Research Article

Analysis of Glutathione S-Transferase M1 and T1 Polymorphism in Samples of Iraqi Children with Autism

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Date Submitted: 5/2/2017
Date Accepted: 23/8/2017

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Abstract

Aims: Is to find relationship between autism and absence of Glutathione-S-transferase (GSTs) genes GSTT1 and GSTM1, which are responsible for detoxification from the body. **Methods:** The present study includes the collection of (sixty) Iraqi children with a psychiatrist autism, the range of their age was 2-10 years. All blood samples were subjected to multiplex polymerase chain reaction (PCR) technique in laboratories of college of science (for Woman) Baghdad University for detection of the presence or absence of the gene encode for glutathione protein, and determination of the level of mercury and lead in blood samples. **Results:** The results of the study showed that the male: female ratio in autistic children is (5.7:1). The percentage of children who have (GSTM1-null) genotype was 55.9% higher than the children 44.0% with (GSTM1-positive) genotype while the frequency of (GSTT1-null) genotype (11.8%) was lower than that of (GSTT1-positive) genotype (88.1%) in the tested cohort. The numbers of parents who have a positive GSTM1 genotype are lower than other groups while the number of parents who have a positive GSTT1 is higher than in other groups. The present study reveals that the presence of high concentrations of mercury in the blood samples for all children with autism compared with their families and with the normal value. **Conclusions:** It is concluded from the present study that the deletion or absence of GSTM1 genotype in autistic children is higher than presence. This indicates that GSTM1-null genotype is related to the ASD but not as a risk factor for autism. The high frequency of GSTT1 positive genotype in autistic children and their parents is an indicator for a relationship between them. Further studies with large size of samples is needed to detect the activity of oxidative stress effect of enzymes encoded by alleles of GSTT1 genes.

Key words: *Genetic Polymorphism, Autism*

INTRODUCTION

Autism spectrum disorders (ASDs) are heterogeneous neurodevelopmental disorders, characterized by social impairments, communication difficulties, and restricted pattern of behavior.[1] The causes of autism remain unknown but it is likely multifactorial arising from the interaction of biological, genetic, and environmental factors. The genetic factors. Like gene mutations, gene deletions and copy number variants (CNVs) are all linked to autism.[2] Early environmental exposure to heavy metals, pesticides, and childhood vaccines, that they may act in concert with genetic vulnerability may account for autism.[3,4] It was hypothesized that variation in the interplay between different environmental exposure and inherited susceptibilities may account for the observed heterogeneity in the autism phenotype.

The prevalence rates of autism were estimated by the Center for Disease Control and Prevention it was 0.2% (20 per 10000) children in the United States.[5] It was 0.14% (14.8 per 10000) children in Asia.[6]

There has been a great interest of genes which encode for enzymes that metabolize xenobiotics it is also relevant to add here few lines about oxidative stress and antioxidants and mutating the activity of healthy normal glutathione. Among the numerous GST genes, the GSTM1 and GSTT1genes have been extensively studied because of the high prevalence of homozygous deletions GSTM1 and GSTT1that were associated with the loss of enzyme activity and consequent with a

decreased ability to detoxify mutagens and carcinogenic compounds resulting in increased vulnerability to genetic, and various health problems.[7,8]

The autism spectrum disorders (ASDs) includes five types of disorders.

1-Typical autism

2-Asperger syndrome

3- Retts Syndrome

4- Childhood disintegrative disorder

5-Pervasive developmental disorder, not otherwise specified(PDD-NOS).

Autism occurs in two patterns; Pattern I which is the simplest and sporadically spread form and Pattern II which is complicated and familial the inherited form.[9]

PATIENTS AND METHODS

1. Patients Collection

The blood samples of autistic children and their families were collected during the period June - July 2013. A total of 5 ml venous blood. was aspirated from each individual in a tube containing ethylenediamine tetraacetic acid (EDTA). The samples were brought to the laboratory in a well-insulated ice box. Each sample was divided into two tubes, one of them for DNA extraction and the other for determination of the serum level of mercury and lead.

2. Genotyping Analysis

2.1 Genomic DNA Extraction from Frozen Blood (Promega DNA Wizard):

A. Principle

The Wizard genomic DNA purification kit (Promega inc., USA) method was used for rapid and DNA isolation from the whole blood cells. The four- step-process was performed according to the procedure of [10].

