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Role of Glutathione-S-transferase M1 (GSTM1) and T1 (GSTT1) Genes on Aluminium Concentration and Oxidative Markers Among Autistic Children

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Abstract

Autism Spectrum Disorder (ASD) is considered a multifaceted neurodevelopmental disorder. The last two decades showed an increase in its prevalence until reached about 1 in 54 children. Autistic symptoms may be exacerbated when the interaction of the genetic and the environmental risk factors occur, **s**uggesting that gene-environment interaction could be a mechanism underlying the aetiology of ASD. Aluminium is a known neurotoxic metal that has known health effects in humans. Glutathione-S-transferase (GST) genes and their enzymes play a major role in the detoxification of many toxic metals.

Data were collected from 76 children aged 2-8 years diagnosed with ASD and 30 sex and age matched healthy children. The aim of this study was to investigate the association of polymorphisms in the two GST genes (GSTM1 and GSTT1) with mean aluminium concentrations (as, gene-environment interaction) and oxidative status markers (GST enzyme, malondialdehyde and nitric oxide) among the studied groups. The study started at December 2019 and last for one year at the clinics of National Research Centre, Egypt.

The results of this study showed that the null GSTM1 and GSTT1 genotype is the most common type in ASD and that genotype may predispose ASD children to decreased antioxidant status (GST enzyme activity) which in term lead to mal detoxification of aluminium. There is marked increase in aluminium concentrations in hair of ASD children and oxidative markers (increase in MDA and NO) leading to oxidative damage that may play an important role in children autistic status. The study recommends adding antioxidant supplements to daily diet of ASD children to improve their antioxidant status and in term improving management of patients with autism spectrum disorders. Further studies are needed to describe other GST gene polymorphisms.

Keywords: Autism Spectrum Disorder (ASD), aluminum; detoxification, glutathione S-transferase (GST), gene-environment interaction, malondialdehyde (MDA), nitric oxide (NO).

Introduction

Autism spectrum disorders (ASD) include a group of symptoms as impairment of social interactions and communication (verbal and non-verbal) with restricted interests and repetitive behaviour [1]. In the past 2 decades, the prevalence of ASD has increased by 23%. The prevalence of autism was about 1 in 54 children [2].

Recent studies show that autistic symptoms could be caused or exacerbated when the interaction of genetic and environmental risk factors occurs, **s**uggested that gene-environment interaction may be a mechanism underlying the etiology of ASD [3, 4]. The environmental factors in ASD might be pollutants such as pesticides and metals like mercury, lead and aluminum [5, 6].

Aluminium is the third most abundant metal in the earth's crust; it comes in the form of rocks of cryolite, bauxite, and silicates [7]. Aluminium could be found in antacids, propellants, astringents, anti-perspirants, airplanes, boats, cars, building materials, alloys, electrical devices, fuel additives, explosives and all households as cooking equipment, cans. We are heavily exposed to aluminium, and exposed to the dark side of aluminium era [8,9].

There have been many studies focused on the role of Aluminium in ASD [9, 10]. It is a neurotoxic agent and destabilizer of cell membranes [11, 12]; moreover, many studies found that the principal target of aluminium toxicity was astrocytes, which

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are responsible for the physical blood-brain barrier [13]. Additionally, to its high reactivity, aluminium is able to interfere the enzymatic activities in many key metabolic pathways. Moreover, aluminium toxicity could be increased by induction of oxidative stress [14] that significantly leads to ASD progression [5]. The response to oxidative stress is a keystone in neuroinflammation, and considered one of the leading causes of ASD [15].

Although aluminum has a direct relationship with increased oxidative stress state; aluminum inhibits the biological management systems of oxidative stress by interfering with the glutathione S-transferase (GST) detoxification system [16]. The superfamily of glutathione-S- transferases (GSTs) enzymes are responsible for detoxification reactions, and important as part of antioxidant defense mechanism that might be contributed to the development of ASD [17]. Glutathione -S-transferase mu *(*GSTM1*)*, and glutathione S-transferase theta *(*GSTT1); both play important roles in detoxification of the xenobiotics [18].

