Oxidative stress has been the important factor in induction and development disease the present study was carried out to detection glutathione S-transferase Mu1 (GSTM1), glutathione S-transferase T1 (GSTT1) gene polymorphisms in breast cancer tissue using multiplex PCR the results show there are significant association between genes sequence deletion and breast cancer The results of present study show the deletion in GSTM gene was 22%, 83.84% for control and patients respectively (P value <0.0001). The deletion in GSTT gene was appeared in 52%, 100% in control and patients respectively (p<0.005).

The association between GSTM and GSTT gene show the two genes in 48% of control were normal (p<0.05), the appearances of GSTM gene without GSTT gene didn’t appear in control while it appeared in 16.16% of cancer tissue (p<0.0506), the GSTT gene was normal without normality of GSTM gene in 40% of control (p<0.0022). The present study concluded that antioxidants enzyme genes strongly linked with breast cancer in Iraqi women.

KEY WORDS: GSTT, GSTM, multiplex PCR, Breast cancer.

1. INTRODUCTION

Oxidative stress become the most important in induced disease and complication of its, oxidative stress known as a balance between reactive species and antioxidant activity of enzymes and vitamins, cell produced Reactive oxygen species (ROS) in normal states for cell functions. Oxidative stress can also be defined as the disordered redox signaling and control. Different types of ROS and RNS are produced throughout the body that are found to be the by-products of cellular aerobic metabolism, ongoing stress, and exposure to UV light or X-rays; Its playing major role in cell signaling and regulation of some cells functions like growth factor cytokine, hormone action, transcription, ion transport, and apoptosis, also it has critical roles in immune system function, proliferation of T cells.

The antioxidant activity in body performed by antioxidant enzymes like catalase, superoxide dismutase, and glutathione S transfers (GSH). There are different isomers of GST in Human are glutathione S-transferase Mu1 (GSTM1), glutathione S-transferase T1 (GSTT1), and glutathione S-transferase P1 (GSTP1), The GSTM1 gene is located at 1p13.3, the enzyme activity was lacked by the deletion in GSTM1 gene. While GSTT1 gene is located at 22q11.2, and some studies reported a homozygous deletion in GSTT1. The polymorphisms of GSTM1 and GSTT1 have been reported with different diseases like schizophrenia, hypertension, diabetes mellitus and capacity for oxidation and detoxification. The review of literature clarified that the deletion in GSTM1 or GSTT1 gene were contributed to the loss of GST enzyme activities.

ROS causes DNA damaged, it causes single- or double-strand breakage, deoxyribose modification, DNA cross-linking, Cell death, DNA mutation, replication errors, and genomic instability may be occurred when damage is not repaired by repair system.

The oxidative DNA damages by ROS produced 8-hydroxydeoxy guanosine (8-OHdG), which is mutagenic in mammalian cells. Some studies have demonstrated that 8-OHdG levels are elevated in various human cancers. Association of GSTs gene polymorphism and cancer type have been reported.

2. MATERIALS AND METHODS

Sample collection about 30 breast cancer embedded tissue was collected from histopathology unit in Al Saader medical city, these samples were diagnosed by specialist physician as a breast tumor tissue, also all samples were used to diagnosis tumors in females that don’t treated with any anticancer therapy, and 30 blood sample of control were collected from healthy female that have age (35-65 years).

DNA extraction: DNA was extracted from embedded tissue according to the leaflet of Gene aid manufacture with modification, in (in DNA lab of biology department) briefly; About 40 mg of tissue was put in eppendorf tube contain 1 ml of xylene, then it mixed and incubate at room temperature for 15 min, then Centrifugation at 14000 for 3 min, and supernatant was removed, Absolute ethanol was added (1 ml) to mixture then Centrifugation at 14000 for 3 min, and supernatant was removed, the mixture was Incubated at 37°C, GT buffer was added (200 µl) with homogenize by micro pestle, then Proteinase K (40 µl) was added and incubate for 20 min at 60°C with inverting every 5 min. GBT buffer was added (200 µl) with mixing, then incubate at 60°C for 20 min and Absolute ethanol was added (200 µl) with mixing, then transfer mixture to GD column after that it Centrifuged at 14000 for 2 min, the flow-through
was discarded and W1 buffer was added to column (400 µl), then Centrifugation at 14000 for 20 sec. the flow-through was discarded also. Wash buffer was added (600 µl) Centrifugation at 14000 for 30 sec. the flow-through was discarded, then it Centrifuged at 14000 for 3 min again, finally DNA was eluted using dH2O (100 µl). Healthy DNA was extracted from whole blood using (Genaid extraction kit), in briefly; 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, then GB buffer was added (200µl) and it shaking vigorously, after this absolute ethanol was added (200 µl) and miter was mixed by shaking, then it centrifuged at 15000 rpm for 5 mints. the Supernatant was transferred to GD column, and centrifuged at 15000 rpm for 1 min. the flow-rate was discarded and 400 µl of W1 buffer was added, then centrifuged at 15000 rpm for 1 min, the Fallow rate was discarded also, about 600 µl of wash buffer was added, then centrifuged at 15000 rpm for 1 min. columns were Re- centrifuged after discarded flow ate for 5 min at the same speed to dry column, finally 100 µl of d H2O was added to column and left 2 min at room temperature to absorb it. DNA eluted in new eppendorf tube by centerfield column for 2 min at15000 rpm.