B. Protocol

DNA extraction was conducted under aseptic conditions and according to the protocol of Wizard Genomic DNA purification kit (Promega Inc. USA) which is supplied by Promega and consisted of the following steps:

1. Cell lysis solution in a volume of 900 µl was added to a sterile 1.5ml microcentrifuge tube. The tube of blood was gently rocked during process of mixing, then 300µl of blood sample transferred to the tube containing the cell lysis solution and the tube was inverted for 5-6 times for mixing.

2. The mixture was incubated for 10 minutes at room temperature (inverted 2-3 times once during the incubation) to lyse the red blood cells. The preparation was centrifuged at 14000rpm for 20 seconds at room temperature.

3. The preparation was removed and as much supernatant as possible was discarded without disturbing the visible white pellet. Approximately 10-20 µl of residual liquid would remain in the 1.5ml tube.

4. The tube was vortexed vigorously until the white blood cells were resuspended (10-15 second).

5. Three hundred microliter of Nuclei lysis solution was added to the tube containing the resuspended cells. The solution was pipetted 5-6 times to lyses the white blood cells. The solution should become very viscous. If clumps of cells were still visible after mixing, the solution was incubated at 37°C until the clumps were disrupted.

6. Protein precipitation solution (100µl for 300µl sample volume) was added to the nuclear lysate and vortexed vigorously for 10-20 seconds. Small protein clumps might be visible after vortexing.

7. The preparation was centrifuged at 14000rpm for 3 minutes at room temperature.

8. The supernatant was transferred to a new 1.5ml microcentrifuge tube containing 300µl of isopropanol at room temperature. This solution was gently mixed until the white thread-like strands of DNA form a visible small white pellet. The preparation was centrifuged at 14000rpm for 1 minute at room temperature.

9. The supernatant was decanted. One sample volume of room temperature 70% ethanol was added to the DNA and the tube was gently inverted several times to wash DNA pellet and the sides of microcentrifuge tube. The tube was centrifuged for 1 min at 14000rpm at room temperature.

10. The ethanol was carefully aspirated by using a pipette tip. The DNA pellet was very loose at this point and care was used to avoid aspirating the pellet into the pipette. The tube was inverted on the clean absorbent paper and the pellet was air-dried for 10-15 min.

11. DNA rehydration solution (100µl for 300µl sample volume) was added to the tube to rehydrat the DNA by incubating at 65°C for 1 hour. The solution was periodically mixed by gently tapping the tube. Alternatively, the DNA was rehydrated by incubating the solution overnight at 4°C.

12. DNA was stored at -20°C.

2.2. Determination of DNA Concentration [11]:

The DNA concentration was determined by two methods:

I- Spectrophotometer Determination:

Five microliters of the DNA solution were diluted with 495µl distilled water. The optical density was determined at 260nm by UV spectrophotometer using distilled water as a reference. The DNA concentration was calculated as the following formula:

DNA conc. µg/ml= O.D 260 nm x Dilution factor x 50

The purity and concentration of the DNA is of decisive important for optimal test results, the purity of DNA was estimated by the ratio of A 260/A 280. Pure preparation of DNA had a ratio of 1.8 or higher. Usually, the ratio below 1.6 indicates the contamination of the DNA by protein and should be re-purified.

II- Agarose Gel Electrophoresis:

The DNA integrity was determined by agarose gel electrophoresis by three steps:

1. Agarose Gel Preparation

Agarose gel 0.08% was prepared by dissolving o.32gm of agarose powder in 40ml of 1xTBE buffer. This solution was heated to boiling on the hotplate for few minutes until the gel particles were dissolved. The agarose solution was allowed to cool to below 60C and then poured onto precleaned-plastic tray placed on plastic stand. The comb was positioned for about 1cm from the top edge of the tray. After hardening (about 30 min at room temperature), the comb was removed from agarose gel leaving wells.

2. Sample Preparation

To prepare the sample for electrophoresis, 10 µl of DNA sample was mixed with 3 µl of loading dye.

