Polymorphism of GSTM1, GSTT1, GSTP1, and GSTA1 genes In Iraqi Population

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Abstract. One of the phase II enzymes that are responsible for detoxification of the body are Glutathione S-transferases (GSTs). Type and frequency of polymorphism of GSTs differ among the population. The current paper was designed to detect the polymorphisms in GSTM1, GSTT1, GSTP1 and GSTA1 genes among the Iraqi population, and the results were compared with other population. Data will be collected in the future to obtain a genetic map of the Iraqi population. To our knowledge, this study is the first done on the Iraqi population. In this study blood samples were collected from 110 healthy individuals (51 males and 59 females) aged between 15-50 years. The presence or absence of GSTM1 and GSTT1 genes was identified by multiplex-PCR. In addition, PCR-RFLP was used to detect polymorphism of GSTP1 (Ile105Val) and GSTA1 (A*/B*). The study revealed the frequencies of GSTM1 null, GSTT1 null, GSTP1 (Ile105Val), and GSTA1 A*/B* were 34.55%, 25.45%, 45.46%, and 41.82% respectively. The most frequently observed combinations were GSTM1 Present/ GSTT1 Present/Ile/Val/A*/A* (18.18%). For the first time in Iraq by this study, four sequences were recorded in NCBI under the following accession numbers LC081235.1, LC090205.1, LC081236.1, and LC090206.1. These findings provide us the basic data for genotypes distribution and allele frequencies of GSTM1, GSTT1, GSTP1 and GSTA1 in the Iraqi population, and this is open a new prospect for further investigations by researchers in identifying differences between individuals in the genetic susceptibility of various diseases caused by environmental gene, rather than depending on results obtained from other populations.

Key words: Iraqi population; Polymorphism; GSTM1; GSTT1; GSTP1; GSTA1; Genes.

1.Introduction

Human glutathione S-transferases belong to supergene family of phase-II isoenzyme be arranged by their substrate specificities, isoelectric points, amino sequence homologies, and immunological connections, into seven classes called Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta [1]. Glutathione S-transferases detoxify a wide range of electrophilic compounds including pesticides, carcinogens, pharmaceuticals, herbicides, chemotherapeutics, industrial chemicals and pollutants, products of oxidative damage and natural plant toxins, through conjugated them to glutathione to form their corresponding glutathione conjugates which often results in less reactive and more water-soluble compounds. This reduces the possibility of damage from xenobiotics and eliminates these compounds from the body [2,3]. Glutathione S-transverses remove toxins from an extensive variety of compounds known as electrophilic compounds, such as cancer-causing agents, herbicides, pesticides, chemotherapeutics, pharmaceuticals, Industrially prepared chemicals contaminants, results of oxidative stress, and naturalistic plant poisonsivalinked these compounds with glutathione to produce their corresponding glutathione couples which are characterized by their low reactivity and high solubility in the water. So the damage that can be caused by vehicles will decrease as a result of being thrown out of the body [2, 3].
Glutathione S-transverses perform many tasks including keep large biomolecules such lipids, nucleic acid, and protein from damaged by reactive oxygen specifies, restoration of S-thiolated proteins, remove toxin from lipid peroxidation products,[4]participate in the production and metabolism of some molecules such as prostaglands, steroids, and leukotrienes, as well as they play an important role in the development of resistance to chemotherapeutic agents [4, 5]. The genetic variation of most GST genes has been studied in much research. Most of the research focused on polymorphism in GSTM1, GSTT1, and GSTP1 genes. The GSTM1 and GSTT1 genes are located on chromosome 1p13.3, and 22q1.2 respectively [6]. Genetic polymorphism of GSTT1 and GSTM1 results from the absence of a gene, therefore, those subjects with GSTT1, and GSTM1 null genotypes suffers from a loss in the activity of the enzyme [7]. As for the polymorphism of GSTP1 gene, it results from replacement Guanine rather than Adenine in exon 5 at nucleotide 313, as a result valine [Val]replace isoleucine [Ile] in the location 105 of amino acid sequence [8, 9, 10]. However, polymorphism of GSTA1 gene occur in three single nucleotide in proximal promoter region, there are two alleles of GSTA1: GSTA*A(-567T, -69C, -52G) and GSTA1*B (-567G, -69T, -52A) [11]. Presence of polymorphism in these genes will reduce the capacity of enzyme in damage chemical carcinogens and mutagens [12]. As well as the variations in therapeutic drugs response are affected by an individual difference in the ability of GST enzymes to metabolize these drugs [13]. The frequency of the above polymorphism differs with the ethnicity of a population at the same place in the world as well as differing in different ethnical groups within the same country. Knowing the frequency of polymorphism in the population could, therefore, be of significance for the planning of health care [14]. In the best of our knowledge, this is the first study on the Iraqi population, therefore, fail due to further investigation. Hence, The objective of the present study was to determine the prevalence of GSTM1, GSTT1, GSTP1 (Ile105Val), and GSTA1(*A/*B) polymorphisms in the Iraqi population, then data will be collected in the future to obtain a genetic map of the Iraqi population as the only country that does not have a genetic map.

