Study of Glutathione-S-transferase (gstm1 and gstt1) Gene Polymorphisms in Down Syndrome Patients

Abstract

Background & aims: Down syndrome is the most common chromosomal abnormalities in chromosome number. Children with Down syndrome are often identified by symptoms such as severe growth and mental retardation and specific facial characteristics. The human glutathione S-transferases (GSTs) are a family of enzymes known to act as the defense systems for neutralize free radicals. These super family of enzymes, are components of metabolic phase II enzymes and play an important role in the immune system of body. The aim of this study was to examine whether an association exists between glutathione S-transferase GSTM1 and GSTT1 genes polymorphism and Down syndrome.

Material and methods: This case-control study conducted between the years 2013 to 2014 in whole of Iran. The study group consisted of 51 patients with Down syndrome and 51 healthy subjects as the control. DNA was extracted by salting out method from peripheral blood and multiplex polymerase chain reaction was performed following agarose gel electrophoresis to detect gstm1 and gstm1 null genotypes. Data were analyzed with SPSS v16 software.

Results: Our findings showed the deletion of both genes and for both groups, is equal to %1/96 or frequency of the presence and absence of these genes in populations of patients and controls group were similar.

Conclusion: It seems that there is no correlation between these two genes and Down syndrome.

Keywords: Down syndrome; Polymorphism; Glutathione-S-Transferase - T1 and M1

Introduction

Downs syndrome (DS), also known as Trisomy 21 is the commonest of congenital anomalies occurring 1 in 800 live births [1]. It is known as one of the most common chromosomal abnormalities. Down syndrome is often the result of lack of proper segregation of chromosomes number 21 during meiosis or in the less frequently in the mitotic phase of the egg cell. By examining artifacts from the Tumaco-La Tolita culture, which existed on the border between current Colombia and Ecuador approximately 2500 years ago [2]. Suspected that certain figurines depicted individuals with Trisomy 21, making these potteries the earliest evidence for the syndrome [3]. Existence of the syndrome is characterized by dysmorphic facies. The incidence of Down’s syndrome increases as the age of mother increases. The syndrome was first described by Dr. John Langdon Down in 1866 [4]. The human GSTs are a family of enzymes known to act in the body as the defense systems for neutralize free radicals [5]. These protein family members are in the form of dimer [6].

GSTs, a superfamily of dimeric phase II metabolic enzymes (molecular mass 17-28 KD), play an important role in the cellular defense system. GST enzymes catalyze the conjugation of toxic and carcinogenic electrophilic molecules with glutathione and thereby protect cellular macromolecules from damage [4]. The loci encoding the GST enzymes located on at least seven chromosomes. This multigene family divided in seven families (Alpha, Mu, Pi, Theta, Sigma, Zeta, and Omega) with functions ranging from detoxification to biosynthesis and cell signaling. Many of the GST genes are polymorphic, therefore, there has been substantial interest in studying the associations between particular allelic variants with altered risk of a variety of diseases. Several GST polymorphisms have been associated with an increased or decreased susceptibility to several diseases. Two of the important members of the GST family, named glutathione-s-transferase mu 1 (gstm1) and glutathione-s-transferase theta 1 (gstt1) have polymorphic homozogous deletion or null genotypes. Persons with homozogous deletions of either the gstm1 or the gstt1 locus have no enzymatic functional activity of the respective enzyme. This has been confirmed by phenotype assays that have demonstrated 94% or greater concordance between phenotype and genotype [7]. The gstm1 locus has been mapped on chromosome 1p13.3, while the gstt1 locus exists on chromosome 22q11.2 [8].

Materials and Methods

In this case-control study conducted between the years 2013 to 2014, Down syndrome patients were selected all over Iran with male gender. Among patients with Down syndrome, 51 patients were selected who were 10 to 25 years old and 51 healthy children aged 12-27 years were selected randomly in 2014. Written informed consent was obtained from the patients’ parents and controllers for the publication of this report and
any accompanying. The criteria of Down syndrome were based on phenotype examination by physician (based on the WHO indexes) and patients karyotypes. The research was carried out in compliance with the WMA Declaration of Helsinki and was approved by the Ethical Committee of Islamic Azad University of Borujerd, Lorestan, Iran. To examine GSTT1 and GSTM1 gene deletion in patients, a sample of 5 ml peripheral blood was taken in tubes and DNA was extracted by salting out method. Molecular examination performed by multiplex PCR using 3 sets of primer pairs for GSTT1, GSTM1 and ß globin gene as internal control (Table 1).

Table 1: Primer sequences for GST multiplex PCR.

| Primer         | Sequencing                                      |
|----------------|-------------------------------------------------|
| GSTM1 Forward  | 5'-GAA CTC CTC GAA AAG CTA AAG C-3'             |
| GSTM1 Reverse  | 5'-GTT GGG CTC AAA TAT AGG GTG G-3'             |
| GST T1 Forward | 5'-TTC CTT ACT GGT CCT CAC ACT TC-3'            |
| GST T1 Reverse | 5'-TCA CCG GAT CAT GGC CAG CA-3'               |
| ß-globin Forward | 5'-CAA CTT CAT CCA GTG TCA GC-3'               |
| ß-globin Reverse | 5'-GAA GAG CCA AGG ACA GGT AC-3'              |