Genetic polymorphism observed in almost all classes of GST superfamily, may increase the individual susceptibility to the environmental factors which could be associated with ASD [19]**,** and to oxidative stress [20]. Considering that identifying new specific gene-environment interactions, and also, elucidating in which way antioxidants contribute to redox imbalance in autism could help resolving the complex etiology of ASD [20]. Therefore, the aim of the present study was to evaluate whether genetic polymorphism in glutathione transferase M1 and T1 genes among ASD children could modulate individual susceptibility to oxidative stress induced by aluminum.

Subjects and Methods

The present study is a descriptive, a cross sectional study. It includes two comparative groups; 76 newly diagnosed with childhood autism (medication free for at least 3 month) with age 2-8 years old compared to 30 healthy control children (after exclusion of any syndromes associated with autistic features). Full medical history and clinical examination for all the patients and the control children.

The control healthy children were matched with the autistic children for age, residential area and socioeconomic status.

The study started at December 2019 to December 2020 at "children with special needs clinics" in National Research Centre hospital, Dokki, Giza, Egypt.

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Hair samples collections

About 1.5-2 cm hair samples were collected by single cutting from the occipital region of each control and cases, for determination of aluminium level using the ICP MASS device**.**

Blood samples collections

Random venous blood samples (5 ml) were collected from all study subjects by sterile disposable syringes. Blood samples were divided into two portions; one left to clot for 30 minutes at 37°C and then centrifuged at 3,000 rpm for 10 minutes to isolate the sera. The sera were kept at -20°C for laboratory investigations. The other portion was collected on EDTA for preparing WBCs for DNA extraction and plasma isolation for determination of glutathione -S-transferase enzyme activity.

The investigated parameters

- ***Determination of aluminum in human hair samples using the ICP MASS device:***

Sample preparation procedure

All glass or plastic ware should be carefully soaked overnight in nitric acid (2M HNO3) then washed and rinsed well three times by deionized water, then kept dry until use. Weigh up to 0.5 g of hair samples into the microwave digestion vessel. Add 10 ml suprapur nitric acid to the digestion vessel and shake gently. Seal the vessel carefully and place it in its holder in the microwave oven and introduce the thermocouple probe in the reference vessel and close the door. Adjust the microwave oven program to temperature at 200 ^{0}C for 15 minutes, finally allow the microwave venting until temperature < 80 °C). After the heating cycle completed, remove the thermocouple probe from the reference vessel to allow the vessels to cool down in a water bath for about 30 min then open the vessels carefully. Rinse down the lid and the walls with deionized water inside the vessel, then transfer the residual solution in 50 mL PMP volumetric flask and then add 0.2 mL from internal standard of Al and completed it with deionized water to the marked volume. Treat the reagent blank with the same way. The samples were kept in polypropylene tubes until measured by Inductively Coupled Optical Emission Spectrometer (ICP-OES). Test values were reported in mg/kg.

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Standards and reagents

Certified reference metals stock standard solutions (1000 mg/L) of Al prepared in 2-3% HNO3 was purchased from Merck- Germany Spiked solutions were prepared from the stock as necessary. All reagents and solvents used in this study were analytical grade. The ICP-MS was calibrated, using aqueous standard solutions (of the same acid

concentration as in the samples and additional internal standards) prepared from the stock solutions by subsequent dilution in the range of 0.01 to 10 mg/L. The operating conditions of the ICP-MS instrument were optimized before the analysis was performed.

- ***Screening of GST gene polymorphisms using Polymerase Chain Reaction (PCR):***

DNA extraction

DNA was extracted from whole blood sample using Genomic DNA Purification kit (Gene JET™/ Fermentas).