2. Materials and Methods

2.1 Specimens:

One hundred and ten subjects (51 males and 59 females) participated in the study. The included subjects age range between 15 - 50 years. All samples were collected from healthy subjects from different regions of Baghdad capital, Iraq. Five mL of blood was collected in EDTA tube. DNA was extracted using genomic DNA extraction kit (Bioneer, Korea). The products of DNA extraction were verified by horizontal electrophoresis (Mupid–One, Japan) in 1% agarose. DNA concentrations were determined using the Nanodrop (QuawellIQ5000, USA).

2.2 Polymorphism of GSTM1 and GSTT1

The GSTM1 and GSTT1null genotypes were detected using a multiplex polymerase chain reaction (PCR)(Agilent Technologies, USA) according to the method of Ben Slah et al.[15] using the same multiple primers with a specific sequence of GSTM1 forward primer (F-5'-CGCCATCTTTGTCTACATGCCC-3') and reverse (R-5'TTCTGGATTGTA GCAGATCA-3'). While GSTT1 forward primer was (F-5'-TTCTTACTGGTCCTCAGATC-3') and reverse (R-5'-TCACCGGAT CATGCCCAGCA-3'). Albumin forward and reverse primers were (F-5'-GCCCTCTGCTAAACATGCT-3') / (R-5'GCCCTAAAAAGAAAAATCGCCATC-3) respectively. Albumin gene was used as an internal control in this study. The 25μL of the multiplex PCR mixture was containing 6 μL genomic DNA, concentration range was 50-100ng, 10 pmol of the above primers, 12.5 μL of Hot start green Master Mix (Promega) and 0.5 μL of free DNAse distilled water. The PCR protocol included an initial denaturation at 95°C for (5 min) followed by 35 cycles of amplification (Denaturation at 94°C for the 50s, Annealing at 56°C for 50s and Extension at 72°C for 50s). A final extension step at 72°C was extended for 10 min. The PCR products were analyzed on 2% agarose gel (Fig.1). The fragments of 230pb, 459pb, and 350pb represent GSTM1, GSTT1, and the positive internal control (albumin) respectively.
2.3 Genotyping of GSTP1 polymorphism (Ile105Val)

Genomic DNA was used to amplify a 176 bp fragment using specific primer sequences, forward primer (F-5’-ACCCCAGGGCTCTATGGGAA-3’) and reverse primer (R-5’-TGAGGGCACAAAGAAGCCCT-3’) according to Korytina et al.[16]. The 25 μL PCR reaction mixture consisted of 100–150 ng of genomic DNA, 0.6 μL [10 pmol/L] of each primer, 12.5 μL of Hot start green Master Mix (Promega) and 6.3 μL of free DNAase distilled water. The conditions of PCR cycling were 5 min. at 95 °C for initial denaturation followed by 30 cycles (denaturation at 94 °C for 30 s, Annealing at 60 °C for 30 s, extension at 72 °C for 30 s), and final extension was done at 72 °C for 10 min. PCR fragment was amplified as a 176bp band (Fig.2A). The detection of GSTP1 (Ile105Val) polymorphism was done using a BsmAI restriction enzyme (Bio labs, United Kingdom) to produce one band at 176bp in Homozygous wild type (Ile/Ile), 3 bands at 176bp, 91bp, and 85bp in Heterozygous mutant (Ile/Val) and two bands at 91bp and 85bp in Homozygous mutant (Val/Val). These fragments were visualized using ethidium bromide staining and 2.5 % agarose gel electrophoresis (Fig.2B).

**Figure 1:** Genotyping of GSTM1 and GSTT1 by Multiplex PCR in our population, the PCR products were analyzed on 2% agarose gel using 100bp DNA marker (M). A fragments of 230pb, 459pb, and 350pb represent GSTM1, GSTT1, and the positive internal control (albumin) respectively. GSTM1present/ GSTT1null can be seen in lane 1 and 5, and GSTM1present/ GSTT1present show in lane 2,8,9, and 10. Lanes 3,4,6,11, and 12 represent the GSTM1null/ GSTT1present and lane 7 shows a GSTM1null/ GSTT1null.