3. Agarose Gel Electrophoresis

The gel mold was placed horizontally onto the electrophoresis tank, which was filled with 1xTBE so that the gel pockets were completely covered with buffer about few milliliters above the surface of agarose. Each well was carefully loaded with 13 µl of sample. The tank was closed and electrophoresis was run at 7vol/cm of the gel until the loading dye was near the end of the gel. The size of DNA fragment was determined by running 100bp DNA ladder with DNA samples. The gel was then stained with ethidium bromide by immersing in distilled water containing the dye in a final concentration of 0.5g/ml for 30-45min. The gel was then transferred to distilled water to remove the dye excess. After that, the DNA bands were visualized by UV transilluminator system and photographed.

2.3- Multiplex Polymerase Chain Reaction:

A. Principle:

Multiplex polymerase chain reaction (PCR) is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction. It consists of multiple Primers sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. Multiplex assays can be tedious and time-consuming to establish, this technique often requires extensive optimization procedures because Primer-dimers and other nonspecific products may interfere with the amplification of specific products [12]. The multiplex PCR protocol was used to simultaneously detect the presence or absence of GSTM1 and GSTT1genes. Although this assay does not distinguish between heterozygous and homozygous positive genotypes, they conclusively identify the null genotypes (the genes are completely deleted) [13].

Primers Selection:

According to the (14), the forward and reverse primers were selected for detecting 480bp, 215bp and 350bp fragments of the genes GSTT1, GSTM1and albumin genes, respectively. Lyophilized forward and reverse primers were suspended with deionized water to reach a final concentration of 30 picomols/µl of suspension. The primers sequences are listed in (Table 1).

Table 1: The sequence of forward and reverse primers used for PCR amplification of GSTM1 & GSTT1 Genes.

Primers	Primer sequences	Length	Tm	Volume for 100 picomles/µl
GSTM1	F- 5-GAA CTC CCT GAA AAG CTA AAG C -3	22	53.2	137.4 µl
	R- 5-GTT GGG CTC AAA TAT ACG GTG G -3	22	57.0	138.0 µl
GSTT1	F- 5-TTC CTT ACT GGT CCT CAC ATC TC -3	23	53.7	151.4 µl
	R- 5-TCA CCG GAT CAT GGC CAG CA -3	20	64.0	105.0 µl
Albumin	F- 5-GCC CTC TGC TAA CAA GTC CTA C-3	22	53.7	151.0 µl
	R-5-GCC CTA AAA AGA AAA TCG CCA ATC-3	24	60.0	124.3 µl

Tm: Melting Temperature

B. PCR Working Solution:

Optimization of polymerase chain reaction was accomplished after several trials. The following mixture of volume was adopted (Table 2).

Table 2: The mixture of working solution.

Working Solution	
Go Taq Green Master Mix	25 µl
Primer Forward	1 µl of each primer
Primer Reverse	1 µl of each primer
Distilled water (D.w)	14 µl
DNA template	5 µl
Final volume 50µl	

C. PCR Program:

Table 3: PCR program that was applied in the thermocycler.

Sr No.	Steps	Temperature	Time	No. of Cycles
I	Denaturation 1	95°C	3 min	1
II	Denaturation 2	94°C	1min	
III	Annealing	59°C	1min	30
IV	Extension 1	72°C	1min	
V	Extension 2	72°C	5 min	1

The PCR products for both genes GSTM1 and GSTT1 were then electrophoresed on 2% agarose gel stained with ethidium bromide. The presence of bands of 480 and 215 bps was indicative of the GSTT1, GSTM1 genotypes; whereas the absence indicated the null genotypes for the gene. Albumin, indicated by a 350 bp product, was used as an internal positive control.

2-3-Determination Level of Mercury in Blood:

The blood samples of blood were analyzed in AAS device to determine the level of mercury in the blood of children with autism and their families. This test performed in the laboratories of Ministry of Science and Technology, Department of Materials Research.

RESULTS

Analysis for GSTM1 Genes in Autistic Children and Their Families. The parents of Autistic children were classified into 5 groups according to the combination of GSTM1 genotypes. The group was classified into subgroups according to the number of children in each family.

Group 1: There are seventeen families in this group. The fathers have a null GSTM1 genotype and the mothers have a positive GSTM1 genotype. There are eighteen autistic children in this group (sixteen males and two females). Ten of them have a positive GSTM1 genotype and the other eight have a null GSTM genotype. This group is classified into:

Subgroup 1: Families have one child, eight autistic males have a positive GSTM1genotype, five autistic males have a null GSTM1 genotype and one autistic female has a positive GSTM1 genotype, no autistic female has a null genotype was recorded.