GSTM1 and GSTT1 genotypes were determined by amplification of each gene with PCR. PCR reaction mixtures (25 μl) contained 5 μl of the extracted DNA, Dream Tag Green PCR master mix (2X)12.5 µl, 2 μl of Forward primer (0.4 μM), 200 µg of each primer and water nuclease completed to 25 µl. The primers used for the GSTM1 gene were 5'-CTGCCCTACTTGATTGATGGG-3' and 5'-CTGGATTGTAGCAGATCATGC-3'. The primers used for the GSTT1 gene were 5' -TTCCTTACTGGTCCTCACATCT C-3' and 5'-TCACC-GGATCATGGCCA GCA-3'. The human B-globin gene (110 bp) was also amplified in each reaction as a positive control to confirm the presence of amplifiable DNA in the samples. The primers used for B-globin were 5'-ACACAACTGTGTTCACTAG-C-3' and 5'-CAACTCATCCACGTTCACC-3'. The PCR were amplified in 35 cycles of 94°C for 1 min 30 seconds for denaturation, 52°C for 1 min for annealing, and 65°C for 1 min for extension; and final elongation at 65°C for 5 mins in an automated thermal cycler. The PCR products were then resolved by electrophoresis in 2% agarose gels, stained with ethidium bromide and photographed under ultraviolet light. Individuals with one or more GSTM1 alleles had a 273-bp fragment, and individuals with one or more GSTT1 alleles had a 480-bp fragment [21].

- ***Determination of Glutathione -S- transferase enzyme (GST) activity:***

GST was determined by colorimetric method according to Habig et al., [22]. GST activity was expressed as µmole/ml.

- ***Determination of serum malondialdehyde (MDA) concentration:***

Malondialdehyde level in Serum was measured at 532 nm using Shimadzu U.V-Visible recorder spectrophotometer model U.V-160 according to Yagi [23]. Malondialdehyde concentration was expressed in nmole/ml.

- ***Determination of serum Nitric Oxide (NO) concentration:***

Nitric oxide was determined at 548 nm using Shimadzu U.V-Visible recorder spectrophotometer model U.V-160 according to **Miranda et al.,** [24]. Final concentration was expressed in µmole/ml.

Statistical analysis

The collected data and the laboratory results were computerized. Statistical analysis was done through SPSS version 18. The quantitative results are expressed as means ± SD. The statistical significance of the data has been determined using independent t-test for the comparisons. Correlation coefficient was used to study the relationships between the quantitative data. Significant was considered when p-value ≤ 0.05.

Ethical approval

Ethical approval was obtained from the "Ethical Committee" of National Research Centre, Egypt.

Results

This study was performed on 76 autistic children including 62 male and 14 female with the mean age (6.11±1.569) years, and 30 control group including 23 male and 7 female with the mean age (7.23±0.858) years. There is no significant difference between autistic and control group regarding to age, sex, and family history. We found that 96% of autistic children used piped water while only 4% used filtered water while the control group used piped water as source of drinking water. All the children of the two studied groups used cooking wares made of aluminium.

Table (1) showed a significant increase in the mean level of aluminum concentrations (mg/kg) in the hair of autistic children in comparison to that of control (p-values < 0.0001). The results of oxidative stress biomarkers showed that there were a significant increase in MDA level and NO (p-values < 0.001) in autistic children compared to controls. Concerning antioxidant status, the results of enzymatic antioxidant showed that there was a significant decrease in GST enzyme (p-values < 0.001) in autistic children compared to controls.

The PCR results for the distribution of GST gene polymorphism among autistic and control children:

The PCR results for GST polymorphism showed that the GSTT1 allele at 480bp and GSTM1 allele at 273bp. The absence of GSTT1 was expressed as null GSTT1 and the absence of GSTM1 was expressed as null GSTM1 (figure 2).

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Table (1): Comparison between autistic children and the control group regarding the levels of Aluminum (mg/kg), the oxidative stress markers and anti-oxidant status

Parameter	Autistic children (76)	Control (30)	Independent t-test	
	Mean± SD	Mean± SD	t-test	p-value
Al (mg/kg)	30.1382±11.32089	14.2740±2.99335	1.470	0.0001
MDA nmole/ml	8.2066±2.06652	3.2000±1.34958	8.784	0.001
NO µmole/ml	2.4868±0.60008	1.7333±0.44747	10.836	0.001
GST µmole/ml	458.5789±126.28700	702.7333±88.3218	9.416	0.001

GST: glutathione-s-transferase enzyme, NO: nitric oxide, MDA: malondialdyhyde, Significant difference at P<0.05

Table (2) showed that the distribution GST gene polymorphism among autistic children and control children. The frequency of null GSTM1&GSTT1 in autistic children compared to controls were significantly higher in autistic children compared to controls p<0.006 (32.9 % *vs* 6.7%). While, the active form of GSTM1 had lower distribution among autistic children compared to the control (22.4% *vs* 46.7%) and this distribution was statistically significant as p<0.018. Also, the active form of GSTT1 had lower distribution among autistic children compared to the control (17.1% *vs* 20%) and the distribution of combined GSTM1&GSTT1 active forms in autistic children compared to controls were (27.6 % *vs* 26.7%).