**Figure 2A:** Electrophoresis of PCR product for GSTP1 gene on 2% agarose gel. M:DNA ladder marker 25bp. Lane 1 shows negative control and Lanes (2-9) show the PCR products for GSTP1 gene.

**Figure 2B:** PCR-RFLP analysis of GSTP1 gene polymorphism using BsmAI restriction enzyme. M:DNA ladder marker 25bp. Lanes 1,2,3,8,9, and 12 represent homozygous(Ile/Ile) producing one band at 176 bp. Lanes 5,6,10, and 11 show heterozygous mutant (Ile/Val) producing 3 bands at 176bp, 91bp, and 85bp. Homozygous mutant (Val/Val) producing 2 bands at 91bp and 85bp can be seen in lanes 4 and 7.
2.4 Genotyping of GSTA1 polymorphism (C69T)

A fragment containing C69T polymorphism in the promoter region of GSTA1 gene was amplified as a 432bp band as shown in (Fig.3A) using specific primers designed by our research which included a forward primer (F-5’-CAGACATCCTCTC CCAGCTA-3’) and reverse primer (R-5’-GGGTGTGAGCAATGTA GAG -3’). The PCR reaction mixture was a volume of 25 μL consisted of approximately 100–150 ng of genomic DNA, 0.6 μL(10 pmol/L) of each primer, 12.5μL of Hot start green Master Mix (Promega) and 6.3 μL of free DNAase distilled water. The optimum conditions of PCR were done according to our research here: 5 min. of initial denaturation at 95°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, then extension at 72°C for 30 s with a final extension was done at 72°C for 10 min. After amplification, the PCR products were digested using an EarI restriction enzyme (Bio labs, United Kingdom) producing one band at 432bp in Homozygous wild type (C*/C*), three bands at 432bp, 290bp, and 142bp respectively in the Heterozygous mutant (C*/T*) (Fig.3B). Resulting fragments were visualized using 2.5 % agarose gel.

![Figure 3A: Electrophoresis of PCR product for GSTA1 gene on 2% agarose gel.](image)

![Figure 3B: PCR-RFLP analysis of GSTA1 gene polymorphism using EarI restriction enzyme. M: DNA molecular weightmarker 100bp, Lanes2,3,4,6,8,10, and 11 represent homozygous wild type (C/C) producing one band at 432bp, Lanes 1,5,7, and 9 represent heterozygous mutant (C/T) producing 3 bands at 432bp, 290bp and 142bp.](image)

2.5 Sequencing and Sequence Alignment

Sequencing of amplified products of GSTP1 gene was done by Macrogen company/USA, using forward and reverse primers for GSTP1 gene in each sequencing reaction. The homology search was conducted using Basic local alignment search tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and Bio Edit program as well as an ExPASY program for the amino acid sequence. The results were compared with reference sequences of the gene obtained from Gene Bank as a control.

2.6 Statistical analysis

The Pearson χ2 criterion (p < 0.05) was used to study the frequencies distribution of the GST genes according to several demographic characteristics, using the SASS program.

3. Results

General characteristics of the two study groups (males and females) like age, tobacco usage, family history of cancer, smoking, and drinkers alcohol are presented in Table 1. The percentage of males was 46.36% vs. 53.64% of females in this study. Most of the population have aged 15-25
only (55.45%). Only (16) 14.55% of the population has a family history of cancer. The Majority of them (82.73%) are not smoking tobacco, while (17.27%) consumed cigarette and only two persons (1.82%) were having alcohol.

Table 1: Characteristics of study population

| Group description          | N     | [%] |
|----------------------------|-------|-----|
| Gender                     |       |     |
| Male                       | 51    | 46.36|
| Female                     | 59    | 53.64|
| Age                        |       |     |
| 15-25                      | 61    | 55.45|
| 26-35                      | 34    | 30.91|
| 36-50                      | 15    | 13.64|
| Family history of cancer   |       |     |
| Yes                        | 16    | 14.55|
| No                         | 94    | 85.45|
| Smoking                    |       |     |
| Yes                        | 19    | 17.27|
| No                         | 91    | 82.73|
| Alcohol Consumption        |       |     |
| Yes                        | 2     | 1.82 |
| No                         | 108   | 98.18|

N: number of subjects

The distribution of GSTM1 and GSTT1 genotypes in healthy subjects according to the demographic factors are shown in Table 2. The frequencies of GSTM1 and GSTT1 null genotypes in the Iraqi population were 34.55 % and 25.45% respectively. Data in Table 2 revealed no significant difference (P > 0.05) in the frequency distribution of GSTM1 and GSTT1 genotypes among sex, age, family history of cancer and drinking alcohol. On the other hand, there was a significant increase (P<0.05) in the frequency of GSTM1 null genotype in the population who had to smoke 57.89% compared to others who had non-smoking 29.67%. In addition Table 2 displayed no significant difference (P>0.05) in the frequency distribution of GSTT1 with a smoking habit.

