic oxidative stress contributes to functional hyporesponsiveness of synovial T lymphocytes. The impaired mitogenic responses of SF T lymphocytes correlated with a significant decrease in the levels of the intracellular redox-regulating agent glutathione (GSH).

Indirect effect of ROS is cartilage degradation resulted from the presence some oxidative stress product in the biological fluids of patients with arthritis like lipid peroxidation products, nitrite, nitrotyrosine, a nitrated type II collagen peptide, modified low-density lipoprotein (LDL) and oxidized IgG. In addition of accumulation nitrotyrosine, nitrated proteins and oxidized LDL (ox-LDL) in cartilage of arthritic patients (15). Rheumatoid arthritis is characterized by irreversible damage to the cartilage matrix caused by enzymatic degradation of the proteins, e.g., collagen type II (CII), and proteoglycans of cartilage (16).

Materials and methods

Sample collections, Healthy and patients DNA was extracted from whole blood using (Genaid extraction kit).

1. A 300 μl of frozen blood was transferred to eppendorf tube, then 40 μl of proteinase k was added and incubated it at 60 C for 20 min.
2. GB buffer was added (200μl) and it shaken vigorously.
3. Absolute ethanol was added (200 μl) then was mixed by shaking, then it centrifuged at 15000 rpm for 5 mints.
4. Supernatant transferred to GD column, and centrifuged at 15000 rpm for 1 min.
5. Flow rate was discarded and 400 μl of W1 buffer was added, and then centrifuged at 15000 rpm for 1 min.
6. Fallow rate was discarded and 600 μl of wash buffer was added, then centrifuged at 15000 rpm for 1 min.
7. Re-centrifuged after discarded flow-rate for 5 min at the same speed to dry columns.
8. 100 μl of d H2O was added to column and left 2 min at room temperature to absorb it.
9. DNA eluted in new eppendorf tube by centerfield columns for 2 mins at15000 rpm.

1. DNA concentration and purity, it detected by nanodrope (optizen).
2. Primers, multiplex PCR was used in present study to detected GSTM and GSTT gene. The primers are GSTM1: forward: 5’-GAACCTCCCTGAAAAGCTAAAGC-3’ , reverse: 5’-GTGGGGCTCAATATACGGTGG -3’ .GSTT1: forward: 5’-TTCCCTACTGGTGCTCATCTAC-3’ , reverse: 5’-TCCCAGGTCA CCGGATCAT-3’. (17)
3. PCR conditions and size products, PCR experiments performed by Multiplex PCR as a following; denaturation for 5 minutes at 94C, then 35 cycles (1 minute at 94C , 1 minute at 58C , one minute at 72C , and finally 10 minutes at 72C). Genotypes were determined by the electrophoresis pattern of PCR products in agarose gel (1.5% agarose, 70 V, 20mA for 45 mints) with ethidium bromide staining, the PCR size product was GSTM1 219 bp and GSTT1 459 bp.
4. Statics, the results were statically analysis using Qi Square at (p value <0.05).

Results

The results of present study were that the mean of age was (48.22±2.564) for patents, and (42.439±1.868) for control.

Electrophoresis pattern for GSTT and GSTTM genes were 215 pb for GSTM and 466 pb for GSTT as in figure (1).

The GSTs gene polymorphism show significant variations in GSTM gene deletion between patients and control it was appeared normally at 63.155, 88% of patients and control respectively while null genotype was at 22%, 36.84% of control and patients respectively with significantly (p<0.05).
The results of GSST genotypewas appeared in 48%, 26.31% in control and patients respectively , while null genotype appeared in 52%, 73.68% in control and patinas respectively with non-significant statically.

Table (1) distribution of GSTM and GSTT genotype in arthritis patients and control group.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Healthy Patients</th>
<th>QI SEQUARE</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM normal</td>
<td>88%</td>
<td>63.15%</td>
<td>3.794</td>
</tr>
<tr>
<td>Null</td>
<td>22%</td>
<td>36.84%</td>
<td></td>
</tr>
<tr>
<td>GSTT normal</td>
<td>48%</td>
<td>26.31%</td>
<td>2.141</td>
</tr>
<tr>
<td>Null</td>
<td>52%</td>
<td>73.68%</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The results of present study suggested that GSTs genes polymorphism may be associated with Romatoid Arthritis, in GSSM there was significant variation between patients and control, while in GSTT there was high percentage of gene deletion in patients, There have been a few reports of an increased risk of RA due to the GSTM1 null genotype, whereas a number of studies have shown the association between GST polymorphisms and various types of diseases. although some small studies have examined the relationship between the polymorphisms of GSTs and the risk of RA the reason for the controversial results of these studies could be associated with the differences in the populations studied and their exposures to agents related to RA development, The data of Morinobu et al., (18) suggests that polymorphism in the GSTM1 gene is associated with disease susceptibility to RA in the Japanese population. Grabare et al., (19)suggested that the presence of the GSTT1-0 genotype contributed to higher disease activity in RA patients. And the risk for developing highly active RA was the highest in smokers with the GSTT1-0 genotype. In korian population study was partial differed from our study, researchers suggest that the deletion polymorphism of GSTM1 was associated with increased susceptibility for RA, particularly among individuals who are not carriers of the HLA-DRB 1 SE (20).

The present study deal with meta-analysis in china study that suggested that GSTT1 null genotype is not association with an increased susceptibility to RA. However, GSTT1 null polymorphism may increase the risk of RA in relation to heavy smokers or seropositive results. (21).

As a results of smoking role in oxidative stress states, all RA studies which deal with GSTs genes clarified its role in genes polymorphisms which concluded from increments of ROS \ RNS productionin RA, the present study excluded smokers from samples in patients and control, this because the present study was suggested to determinate oxidative stress gene polymorphisms role in disease and its developments in Iraqi population which suffer from unbalance of oxidative stress (data not show).

The association between oxidative stress and RA has been reported, but this association still unclear because indirect association of the genetic susceptibility of RA genetic with genetic regulation of antioxidant mechanisms systems, Veselinovic et al., (22)Suggested that the GSTM1 null genotype is a risk factor for susceptibility to RA in a sample of Iranian population.

As a results of oxidative stress effects in human life, Nutritionists recommended that the life style must be included antioxidant activities like medical plant (23, 28), for ROS scavenger (24, 29) and detoxification
We conclude from this study that the GSTM deletion was association with RA while there was strong association of GSTT gene deletion with RA patients in Iraq. The study need more investigation about gene sequences and epigenetic study.

Acknowledgments

This study performed in DNA lab in biology departments\Unv. of Babylon by helping prof. Dr. Ali Al-Saadi, Dr. Mona Al-Terehi, and prof. Dr. Haider K. Zaidan.

References


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