A total of 100 ng of genomic DNA was used for PCR amplification, in 30 µL of reaction mixture that contained 2 mM MgCl₂ (Sigmaaldrich-USA) and 12.5 pM each of the forward and reverse primers (Genfanavaran-Iran) and 0.5 U Taq DNA polymerase (Kawsar-Iran) (Table 2). The PCR condition was one cycle of 94°C for 5 minutes followed by 35 cycles of 94°C, 59°C, and 72°C for 1 min each (FlexCycler-Germany) (Table 3). The PCR products were visualized using 1/5% agarose gel electrophoresis (Merck-Germany) in the electric current is 100 volts and amps 1 MA for 55 minute. DNA bands for GSTM1 and GSTT1 gene sequence of 480 and 215 base pairs in length, and DNA fragments gene amplification of derived from β-globin gene 268 base pairs long. Negative examples, GSTM1 and GSTT1 genes lack either separately or together in the presence of B-Globin gene null genotype for each is indicated. In positive samples of each gene separately or together in the presence of B-Globin gene expression of wild genotype. In Fig. 1, M represents a Ladder or molecular marker-fermentase 100bp, column PC is positive control, column NC is negative control and lanes 1-3 are patients Multiplex PCR samples (Figure 1). Using the chi-square test showed no significant relationship between the variables. The removal rates for both genes and for both groups, equal 1/96 percent (1 of 51), respectively. Fisher’s exact test for both genes had the same results with the P-Value of 1 indicates that there is no a significant association between the absence or presence of genes and Down syndrome. Checking for receiver operating characteristic (ROC) curve for both the gene and the same cannot be said that these genes can be diagnostic for the disease, Down syndrome (Table 4).

Result

From 51 Down syndrome patients and 51 healthy children as control group that involved in this study, the GSTT1 and GSTM1 gene deletion in the patients group and controls was identical. DNA fragments amplification GSTM1 and GSTT1 gene sequence of 480 and 215 base pairs in length, and DNA fragments gene amplification of derived from B-globin 268 base pairs long. Negative examples, GSTM1 and GSTT1 genes lack either separately or together in the presence of B-Globin gene null genotype for each is indicated. In positive samples of each gene separately or together in the presence of B-Globin gene expression of wild genotype. In Fig. 1, M represents a Ladder or molecular marker-fermentase 100bp, column PC is positive control, column NC is negative control and lanes 1-3 are patients Multiplex PCR samples (Figure 1). Using the chi-square test showed no significant relationship between the variables. The removal rates for both genes and for both groups, equal 1/96 percent (1 of 51), respectively. Fisher’s exact test for both genes had the same results with the P-Value of 1 indicates that there is no a significant association between the absence or presence of genes and Down syndrome. Checking for receiver operating characteristic (ROC) curve for both the gene and the same cannot be said that these genes can be diagnostic for the disease, Down syndrome (Table 4).

Table 3: PCR program.

| Reaction Components | For a Total Volume of 25 µl |
|---------------------|---------------------------|
| Sterilzed ddH₂O      | -                         |
| PCR Buffer           | 10 X                      |
| MgCl₂                | 50 mM                     |
| dNTP                 | 10 mM                     |
| Primers              | 5 µM                      |
| Taq Polymerase       | 1.6 µg/µl                 |

Figure 1: Gel electrophoresis (%1/5 Agarose) showing Multiplex PCR products.
Discussion

Fifty years ago, Lejeune et al. [9] discovered that DS results from the presence of an additional Chromosome 21. A common defect present in about 1 in 700 liveborn children, it is the most frequent cause of mental retardation and a recognized genetic etiology of Alzheimer disease (AD) [9]. Down’s syndrome constitutes one of the most common chromosomal disorders [10]. Down syndrome is the leading chromosomal defect in the United States and has a national estimated prevalence of 13.65 per 10,000 live births [11]. The glutathione-S-transferase gene family encodes genes that are critical for certain life processes, as well as for detoxification and toxification mechanisms, via conjugation of reduced glutathione (GSH) with numerous substrates such as pharmaceuticals and environmental pollutants [12]. GSTs are dimeric, mainly cytosolic enzymes that have extensive ligand binding properties in addition to their catalytic role in detoxification [13,14]. The glutathione-S-transferase gene family encodes genes that are critical for certain life processes, as well as for detoxification and toxification mechanisms, via conjugation of reduced glutathione (GSH) with numerous substrates such as pharmaceuticals and environmental pollutants [12]. GSTs are dimeric, mainly cytosolic enzymes that have extensive ligand binding properties in addition to their catalytic role in detoxification [13,14]. The glutathione-S-transferase gene family encodes genes that are critical for certain life processes, as well as for detoxification and toxification mechanisms, via conjugation of reduced glutathione (GSH) with numerous substrates such as pharmaceuticals and environmental pollutants [12]. GSTs are dimeric, mainly cytosolic enzymes that have extensive ligand binding properties in addition to their catalytic role in detoxification [13,14]. The glutathione-S-transferase gene family encodes genes that are critical for certain life processes, as well as for detoxification and toxification mechanisms, via conjugation of reduced glutathione (GSH) with numerous substrates such as pharmaceuticals and environmental pollutants [12]. GSTs are dimeric, mainly cytosolic enzymes that have extensive ligand binding properties in addition to their catalytic role in detoxification [13,14].

Table 4: The relationship between genotypes gstm1 and gstt1 and the risk of suffering from Down syndrome.

| gstm1 & gstt1 Combined | Control | Cases (Down Syndrome) | OR (95%CI) |
|------------------------|---------|-----------------------|------------|
| Both Present           | 49 (96.08) | 49 (96.08)           | 1 (reference) |
| Either One Null        | 50 (98.04) | 50 (98.04)           | (0.573 to 1/746) |

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