Table 2: The frequency distribution of GSTM1 and GSTT1 genotypes according to demographic characteristics

| Factor                  | GSTM1   | GSTM1   | GSTT1   | GSTT1   |
|                        | Present | Null    | Present | Null    |
|                        | N  [ % ]| N  [ % ]| N  [ % ]| N  [ % ]|
| Total                  | 72 (65.45) | 38 (34.55) | 82 (74.55) | 28 (25.45) |
| Gender                 |         |         |         |         |
| Female, N=59 (53.63%)  | 41 (69.49) | 18 (30.51) | 45 (76.27) | 14 (23.73) |
| Male, N=51 (46.36%)    | 31 (60.78) | 20 (39.22) | 37 (72.55) | 14 (27.45) |
| Chi-square(x²)         | 0.9172  |         | 0.1997  |         |
| Age                    |         |         |         |         |
| 15-25, N=61            | 39 (63.93) | 22 (36.07) | 43 (70.49) | 18 (29.51) |
Comparisons in GSTM1 and GSTT1 genotypes among Iraqi population and different populations were performed (Table 3). We observed GSTM1-null frequency (34.55%) in our population was in agreement with Iranians (40.46%), Japanese (44.06%) and Cameroonians (27.78%) whereas significantly lower-frequency value was observed with respect to Bahrainis (49.7%), Lebanese (52.48%), Egyptians (50.98%), Tunisians (63.44), Turkish (51.88%), French (52.33%), Italian (49.17%), Spanish (55.32%), and Chinese (56.46%). On the other hand, we found that GSTM1-null frequency was significantly higher than Saudi populations (16.5%). As for comparisons of GSTT1-null frequency (25.45%), this study observed there was no significant differences related to Bahrainis (28.74), Egyptians (35.29%), Turkish (17.29%), Italian (28.33%), Spanish (27.66%), Indians (31.30%). Whereas significant differences in frequency GSTT1-null respect to some country such as Saudi, Lebanese, Tunisians, French, Chinese, Japanese, and Cameroonians.

**Table 3:** Frequency of GSTs null genotypes in various ethnic groups

| Population | n | GSTM1 Present | GSTM1 null | p | GSTT1 present | GSTT1 null | p | References |
|------------|---|---------------|------------|---|--------------|------------|---|------------|
| Iraqi      | 110 | 72 (65.45) | 38 (34.55) | 0.000297 | 82 (74.55) | 28 (25.4) | 0.000012 | [21] |
| Saudi      | 200 | 167 (83.5) | 33 (16.5) | 0.012836 | 119 (71.26) | 48 (28.74) | 0.548445 | [22] |
| Bahrainis  | 167 | 84 (50.3) | 83 (49.7) | 0.004562 | 88 (62.41) | 53 (37.59) | 0.041325 | [21] |
| Lebanese   | 141 | 67 (47.52) | 74 (52.48) | 0.047437 | 33 (64.71) | 18 (35.29) | 0.198545 | [26] |
| Egyptians  | 51  | 25 (49.02) | 26 (50.98) | 0.000001 | 117 (62.90) | 69 (37.10) | 0.039192 | [22] |
| Tunisians  | 186 | 68 (36.56) | 118 (63.44) | 0.00674 | 110 (82.71) | 23 (17.29) | 0.11993 | [23] |
| Turkish    | 133 | 64 (48.12) | 69 (51.88) | 0.03442 | 145 (84.3) | 27 (15.70) | 0.043698 | [24] |
| French     | 172 | 82 (47.67) | 90 (52.33) | 0.024899 | 86 (71.67) | 34 (28.33) | 0.623084 | [20] |

N: number of subjects, PFE: Probability by fisher extract test
This is the first study was done to our knowledge to give frequency distribution genotypes of the GSTP1 gene in the Iraqi population. Table 4 displayed that 54.55% of individuals had GSTP1 (Ile/Ile), 44.55% had GSTP1 (Ile/Val) and only one person (male 0.91%) had GSTP1 (Val/Val) genotype. The distribution frequency of GSTP1 (Ile allele) and GSTP1 (Val allele) were 0.7682 and 0.2318 respectively. The results in Table 4 demonstrate no significant differences (p>0.05) of GSTP1 genotypes among the factors in Table 4. In order to confirm the results of electrophoresis of PCR-RFLP products for GSTP1 gene, all samples of amplified products of the GSTP1 gene (forward and reverse strand) from Iraqi population were further analyzed by direct sequencing for detecting SNPs within these sequences. After alignment of these sequences of nucleotide for the amplified product with the GSTP1 of Homo sapiens from the Gen Bank using the Bio Edit software. Two of these sequences with homozygous genotype A/A in forward and reverse strand was recorded in Gene Bank National Center Biotechnology Information (NCBI), DNA data bank of Japan (DDBJ) and European Bioinformatics Institutes (EMBI) under the accession number LC081235.1, and LC090206.1 respectively, as well as the two others sequences with homozygous mutant genotype G/G in forward and reverse strand were submitted under the accession number LC081236.1, and LC090206.1 respectively and for acknowledgment these sequences were recorded for the first time in Iraq by this study.

