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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 in the body 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 gstt1 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

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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 Downs'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 17-28KD), 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 homozygous deletion or null genotypes. Persons with homozygous 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 25years old and 51 healthy children aged 12-27years were selected randomly in 2014. Written informed consent was obtained from the patients' parents and controles 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 5ml peripheral blood was taken in tubes and DNA was extracted by salting out method. Molecular examination preformed by multiplex PCR using 3 sets of primer pairs for *gstt1*, *gstm1* and β *globin* gene as internal control (Table 1).

A total of 100 ng of genomic DNA was used for PCR amplification, in 30µL of reaction mixture that contained 2mM $MgCl_2$ (Sigmaaldrich-USA) and 12.5pM each of the forward and reverse primers (Genfanavaran-Iran) and 0.5UTaq DNA polymerase (Kawsar-Iran) (Table 2). The PCR condition was one cycle of 94°C for 5minutes followed by 35 cycles of 94°C, 59°C, and 72°C for 1min each (FlexCycler-Germany) (Table 3). The PCR products were visualized using 1/5% agarose gel electrophoresis (Merck-Germany) in the electric current is 100volts and amps 1 MA for 55minute. DNA bands for *gstm1*, *gstt1*, and β globin alleles were 219bp, 480bp, and 268bp, respectively. The absence of bands for *gstm1* or *gstt1* in the presence of β *globin* PCR product indicates null genotype for each (Figure 1). In order to determine the optimum temperature connection, the online software Tm Calculator was used. The color used for detection of bands was GelRed (Sinacolon-Iran) that the staining was performed Pre-cast. Samples positive for all three PCR products were considered wild-type. The data were analyzed by SPSS v.16 software and Chi-Square test.

Results

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 Figure 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 1 Primer sequences for GST multiplex PCR

Primer	Sequensing
GSTM1 Forward	F: 5'-GAA CTC CCT GAA AAG CTA AAG C-3'
GSTM1 Reverse	R:5'-GTT GGG CTC AAA TAT ACG GTG G-3'
GST T1 Forward	F:5'-TTC CTT ACT GGT CCT CAC ATC TC-3'
GST T1 Reverse	R:5'-TCA CCG GAT CAT GGC CAG CA-3'
β-globin Forward	F:5'-CAA CTT CAT CCA CGT TCA CC-3'
β-globin Reverse	R:5'-GAA GAG CCA AGG ACA GGT AC-3'

Table 2 Volume and concentration needed to multiplex PCR

Reaction components	Stoke concentration	For a total volume of 25 µl
Steriled ddH_2O	-	15µl
PCR Buffer	10X	2/5µl
MgCl2	50mM	1/5µl
dNTP	10mM	0 /5µl
Primers	10µM	6×0 /5µl
Taq Polymerase	5µl/U	0.5µl
DNA	1.6µg/ml	2µl

Table 3 PCR program

Pre Denaturation	4 minute	94°C
Denaturation	1 minute	94°C
Annealing	1 minute	59°C
Polymerization	70 Second	72°C
Final Polymerization	5 minute	72°C

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Table 4 The relationship between genotypes gstt1 and gstm1 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)

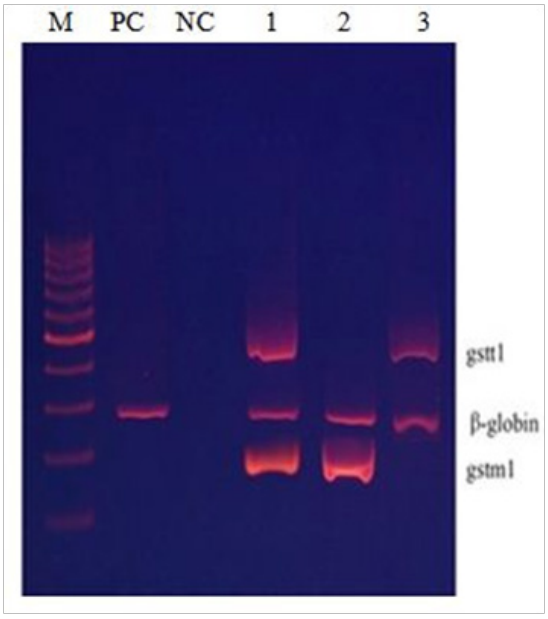

Figure 1 Gel electrophoresis (%1/5 Agarose) showing Multiplex PCR products.

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] Downs syndrome constitutes to be the most common chromosomal disorder.[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 detoxication 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 are a group of enzymes that add sulfur molecules (as glutathione) to a wide range of acceptor molecules.

Xenobiotic acceptors include halogenated and nitro compounds, organophosphates (including pesticides), alkylating agents, epoxides, and polycyclic aromatic hydrocarbons.[15,16] So far several studies have been done in the field of relationship between polymorphism glutathion-S- transferase gene family with various diseases for example studies like: Haghirasadat et al in 2013 showed that a mutation or inherited deletion of gstt1 and gstm1 genes or removal of at least one gene in particular gstm1 gene increases lung cancer risk.[17] Dehghani and his colleague's identified the lack of enzymatic activity associated with gstm1 null genotype caused a significant decrease in sperm count but gstt1 null genotype dose not display effect on sperm indexes.[18] Alidoust and his colleagues found there is no connection between gene mutation Glutathion S -Transfrase and autoimmune hepatitis type 1.[19] This lack of correlation is consistent with our present study. Studies of Cilensek et al.,[20] showed that homozygous individuals for the deletion of gstt1 two times more likely to develop diabetes type 2.[20]

In 2007 Mirfeizollahi et al.,[21] gene polymorphisms Glutathion-S-transferase isoenzymes m1, p1 and Glutathion-S-transferase enzyme activity examined in Iranian infertile men. The results showed a lack of enzymatic activity associated with the genotype null *gstm1* has no effect on sperm parameters and the rate of enzymatic activity.[21] Davies et al.,[22] stated that GSTM1 genotype null is a major risk factor for developing Myeloid Leukemia in the childhood.[22] Helzlsouer et al.,[23] in their research entitled association between glutathione-S-Transferase *M1*, *P1*, and *T1* Genetic Polymorphisms and Development of Breast Cancer which was conducted in 1998 stated that genetic variation in the member gene of GST family is associated with increased susceptibility to breast cancer.[23] Although these studies report association between Down's syndrome and cancer and relationship of cancer with gene mutations glutathion-S-transferase *m1* and *t1* but there was no report on the relationship Glutathion S- transferase gene mutations *m1* and *t1* with Down syndrome in previous studies. In our study, we didn't find evidence that indicates the relationship between genotype and allelic polymorphisms of Glutathion -S-transferase gene *m1* and *t1* with Down syndrome. The results obtained can be explained in several forms: It is believed that Down syndrome has multiple etiologies and genes involved in the disease among populations have differences in allele and genotype level. Gstm1 and gstt1 may be the result of genes involved in susceptibility to disease and Down syndrome in our society is different from other communities. It is possible that Geographic and climatic conditions have effect on polymorphism. It is likely that glutathione-S-transferase alone has not shown it is the cause of this syndrome. But also as an auxiliary or supplementary factor contributing to the occurrence of this syndrome. Who had never been in the field of molecular theory. The low number of patients in this study cannot be ineffective, therefore the most number of patients in this area is recommended.

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Conflict of interest

The author declares no conflict of interest.

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