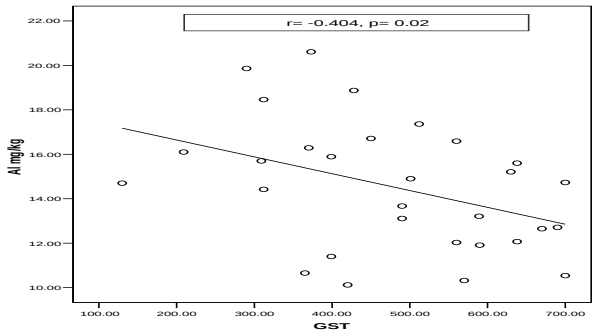

Figure (1) the correlation between Aluminum hair levels and antioxidant enzymatic activity of glutathione -S-transferase among autistic children

Figure (1) showed a significant negative correlation between aluminum hair levels and antioxidant enzymatic activity of glutathione -s-transferase among autistic children.

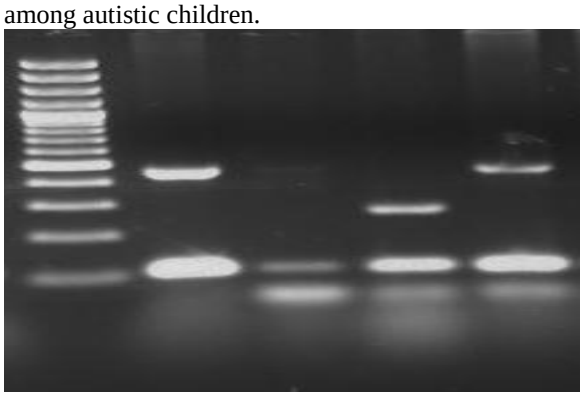

Figure 2: PCR product for GSTT1, GSTM1 and β globulin genes separated by agarose gel electrophoresis stained with ethidium bromide. Lane 1: is the size marker (100 bp). Lane 2& 5: represent wild genotypes for the GSTT1 gene at 480 bp. Lanes 3: represent null genotypes for both GSTM1 and GSTT1 genes. Lanes 4: represent wild genotypes for the GSTM1 gene at 273 bp. All lanes (2-5) are positive for β globulin (positive control) at 110bp.

Table (2): Distribution of GST gene polymorphism among autistic and control children

GST gene polymorphisms	**autistic children (76)**		**Control children (30)**		**p-value**
	Count	**Percent%**	**Count**	**Percent%**	
Null M1&T1	25	32.9	2	6.7	0.006
M1 +ve	17	22.4	14	46.7	0.018
T1 +ve	13	17.1	6	20	1.000
M1&T1(+ve)	21	27.6	8	26.6	1.000

NB: Null =Null GSTT1&GSTM1, T1= GSTT1 active form, M1= GSTM1active form, M1&T1 active form

Significant differences at P<0.05

Levels of Aluminium excreted with respect to GSTM1 and GSTT1 genotypes among autistic and control children:

The levels of Aluminium were significantly higher in the autistic children with GSTT1 allele when compared to the controls and to the autistic children with other types of GST alleles. Also, the aluminium levels of the autistic children with GSTM1 allele and those with Null (GSTM1 and GSTT1) allele were significantly higher when compared to the controls (Table 3).