Table 4: The frequency distribution of GSTP1 genes according to demographic characteristics

| Factor          | GSTP1     |           | GSTP1     |           | GSTP1     |           |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                 | Ile /Ile  | Ile/Val   | Val/Val   |           | N [%]     | N [%]     | N [%]     |
| Total           | Ile =0.7682 |           |           |           | 60(54.55) | 49(44.55) | 1(0.91)   |
| Allele          | Val=0.2318 |           |           |           |           |           |           |
| Gender          | Female, N=59(53.63%) |           |           |           | 34,(57.63) | 25,(42.37) | 0,(0.00)  |
|                 | Male ,N=51(46.36%) |           |           |           | 26,(50.98) | 24,(47.06) | 1,(1.96)  |
| Chi-square [x²]| PFE 0.503 |           |           |           |           |           |           |
| Age             | 15-25, N=61 |           |           |           | 34,(55.74) | 26,(42.62) | 1,(1.64)  |
|                 | 26-35, N=34 |           |           |           | 18,(52.94) | 16,(47.06) | 0,(0.00)  |
|                 | 36-50, N=15 |           |           |           | 8,(53.33)  | 7,(46.67)  | 0,(0.00)  |
| Chi-square [x²]| PFE 0.9532 |           |           |           |           |           |           |
| Family history  | Yes, N=16 |           |           |           | 7,(43.75)  | 9,(56.25)  | 0,(0.00)  |
|                 | No, N=94 |           |           |           | 53,(56.38) | 40,(42.55) | 1,(1.06)  |

N: number of subjects, P<0.05, means significantly different from Iraqi population. P>0.05, refers to significantly different from Iraqi population.
Table 5 show comparison of GSTP1 gene frequencies in our population with the different ethnic population. Most of subjects in this study were having GSTP1AA (54.54%), GSTP1 AG (44.54%) and GSTP1 GG (0.009%) genotypes. These results pointed to that GSTP1 G genotype in this study resemble Turkish (52.0%), but there were significant differences in the frequency of GSTP1G genotype compared to the other population in this study. Where the frequency of GSTP1G genotype was less than all countries that found in the current study except Egyptian (25.49%), Chinese (29.41%), and Japanese (28.4%).

Table 5: Frequency distribution of GSTP1 genotypes in various ethnic groups

| Population          | n   | A/A N(%) | A/G N(%) | G/G N(%) | p        | References |
|---------------------|-----|----------|----------|----------|----------|------------|
| Iraqi               | 110 | 60 54.54 | 49 44.54 | 1 0.009  |          | Present study |
| Saudi               | 87  | 35 40.23 | 41 47.13 | 11 12.64 | 0.001    | PFE        |
| Egyptian            | 51  | 38 74.51 | 11 21.57 | 2 3.92   | PFE0.0057|            |
| south Tunisia       | 154 | 73 47.40 | 63 40.91 | 18 11.69 | 0.0016   | PFE        |
| Center of Tunisia   | 182 | 84 46.15 | 79 43.41 | 19 10.44 | 0.0046   | PFE        |
| North Tunisia       | 103 | 35 33.98 | 48 46.60 | 20 19.42 | 0.000019 | PFE        |
| Italian             | 153 | 77 50.32 | 63 41.18 | 13 8.50  | 0.021    | PFE        |
| German              | 622 | 298 47.91| 254 40.84| 70 11.25 | 0.003231 |            |
| Spanish             | 337 | 177 52.52| 136 40.36| 24 7.12  | 0.04621  |            |
| French              | 124 | 59 47.58 | 56 45.16 | 9 7.26   | 0.042    | PFE        |
| Turkish             | 50  | 22 44.0  | 26 52.0  | 2 4.0    | 0.167    | PFE        |
| Chinese             | 119 | 84 70.59 | 34 28.57 | 1 0.84   | 0.002    | PFE        |
| Japanese            | 257 | 184 71.6 | 65 25.29 | 8 3.11   | 0.000872 |            |
| African-Americans   | 247 | 54 21.86 | 135 54.66| 58 23.48 | 0        |            |
| Whites              | 348 | 141      | 169      | 38       | 0.000973 |            |
The results of Table 6 demonstrate the distribution of the GSTA1 genotypes among the population in this study was 58.18% in wild-type (A*/A*), 41.82% in heterozygous (A*/B*) and 0% in the homozygous mutant (B*/B*). The allele frequencies were (0.790, 0.209) for the A* and B* alleles respectively. There was no significant association of GSTA1 genotypes among the factors in Table 6.