Subgroup 2: One family has two children: The first child is autistic female, her age is three years and has a positive GSTM1 genotype, the second child is non-autistic male, his age was one year and seven months, he has a null GSTM1genotype.

Subgroup 3: One family has a twin: Both of them are autistic and have a null GSTM1 genotype, their age is two years and seven months.

Subgroup 4: Family has three children: The first child is non-autistic female whose age is nine years, she has a null genotype. The second child is non-autistic female whose age is eight years and she has a null genotype. The third child is autistic male. His age was four years and 3 months, he has a null genotype.

Group 2: There are fourteen families in this group. The fathers have a positive GSTM1 genotype and the

mothers have a null GSTM1 genotype. There are seventeen autistic children in this group (fourteen males and three females) seven of them have a positive GSTM1 genotype and nine of them have a null GSTM1 genotype. The genotype of one child was not determined.

Subgroup 1: Families have one child. Two autistic males have a positive GSTM1 genotype, six autistic males have a null GSTM1 genotype, one autistic female has a null GSTM1 genotype, no autistic female has a positive GSTM1 genotype was recorded.

Subgroup 2: Two families have two children. In one family, the first child is non-autistic female whose age is four years and five months; she has a positive GSTM1 genotype. The second child is autistic male, his age was three years, he has a positive GSTM1 genotype. Another family, the first child is non-autistic female whose age is seven years, and she has a positive GSTM1 genotype. The second child is autistic male, whose age is four years and four months, he has a positive GSTM1 genotype.

Subgroup 3: Nil

Subgroup 4: One family has three children, all of them autistic, the first child is a male, whose age is ten years, his genotype was not determined. The second child is a female, whose age is four years and ten months, she has a null GSTM1 genotype. The third is a male, whose age is three years and three months, he has a positive GSTM1 genotype.

The second family has three children, the first child is an autistic male whose age is three years and nine months, he has a positive GSTM1 genotype. The second child is non-autistic male whose age is two years and seven months, he has a positive GSTM1 genotype. The third child is not characterized as autistic yet, his age is one year and five months, he has a null GSTM1genotype.

The third family has three children. The first child is autistic female, whose age is four years and one month, she has a null GSTM1 genotype. The second child is an autistic male, whose age is three years, he has a positive genotype. The third child is not autistic, his age was eight months, he has a positive GSTM1 genotype.

Group 3: There are fourteen families in this group, both of the parents have a null GSTM1 genotype. There are fifteen autistic children in this group.

Subgroup 1: The families have one child. There are ten autistic children in this subgroup, eight of them were males, they have a null genotype and two of them were females, they have a null genotype.

Subgroup 2: One family has two children both of them have a null genotype, the first child is autistic male, his age is three years. The second child is non-autistic male, whose age is one year.

The second family has two children both of them have a null genotype. The first child is non-autistic male whose age is four years and five months, the second child is an autistic male whose age is three years and five months.

Subgroup 3: two families have a twin, both of them have a null genotype, the first family has one autistic male child and the other child is an autistic female, their age is three years and eight months. The second family has one autistic male child and the other child is non-autistic male, their age is four years and two months.

Group 4: There are nine families in this group. Both parents have a positive GSTM1 genotype, there are nine autistic children in this group, eight of them have a positive GSTM1 genotype and only one autistic child has a null GSTM1 genotype, this group is classified into:

Subgroup 1: Families have one child: Seven autistic males have a positive genotype, one autistic male has a null genotype, one autistic female has a positive genotype, no autistic female has a null genotype was recorded.

There is only one child who has a null genotype, so the genotype of both parents in one family is heterozygous. The homozygous or heterozygous genotypes of the other parent cannot be determined depending on one child with a positive GSTM1 genotype.

Group 5: The genotype for a dead father is unknown, the mother has a positive GSTM1. The child is a male and he has a positive GSTM1 genotype.

Analysis for GSTT1 Genotypes in Autistic Children and Their Families

The parents of autistic children were classified into three groups regarding the combination of their GSTT1 genotype. The frequency of group (2) was the highest (53). There is only one child in the first group and third group and second group (57). These groups are.

Group 1: The father has a null and the mother has a positive GSTT1 genotype.

Subgroup 1: one autistic male has a null genotype.