1. Primers, multiplex PCR was used in present study to detected GSTM and GSTT gene. The primers are GSTM1: forward: 5'-GAACTCCCTGAAAAGCTAAAGC-3', reverse: 5'-GTTGGGCTCAAATATACGGTGG-3'.GSTT1: forward: 5'-TTCCTTACTGGTCCTCACATCTC-3', reverse: 5'-TCCCAGGTCACCGGATCAT-3'.

2. PCR conditions and size products, PCR experiments performed by Multiplex PCR as a following; denaturation for 5 minutes at 94ºC ,then 35 cycles (1 minute at 94ºC, 1 minute at 58ºC, one minute at 72 ºC, and finally 10 minutes at 72 ºC). 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 215bp and GSTT1312bp.

3. Statics, the results were statically analysis using Qi Square analysis at p value <0.05).

3. RESULTS
The results of present study show that there are significant variations between patient and control in GST genotypes, the deletion in GSTM gene was 22% in control while in cancer tissue was 83.84% at (P value <0.0001). The deletion in GSTT gene was appeared in control 52% while in cancer was appeared in all samples in present study with significant variation at (p<0.005) as show in table 1.

Table.1.GSTT and GSTM gene in study subjects groups

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal</th>
<th>Deletion</th>
<th>Deletion</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM</td>
<td>88%</td>
<td>16.66%</td>
<td>21.88%</td>
<td>21.88%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>GSTT</td>
<td>48%</td>
<td>0</td>
<td>11.985</td>
<td>11.985</td>
<td>0.0005</td>
</tr>
<tr>
<td>GSTM+GSTT</td>
<td>48%</td>
<td>0</td>
<td>11.985</td>
<td>11.985</td>
<td>0.0005</td>
</tr>
<tr>
<td>Null</td>
<td>52%</td>
<td>100%</td>
<td>3.821</td>
<td>3.821</td>
<td>0.0506</td>
</tr>
<tr>
<td>GSTM-GSTT</td>
<td>0%</td>
<td>100%</td>
<td>9.382</td>
<td>9.382</td>
<td>0.0022</td>
</tr>
<tr>
<td>Null</td>
<td>60%</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The association between GSTM and GSTT genes show that the normality of the two genes was appeared in 48% of control while it disappeared in cancer patients at (p< 0.0005), the appearances of GSTM gene without GSTT gene didn’t appear in control while it appeared in 16.16% of cancer tissue at (p<0.0506), the GSTT gene was normal without normality of GSTM gene in 40% of control, while it null in patients at (p< 0.0022).

Figure.1.Electrophoresis pattern of GSTT and GSTM polymorphism in breast cancer tissue and control group lane 1 DNA marker, lane2, 7,10,11,12,13,14 show GSTT only , lane 4,6, show GSTM>
DISCUSSION

The results of present study show that the deletion in GSST and GSTM gene were more frequent in patients than control group. In Iraq there were more factors effect in human health especially in induction and development disease like cancer in last decades, one of the important factors is oxidative stress which had major role in development disease, the deletion in large percentage of patients in present study may be because the accumulation effect of defect redox regulation in Iraqi population which induced cancer, the previous studies show that The ROS which is associated with cell function as secondary messengers in intracellular signaling cascades, induce and maintain the oncogenic phenotype of cancer cells, also accumulation of ROS/RNS production was found in many types of cancer cell are linked with altered redox regulation of cellular signaling pathways, Thus it may be related to oncogenic stimulation. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions (8-OHG) have been noted in various tumors.

Multiplex PCR was used in present study because it easy, less time and low cost than other techniques had been used to detected mutation in GSTs gene.

The deletion in antioxidant genes GSST and GSTM may be resulted from genetic predisposition in Iraqi family especially which have this important factor in breast cancer pathogenicity, however this deletion may be because high radius pollution in environment which induced genetic alteration. The heavy metals have been recorded as a factor induced genetic alteration and induced oxidative stress in cells, in to study carried out on tumor tissue of Iraqi patient show high level of heavy metals in tumor tissue it was Mn, Cd, Co and Zn.

The present study performed using multiplex PCR, this technique has low cost, short time for detection more than one gene in the same PCR tube. GSTs enzymes are used in the biotransformation of exogenous substances, including mutagens, carcinogens, also it play a major role in the detoxification process, thereby protecting cells from these compounds. Our study agreements with other studies deal with different cancer types. Srivastava, 1998, studied GSTP1313 G/G polymorphism in bladder cancer, they found that a strong predisposing of GSTs genes was a risk factor of bladder cancer, however the Combination of three GST genotypes association exhibiting gene–gene interaction further substantiates the increased risk of bladder cancer. In meta-analysis study Lu et al., found variation in association of GSTs gene with breast cancer in population study, they found that GSTP1 Ile105Val polymorphism was not associated with breast cancer susceptibility in overall population but in subgroup analysis by ethnicity, they found a significant association among Asian population and When stratified by study design, significantly elevated susceptibility to breast cancer was found among hospital-based studies.

4. CONCLUSION

The present study was aimed to detect the normality of GSST and GSTM gene in breast cancer tissue, we conclude that these genes were altered by deletion DNA sequence, and thus there was imbalance in redox regulation in cells which lead to increase the disease severity. We need more investigations for detection deletion sequence by DNA sequence and detection GSTT and GSTM gene expression in patients.

REFERENCES