Table 6: The frequency distribution of GSTA1 genes according to demographic characteristics.

| Factor       | GSTA1 A*/A* N(%) | GSTA1 A*/B* N(%) | GSTA1 B*/B* N(%) | N: |
|--------------|------------------|------------------|------------------|----|
| Total        | 64(58.18)        | 46(41.82)        | 0,(0,00)         |    |
| Gender       |                  |                  |                  |    |
| Female, N=59 | 34(57.63)        | 25,(42.37)       | 0, 0.00          |    |
| Male, N=51   | 30, (58.82)      | 21, (41.18)      | 0, 0.00          |    |
| Chi-square [x^2] | 0.0161           |                  |                  |    |
| Age          |                  |                  |                  |    |
| 15-25, N=61  | 34, (55.74)      | 27,(44.26)       | 0, 0.00          |    |
| 26-35, N=34  | 18, (52.94)      | 16, (47.06)      | 0, 0.00          |    |
| 36-50, N=15  | 12, (80.00)      | 3, (20.00)       | 0, 0.00          |    |
| Chi-square [x^2] | 3.463            |                  |                  |    |
| Family history |                  |                  |                  |    |
| Yes, N=16    | 9, (56.25)       | 7, (43.75)       | 0, 0.00          |    |
| No, N=94     | 55, (58.51)      | 39, (41.49)      | 0, 0.00          |    |
| Chi-square [x^2] | 0.0287           |                  |                  |    |
| Smoking      |                  |                  |                  |    |
| Yes, N=19    | 11, (57.89)      | 8, (42.11)       | 0, 0.00          |    |
| No, N=91     | 53, (58.24)      | 38, (41.76)      | 0, 0.00          |    |
| Chi-square [x^2] | 0.0008           |                  |                  |    |
| Alcohol      |                  |                  |                  |    |
| Yes, N=2     | 0, (0.00)        | 2, (100)         | 0, 0.00          |    |
| No, N=108    | 64, (59.26)      | 44, (40.74)      | 0, 0.00          |    |
| Chi-square [x^2] | PFE 0.172644     |                  |                  |    |

number of subjects, PFE: Probability by fisher extract test

Table 7 illustrates the frequency of GSTA1 A/B genotype [41.82%] in Iraqi population with absent of GSTA1 B/B genotype, all studies in table 7 have significant high frequency of GSTA1 A/B and B/B genotypes compared to our study except Chinese which have GSTA1 A/B [24.29%] and B/B [0.71%] genotype.
Table 7: Comparison of GSTA1 genotype frequencies among different ethnic populations and Iraqi populations.

| Population | n  | GSTA1 A/A N(%) | GSTA1 A/B N(%) | GSTA1 B/B N(%) | p          | References |
|------------|----|-----------------|----------------|----------------|------------|------------|
| Iraqi      | 110| 64              | 46             | 0              |            | Present study |
| Serbian    | 66 | 23              | 36             | 7              | 0.0007PFE  | [40]       |
| Czech      | 218| 76              | 108            | 34             | 0.000001   | [41]       |
| Central European population | | | | | |
| Netherlands | 698 | 243 | 329 | 126 | 0 | [42] |
| Italian | 200 | 113 | 74 | 13 | 0.022259 | [43] |
| Caucasian | 81 | 24 | 44 | 13 | 0.000001 | [44] |
| Chinese    | 140| 105             | 34             | 1              | 0.00405   | [45]       |
| Brazilian  | 199| 76              | 99             | 24             | 0.000036   | [11]       |
| African–American | | | | | |
| South Tunisian | 154 | 38 | 83 | 33 | 0 | [15] |

N: number of subjects, PFE: Probability by fisher extract test

The results of Table 8 demonstrate the distribution of combined genotypes of GSTM1, GSTT1, GSTP1 and GSTA1 polymorphisms, and the data showed that 17 of the 36 possible genotypes are present in Iraqi population. More frequently observed combinations were GSTM1 Present/ GSTT1 Present /Ile/Val/A*/A* (18.18%), GSTM1 Present/GSTT1 Present / Ile/ Ile / A*/A* (14.55%) and GSTM1 Present/ GSTT1 Present / Ile/ Ile / A*/B* (11.82%).