Group 2: The parents of (57) children have a positive GSTM1 for both of them, this group is classified into:

Subgroup 1: The family has one child (31) single autistic males have a positive genotype, five single autistic males have a null genotype, five single autistic females have a null genotype, no single autistic female has a positive genotype was recorded.

Subgroup 2: Families have two children. Two families have first child is non-autistic female, she has a positive genotype. The second child is an autistic male who he has a positive genotype.

The age of the girl in the first family is four years and five months, and the boy is three years. In the second families, the girl is seven years and the boy is four years and four months

One family has two children, the first child is an autistic female. Her age is three years and she has a positive genotype. The second child is a non- autistic male. His age is one year and seven months, he has a positive genotype.

One family has (2) children, both of them have a positive genotype. The first child is an autistic male whose age is three years. The second child is non–autistic male whose age is one year, and one family has (2) children, the first child is a non-autistic male whose age is four years and five months, he has a positive genotype, the second child is an autistic male, his age is three years and five months, he has a null genotype.

Subgroup 3: Two families have twins, both of them are males. Their genotypes are positive GSTT1.The age of the first twin is two years and seven months, both of them is autistic. In second twin, one child is autistic and the other child is non-autistic. There is four years and two months.

One family has a twin both of them have a positive genotype, one child is an autistic male, the second child is an autistic female, their age is three years and eight months.

Subgroup 4: The first family has 3 children, all of them are autistic, one child is a male whose age is ten years, his genotype was not determined. The second child is a female, her age was four years and ten months, she has a positive genotype. The third is a male whose age is three years and three months, he has a positive genotype.

The second family has 3children, all of them have a positive genotype, the first child is a non-autistic female whose age is nine years, the second child is a non-autistic female whose age is eight years, the third child is an autistic male whose age is four years and three months.

The third family has 3children; all of them have a positive genotype. The first child is an autistic female whose age is four years and one month, the second child is an autistic male whose age is three years, the third child is a non-autistic male whose age is eight months.

The fourth family has 3 children, all of them have a positive genotype, the first child is an autistic male whose age is three years and nine months. The second child is a non-autistic male, whose age is two years and seven months. The third child is a non-autistic male whose age is one year and five months.

Group 3: GSTT1 genotype for, the dead father is unknown, the mother has a positive GSTT1 genotype. The child is a male who has a positive GSTT1 genotype.

The Levels of Mercury in Blood of autistic children and their families.

Tables show data for the range and the means of concentration of mercury in blood of autistic children, sibling and their parents. The autistic children were divided into two groups:

Group I: The range was 16-37 (ng/ml) in 34 autistic children. The mean of mercury concentration was 25.03. The frequency of children who had the GSTM1-positive genotype was 16(32%), GSTM1-null 17(34%) children.

The frequency of GSTT1-positive genotype was 29(58%) which was higher than that of GSTT1- null genotype 4(8%).

Group II: The range was 38-82 (ng/ml) in 16 autistic children. The mean of mercury concentration was 50.8. The frequency of positive GSTM1 genotype is 8(16%), null-GSTM1 genotype 9(18%).

The frequency of positive GSTT1genotype is 14(28%) higher than frequency of null-GSTT1 genotype 3(6%).

In sibling, the range was1.3-4 (ng/ml) in 12 children. The mean of mercury concentration was 3.3

The parents were divided into two groups:

Group I: The range was 1.1-3.1(ng/ml), mean was 2.11.

Group II: The range was 3.1 – 4.5(ng/ml), mean was 4.08.

The results of the present study revealed that the children diagnosed with an ASD had significantly increased mean of total Hg levels in comparison to sibling and their family and higher than the acceptable laboratory limits in the patients (0-9 ng/ml).

Table (3-5) –Analysis for GSTM1 genes in autistic children and their families.

