EXPERIMENT : 3

DETERMINATION OF GSTM1 and GSTT1

GENERAL INFORMATION

A) Genetic Polymorphism

Polymorphism is made of *poly (multiple)* and *morphismos(form)* came from the Latin language. It means that "multiple form". Genetic polymorphism is defined as "The occurrence in the same population of two or more alleles at one locus, each with appreciable frequency".

Polymorphisms result from mutations. Mutations can be defined as the exchange of one nucleotide (single nucleotide polymorphism-SNP) or the addition or the deletion of nucleotide from the DNA alignment. The alignment of amino acids in proteins depends on the genetic information located in the genes. If there are different DNA formations of a gene, this gene is called as polymorphic. This different gene forms are called as **alleles**. If the alleles lead the different amino acid alignments of a protein, the new protein forms that are coded by alleles are called as **isozymes**. There are several molecular genetic mechanisms that cause polymorphisms on the metabolic enzymes. One of these mechanisms is **gene deletion**. In gene deletion, the gene that is coding the biotransformation enzyme is missing. The mutations of the amino acids that are not critical in protein alignment may alter or inhibit the enzyme activity. In table 1, the results of metabolic gene mutations are given.

Table 1. The results of gene mutations

Polymorphic gene	Result
In gene is absent	No metabolic activity
If there is function alteration	The alteration of substrate selectivity and increased or decreased activity
If gene is amplified	Increased metabolic activity

B) Glutathione and Glutathione S-Transferases

Glutathione is a tripeptide made of glycine, cysteine and glutamic acid. It is formed in liver then excreted by kidneys via bile or mercapturic acid after transformation. In the first stage of mercapturic acid formation, the nucleophilic sulphydryl group of glutathione reacts with the electrophilic carbon atom of substrate. Thus, a polar thioether structured conjugate forms. Glutathione S-transferases (GST) catalyze this conjugation reaction. In humans, GSTs can be found in lots of tissues and they are multiple functioned enzymes having large substrate selectivity. Because of this property, GSTs have the defence duty in humans exposed to the potential toxic chemicals. They functioned this detoxification property by a neutralization reaction of the thiol (-SH) groups of the reduced glutathione (GSH) with the electrophilic regions of the xenobiotics (chemicals causing oxidative stress, environmental pollutants and carcinogens) having electrophilic functional groups. The reaction product is the mercapturic acid (N-acetyl cysteine) which is very polar metabolite. It can be easily excreted with urine.

Cytosolic GSTs can be classified into 7 groups by their biochemical properties. These are; GSTA, GSTM, GSTP, GSTO, GSTS, GSTT and GSTZ. The similarity of the amino acid alignment

is more than 50% between the members of each group and less than 30% between the members of different groups. GSTs are responsible from the drug resistance because they play role in the inactivation of the antineoplastic drugs.

C) GST polymorphism

Nearly all of the GSTs have genetic polymorphism. The major type of polymorphism is SNPs and less is the gene deletion. The relation between GSTM1, GSTT1 and GSTP1 polymorphisms and the response for the chemotherapy of the patients with cancer are widely searched with lots of studies. In table 2, the important GST polymorphisms and the alterations in the enzyme activities following these polymorphisms are shown.

Table 2. Important GST polymorphisms and the alterations in the enzyme activities

Class	Chromosome	Subunit	Codon-nucleotide alteration	Enzyme activity
M	1q13	GSTM1	Deletion	-
T	22q11	GSTT1	Deletion	-
P	11q13	GSTP1	Ile105Val, Ala114Val	Decrease

GSTs can inactivate the several xenobiotics such as chemotherapeutic drugs, environmental carcinogens and endogenous molecules. The important substrates of the GSTM1, GSTT1 and GSTP1 enzymes are shown in Table 3.

The polymorphisms in GST can affect the sensitivity of the individuals to cancer and toxic chemicals also the drugs efficiency and toxicities. GSTM1 gene is 50% and GSTT1 gene is 15-20% missing in Caucasians. The individuals carrying GSTP1İle105Val mutant allele is about 45-50% and carrying GSTP1Ala114Val mutant allele is about 10-15%.

Table 3. The important substrates of the GSTM1, GSTT1 and GSTP1 enzymes

	Drugs	Other xenobiotics
GSTM1	1,3-bis(2-chloroethyl)-1-nitrous urea (BCNU), brostacilline, thiopurines, ethacrynic acid	PAHs, chemical epoxydes, aromatic nitrous compounds, the by products of reactive oxygen species
GSTT1	BCNU	Halomethanes, organic peroxides, the by products of reactive oxygen species
GSTP1	Brostacilline, cisplatine, chlorambucil, docetaxel, doxorubicin, etoposide, cyclophosphamide, iphosphamide, thiotepa, ethacrynic acid	PAHs, chemical epoxides and diol epoxides, aromatic nitrous compounds

A) Materials, devices and solutions used in the assay

For PCR

1) PCR main mixture: PCR buffer, $MgCl_2$, nucleotide mixture, *Taq* polymerase enzyme.

2) Primers: They are synthesized specifically for the amplified gene region

3) DNA solution

4) Thermal cycler

5) Micropipette

6) Centrifuge

7) PCR tubes

For Gel

1) Electrophoresis tank

2) Agarose gel

3) Electrophoresis buffer (TBE)

4) DNA marker

5) Electrophoresis power supply

6) Gel imaging system

B) Procedure

Polymerase chain reaction: After adding primers and DNA solution into the PCR main mixture, PCR cycle programme have to be programmed in the thermal cycler as: 2min. at 95^{o}C for first denaturation, 35 cycles of 2 min. at 94 ^{o}C (melting), 1 min. at 59 ^{o}C (annealing) and 2 min. at 72 ^{o}C (synthesis). At last 10 min. at 72 ^{o}C for a synthesis stage. During the running the gel is need to be prepared. After the run, the PCR products have to be applied to the gel.

1. Preparation of the gel: Prepare agarose at 1.5 % concentration in 40 ml of buffer. Add the weighed amount of the agarose into the 40 ml TBE buffer and then mix. Agarose must be in gel form so heat it. After heating, wait it to be cooled and lucid. Put the gel into the template and the put to comb in order to apply the PCR products easily. After the gel become frozen, fill the tank with the buffer solution.

2. Electrophoresis: After the application of PCR products into the gel, close the lid of the tank and connect to the power supply. Apply 100V electricity for 65 min. for electrophoresis. After the run, bring out the gel delicately.

3. To make the PCR products visible by nucleic acid gel dye: Incubate the gel with a help of low-speed shaker in a plate with diluted SYBR Green nucleic acid gel dye (Dilution ratio: 1/10000) for 30 minutes so that the lines of PCR products will be visible under UV light.

4. Gel imaging: Following the incubation, place the gel on the UV tray and take the photograph of the lines of the PCR products. Then compare these lines with the DNA marker in order to confirm whether the studied gene region is amplified or not.