Table 8: Combined genotype analysis of GSTM1, GSTT1, GSTP1, and GSTA1 polymorphisms.

| GSTM1 | GSTT1 | GSTP1 | GSTA1 | Frequency |
|-------|-------|-------|-------|-----------|
| Present | Present | Ile/Ile | A*/A* | 16 (14.55) |
|        |        |       |       | 13 (11.82) |
|        |        |       |       | 0         |
| Present | Present | Ile/Val | A*/A* | 20 (18.18) |
|        |        |       |       | 6 (5.45)  |
|        |        |       |       | 0         |
|        |        |       |       | 0         |
|        |        |       |       | 0         |
### Table 3

| Present | null | Ile/ Ile | A*/A* | 8 (7.27) |
|---------|------|---------|-------|----------|
|         |      | Ile/ Ile | A*/B* | 1 (0.91) |
|         |      | Ile/ Ile | B*/B* | 0        |
| Present | null | Ile/Val  | A*/A* | 4 (3.64) |
|         |      | Ile/Val  | A*/B* | 4 (3.64) |
|         |      | Ile/Val  | B*/B* | 0        |
| Present | null | Val /Val | A*/A* | 0        |
|         |      | Val /Val | A*/B* | 0        |
|         |      | Val /Val | B*/B* | 0        |
| null    | Present | Ile/ Ile | A*/A* | 5 (4.55) |
|         |      | Ile/ Ile | A*/B* | 9 (8.18) |
|         |      | Ile/ Ile | B*/B* | 0        |
| null    | Present | Ile/Val  | A*/A* | 7 (6.36) |
|         |      | Ile/Val  | A*/B* | 5 (4.55) |
|         |      | Ile/Val  | B*/B* | 0        |
| null    | Present | Val /Val | A*/A* | 1 (0.91) |
|         |      | Val /Val | A*/B* | 0        |
|         |      | Val /Val | B*/B* | 0        |
| null    | null  | Ile/ Ile | A*/A* | 2 (1.82) |
|         |      | Ile/ Ile | A*/B* | 6 (5.45) |
|         |      | Ile/ Ile | B*/B* | 0        |
| null    | null  | Ile/Val  | A*/A* | 1 (0.91) |
|         |      | Ile/Val  | A*/B* | 2 (1.82) |
|         |      | Ile/Val  | B*/B* | 0        |
| null    | null  | Val /Val | A*/A* | 0        |
|         |      | Val /Val | A*/B* | 0        |
|         |      | Val /Val | B*/B* | 0        |
|         |      |          | Total | (100)    |

N: number of subjects.