NO.	Parents			Children														Number of AutisticChildren genotype		number Autistic. Children
				SUB-G-1-				SUB-G-2-				SUB-G-3-			SUB-G-4					
	Genotype		NO.																+	–
1			17	8	5	1	0	1				2			1				10	8	18
2			14	2	6	0	1		2*							3?	1	2	7	9	17
3			14	0	8	0	2			1	1		2	1					0	15	15
4			9	7	1	1	0												8	1	9
5			1	1															1	0	1
	+	-																			
total	50	59	55																26	33	60
%	45.9	54.1																	44.0	55.9	

(*)-This sub-group was recorded in children of two families, (?)-The genotype of one child was not determined

Normal male
Autistic male
Normal female
Autistic female

Analysis for GSTT1 genes in autistic children and their families

NO.	parents			Children														Number of Autistic Children genotype		Number of Autistic Children
				SUB-G-1-				SUB-G-2-				SUB-G-3-			SUB-G-4					
	Genotype		NO.																+	–
1			1		1														0	1	1
2			53	31	5	5	0	2	1	1	1	2	2	1	3?	1	2	1	52	5	58
3			1	1															1	0	1
	+	-																	+	-	
total	108	1	55																53	6	60
%	99.1	0.90																	89.8	10.1	

The levels of mercury in Blood of autistic children and their families

	Mercury Groups(ng/ml)		Mean ng/ml	Number of samples
AUTISTIC	I	16-37	25.0303	34
CHILDREN	II	38-82	50.8125	16
Total			37.553	50
Sibling	I	1.3-4	3.3	12
Total			3.3	12
Parents	I	1.1-3.1	2.11	79
	II	3.1-4.5	4.08	17
Total			3.192	96
acceptable laboratory limits	Normal value mercury blood (0-9) ng/ml MAYO CLINC			

The relationship between levels of mercury and frequency of GSTM1 and GSTT1genotypes in a sample of Iraqi autistic children

Groups	Mercury Level	GSTM1(+)		GSTM1(-)		GSTT1(+)		GSTT1(-)		Total
		No.	%	No.	%	No.	%	No.	%	
Group-I-	16-36 ng/ml	16	32	17	34	29	58	4	8	33
Group -II-	38-82 ng/ml	8	16	9	18	14	28	3	6	7
		24	48	26	52	43	86	7	14	50

DISCUSSION

The null genotype of children in group one and group two indicates that almost the positive GSTM1 genotype of the parents is heterozygous.

The frequency of the combination of the parent's genotypes group one, (null GSTM1genotype, positive GSTM1 genotype) was higher,[17] than the second and the third group.[14] The frequency of the positive genotypes of both parents is the lowest.[9]

In both, the children and the parents, the frequency of the null GSTM1 genotype 33 (55.9%), 59 (54.1% respectively) which was higher than the frequency of the positive genotype 26 (44%), 50 (45.9% respectively).

The genotypes of the parents in group one and group two can be considered as reciprocal crosses; there are slight differences in the number of children with positive and null genotypes. In group one, the mothers have positive GSTM1 genotype, the number of children with positive genotype was higher than the number of children with null genotype while. in group two, the mothers have a null genotype, the number of children with a null GSTM1genotype was slightly higher than the number of children with a positive genotype. The differences should be investigated in future studies in a large sample of parents and children.

There are three cases of twins. Regarding the sex: They are boys in two cases and in the third case they are a boy and a girl. In the first case, the mother has a positive GSTM1 genotype and the father has a null genotype. In the second case, both parents have a null genotype. The boys in these two cases have a null genotype. But in the first, both of them are autistic and in the second family, one of them is autistic and the other is non-autistic. In the third case, they are a boy and a girl. Both of them are autistic. They have a null GSTM1 genotype.

Regarding ASD Monozygotic (MZ), twins show > 70% concordance much higher than dizygotic (DZ) twins.[15]

The glutathione S- transferase (GSTM1) are known to be highly polymorphic. Polymorphic alleles of GSTs have been reported to contribute to a number of human diseases.[16,17] Homozygosity of the null GSTM1 is complete gene deletion that lacks function of the GSTM1enzyme and associated with an increased risk of prostate cancer in the presence of either the val/val or the ile/val genotypes of the Phase I enzyme CYP1A1.[18] Another study has found that the Homozygosity for null GSTM1 genotype was associated with increased risk of bladder cancer.[19]

Another study reported that null GSTM1 genotype contributed to the risk of hepatocellular cancer in conjunction with environmental factors,[20] Others revealed that null GSTM1 genotype also contributed to the risk of small cell lung cancer,[21] and asthma,[22] GSTM1 is located on 1pl3.3. Evidence of genetic linkage of autism to this region was shown in three studies.[23-25]

The association of GSTM1null genotype with autism was reported but was not considered as a risk factor of ASD.[26]

In the present study, the autistic children have the genotype of both null and positive GSTM1, null genotype was significantly higher than the positive genotype. This result is in agreement with study has reported that the null GSTM1 was significantly increased among autistic children.[27]

In addition to that, the gene GSTM1 are polymorphic alleles, the difference in their activity of their product, so it is probable that positive GSTM1 includes the weakest alleles product.