## 4. Discussion

The current study investigates the polymorphism of GSTs genes among 110 healthy subjects from different areas of Baghdad city in Iraq and different ethnic groups from different areas of the world. The prevalence of individuals is not expressing the GSTM1 enzyme due to a homozygous gene deletion which is reported to be higher in Europeans and Asians when compared to Africans [17]. The results of Table 3 indicate that the frequencies of GSTM1 null genotype were close to Iranians [18], Japanese [19], and Cameroonians [20], whereas there was statistically differences with other population involved in Table 3 (Saudi [21], Bahrainis [22], Lebanese [21], Tunisians [22], Turkish [23], French [24], Italian [20], Spanish [20], Chinese) [25], Egyptians [26]. As for distribution of GSTT1 null genotype were near to Bahrainis [22], Egyptians [26], Turkish [23], Italian [20], Spanish [20], Iranians [18], but statistically differ from Saudi [21], Lebanese [21], Tunisians [22], French [24], Chinese [25], and Japanese [19] and Cameroonians [20]. Epidemiological studies have suggested that individual differences in the expression of allelic variants of the GSTP1 gene, which could lead to differences in sensitivity to various cancer-causing chemicals and differences in ability to metabolize anti-cancer agents [6]. Therefore, investigation of this polymorphism considered a predictor for some disease and will provide an indicator of response to chemotherapy. This is the first study of our knowledge to give frequency distribution genotypes of the GSTP1 gene in Iraqi population [Table 3]. When you see the frequency comparison results of GSTP1 val genotypes frequencies, we observed that GSTP1 val genotype was statistically different from all studies in Table 5 (Egyptian [26], Saudi
[27], south Tunisia [15], Center of Tunisia [28], North Tunisia [29], Italian [30], German [31], Spanish [32], French [33], Chinese [34], Japanese [35], African-Americans and Whites - Americans [36], Delhi [6], but Turkish [37]. As for studying the polymorphism of GSTA1 gene in Iraqi, we put the first step in this field. There are few studies about GSTA1 in the Arab world; in Tunisia [15] and in Egypt [38]. Coles and Kadlubar, [39] reported variant genotype of GSTA1 was associated with the decreased specific activity of GST. Therefore, it is reasonable to suppose that the variant genotype of this gene may be associated with the impairment of the health situation in the human and susceptible to some diseases [15]. In the current study the frequency distribution of GSTA1 B* allele was lower than all studies in Table 7 (Serbian [40], Czech Central European population [41], Netherlands [42], Italian [43], Caucasian [44], Brazilian [11], African–American [44], South Tunisian [15] except Chinese [45]. The reason to differences for distribution of GST genotypes in our population from other countries may belong to different sample size in addition to limited immigration flow from these countries. The study also studied the correlation of the polymorphism of GSTs genes among various factors. Our results are in agreement with Ben Slah et al. [15] who reported that the lack of any effect of gender in genotype distribution because none of these genes is located on the sex chromosomes. On the other hand, studies on the relationship between GSTM1 and GSTT1 gene polymorphisms and age groups were carried out, but their data were contradictory. We found there was no significant association between different age groups and distribution of GSTM1 and GSTT1 genotypes. These results were agreed with the previous report of Garte et al. [14] but differ from Santovito et al. [46] who observed that in north Iranian population a decreasing gradient of GSTs null genotypes between younger and older groups with the 80-100 age group. This contradictory among studies may be due to the difference in sample size, population, as well as the difference in the age range to be identified by individuals as an inclusion criterion. As for the relationship between polymorphism of GSTM1, GSTT1 and family history of cancer in our population, were no significant differences in frequencies of GSTM1 and GSTT1 genotypes regarding to family history of cancer. Gong et al. [47] reported up the growth and progression of cancer that can be attributed to unknown genetic or environmental factors which interact with GST genes to increase the risk of cancer. Some studies found that smokers with GST-null genotype had a higher risk of bladder cancer [48], diabetic patients [49] and colorectal cancer [50]. Our results demonstrated that GSTT1 null genotype was 100% in subjects consuming alcohol and was 24.07% in others not consuming alcohol. It cannot depend on this result because of the small numbers of individuals who consumed alcohol in this study due to the social customs and traditions. Recently, it was observed that polymorphisms of GSTT1 might play role in increasing the susceptibility to hepatocellular carcinoma among smokers [51]. The results in Table 4 showed that no significant association [p>0.05] of GSTP1 genotypes among gender, age, alcohol consumption, smoking, and family history of cancer. Also, we observed that heterozygous genotype of the GSTP1 gene [Ile/Val] in individuals who have a positive history of cancer [56.25%] was higher than others who don’t have a family history of cancer 42.55%. These results appointed to GSTP1 [Ile/Val] genotype may be associated with several types of cancer. However, in a study by Shang Xia et al. [52] on Hakka population, reported that smokers had higher distributions of variant GSTP1 Ile/Val 29.2% and Val/Val 8.3% compared to none-smokers had GSTP1 Ile/Val 28.1% and GSTP1 Val/Val 1.4%, suggested that the smokers might be at a higher risk of lung cancer [52].

As for the correlation between GSTA1 genotypes and some factors, this study is in the same line with Shang Xia et al. [52] and Pan et al. [53] who reported no significant associations between genetic polymorphisms of GSTA1 and each of smoking, drinking alcohol, different age groups, and gender. The present study shows that alcohol drinkers have higher frequencies of a heterozygous mutant of GSTA1 [A*/B+] genotypes 100% than the nondrinkers 40.7%. Drinking alcohol is known to be an important risk factor for many diseases. Oxidative metabolism of ethanol in alcohol produces acetaldehyde and various free radicals, causing hepatic damage [52]. The variant *B of GSTA1 gene might reduce the detoxification ability and increase individual susceptibility to related diseases. Some studies revealed that single GST gene polymorphism does not significantly increase risk of cancer

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whereas numerous studies have reported a relationship between the combination of GSTM1, GSTT1, and GSTP1 genotypes and the risk of different diseases, such as chronic lymphocytic leukemia, lung cancer, thyroid cancer, breast cancer and they suggested a possible synergistic effect between GST genotypes. Those results encouraged us to examine the distribution and the frequency of the possible combinations in our population, in order to give such information for the future epidemiological and clinical studies. To examine the effect of these combinations in drugs metabolism and cancer predisposition, the further largest group would be needed, since their frequencies are quite low.

5. Conclusion: The Present study showed the basic data for genotypes distribution and allele frequencies of GSTM1, GSTT1, GSTP1, and GSTA1 in Iraqi population. We suppose it opens a new prospect for further investigations by researchers in determining inter individual variation in genetic susceptibility to various diseases caused due to environmental gene, rather than depending on the results obtained from other populations.

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