The fathers have a positive and the mothers have a null. It was not found autistic child. The null genotype for both of parents was not found.

In group one the fathers have a null GSTT1 genotype, the mothers have a positive GSTT1 genotype, only one autistic child has a null GSTT1, so the genotype of mother is heterozygote while in group two, both of the parents have a positive GSTT1 genotype. The number of a single child with positive GSTT1genotype was.[36] The number of a single child with null GSTT1 genotype was.[5] The children in sub group [2,3,4] are all with a positive GSTT1 genotype. This indicates that only a few parents in this group are heterozygous for the GSTT1 genotype and almost the positive GSTT1 genotype of the parent association direction or indirectly with autism.

Genetic polymorphism of GSTT1 results from gene deletion. In human population, two genotypes GSTT1 positive with proper activity and GSTT1null with lack of expression.

Study has elucidated that GSTT1 null is not a risk factor of ASD.[28]

The present study shows a higher percentage of positive GSTT1 genotype in autistic children and their parents to maintain intracellular redox balance and protect against oxidative stress. The cells produce reducers such as Glutathione. It was reported that the Mercury, mutagenic effect and toxic to cells by depleting.

Intracellular levels of anti-oxidative glutathione and binding to sulfhydryl groups of proteins.[29,30] GSTT1gene has multiple alleles, that is strongly indicated a mendelian intermediary inheritance, in which a gene-dosage effect results in a doubled enzyme expression in the presence of 2 functional alleles.[31]

The elevated Hg levels in blood samples that observed in the present study are consistent with the previous studies that showed increased brain Hg levels,[32] increased Hg levels in baby teeth;[33] increased hair Hg levels,[34] increased Hg in the urine/fecal samples following chelation therapy.[35,36]

The present study shows that autistic children have both genotypes: (positive/null) GSTM1 and (positive/null) GSTT1genotype, but most frequency genotype in all samples is positive- GSTT1 43(82%), null-GSTM1 genotype 26 (52%). and high levels of mercury in blood.

It was that mercury (Hg) leads to depletion of (low glutathione and antioxidant enzymes) leading to the increases in the production of free radicals. This can cause an increased oxidative stress.[37] Oxidative stress could interact with common functional polymorphic variants of genes that protect against oxidative stress and could thus affect brain development during gestation or possibly after gestation, contributing to expression of autism.[38]

The Glutathione S-transferase GSTs- are known to be highly polymorphic. Homozygous deletions of these genes result in lack of enzyme activity and impaired the ability to excrete metals including mercury. Combined effects of mercury (Hg) accumulation coupled with decreased levels of antioxidants (low glutathione and antioxidant enzymes) contribute to the phenotypic presentation of autism spectrum disorder (ASD), and association of GSTM1 null genotype with autism was reported but not risk factor of ASD.[39]

The reduced of neuronal glutathione and acetyl cholinesterase activities were recorded, it was shown that glutathione levels tend to be lower in autistic and mercury levels are much higher.[40] A study has mentioned that glutathione-s-transferase M1(GSTM1), which conjugates GSH to toxic electrophiles is reduced or absent in individuals carrying the GSTM1*0 null alleles, increasing their sensitivity to xenobiotics [41]. This mercury cannot be eliminated because GSH or GSST is lacking, then it a accumulates in tissues and does damage and inhibits methionine synthase and methyl reductase [SAM (S-adenosyl methionine)], and thus has effects on the body's ability to methylate and to produce glutathione that leads to increased oxidative stress.[42-44]

Another study has found that children with autism have low cysteine and low glutathione, probably due to low SAM.[45] In some studies, it was pointed that glutathione levels tend to be lower in autistics and mercury levels are much higher.[46] In other studies, it was shown that HST (hydroxyl steroid transferase) is known to be inhibited by mercury compound. Normally most of the DHEA that is produced in the testosterone synthesis pathway is stored as DHEA –S, reducing the amount that goes on to be made in to and rostrenediol and then in to testosterone and subsequently more testosterone breakdown products.[47, 48]

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