Table (3): levels of Aluminium excreted with respect to GSTM1 and GSTT1 genotypes among autistic and control children

GST polymorphism	Autistic children (76)	Control children (30)	p-value
	Aluminum level concentrations(mg/kg)		
	Mean± SD	Mean ±SD	
NULL M1&T1	29.0222±10.53002	19.3000±1.69706	0.001
M1(+ve)	29.2918±12.80379	13.5700±2.71726	0.001
T1(+ve)	36.7479±11.40520	16.4517±2.75392	0.001
M1&T1(+ve)	27.7024±9.63927	12.6163±1.47157	0.001

NB: Null =Null GSTT1&GSTM1, T1= GSTT1 active form, M1= GSTM1active form, M1&T1 active form
Significant differences at P<0.05

Table (4) showed that there was no significant difference in MDA &NO levels between null GSTM1 and T1 and other active forms of GSTT1 or GSTM1 among autistic group. While, the levels were significantly lower in the autistic children with Null (GSTM1 and GSTT1) allele, active GSTM1, and active forms of GSTT1&M1 compared to the controls. In contrast, GSTT1 active form has no significant difference comparing to control.

The levels of GST were significantly lower in the autistic children with Null (GSTM1 and GSTT1) allele compared to the controls and to the autistic children with the other types of GST alleles p<0.01 (Table 5).

Table (4): Blood levels of oxidative stress biomarkers (MDA& NO) with respect to GSTM1 and GSTT1 genotypes among autistic and control children

Oxidative stress biomarker	Autistic children (76) Mean± SD		control children (30) Mean± SD	p-value
MDA nmole/ml	NULL M1&T1	7.9200±1.86190	2.3500±0.07071	0.001
	M1(+ve)	8.0047±2.29971	2.6571±0.56257	0.01
	T1(+ve)	7.7500±2.25994	5.1500±1.82839	0.23
	M1&T1(+ve)	7.5524±1.93355	2.9000±.64143	0.001
NO µmole/ml	NULL M1&T1	2.5040±0.57408	1.4000±0.14142	0.001
	M1(+ve)	2.5412±0.56905	1.6714±0.49370	0.01
	T1(+ve)	2.3143±0.62617	2.1667±0.22509	0.5
	M1&T1(+ve)	2.5190±0.65086	1.6000±0.34226	0.001

NB: Null =Null GSTT1&GSTM1, T1= GSTT1 active form, M1= GSTM1active form,
M1&T1 active form NO: nitric oxide, MDA: malondialdyhyde, Significant difference at P<0.05

Table (5): Blood levels of glutathione-s-transferase (GST) enzyme with respect to GSTM1 and GSTT1 genotypes among autistic and control children

GST gene polymorphism	Autistic children (76) Mean± SD	Control children (30) Mean± SD	p-value
	GST enzyme levels(µmole/ml)		
NULL M1&T1	339.5600±97.52225	520.5000±27.57716	0.016
M1(+ve)	501.9412±75.23669	710 ±76.80745	0.01
T1(+ve)	494.7857±77.36545	695.8333± 103.42	0.01
M1&T1(+ve)	544.4286±110.76307	740.7500±48.22788	0.01

NB: Null =Null GSTT1&GSTM1, T1= GSTT1 active form, M1= GSTM1active form, M1&T1 active form
Significant differences at P<0.05

Discussion:

Aluminium is third most common abundant metal on earth, so, it has been used extremely in the past five decades in every product, and we became exposed to it from many sources like aluminium cookware, aluminium foil, food additives, medications, tap water and cosmetics, all of which raise our exposure to this metal [25,6]. It has been proposed that aluminium exposure may contribute as an environmental factor to cause ASD [9, 26]**,** and the increased frequency of ASD occurrence may be attributed to the exposure to this era. Many studies highlight the prevalence of aluminium exposure and development of ASD [27, 28] and has been proposed that the remarkable higher aluminium concentration was reported in hair of ASD children compared to neuro typical one [29].

In this study, we use hair analysis to evaluate the long-term aluminium exposure as a non-invasive best indicator of a given mineral in the body and this was in agreement with **Mohamed et al [**6] for the use of this technique.

The present found that aluminium hair levels in the ASD children were significantly higher compared to controls (Table 1). The same results documented by **Mohamed et al** [6] in Egypt and **Al-Farsi et al** [30] in Oman, all of them reported a similar increase in the mean of aluminium level in hair of autistic children in comparison to neuro typical children. Also, **Metwally et al** [9] found that the mean level of aluminium measured in urine was higher than in group of children suffering from autism compared to their control. As aluminium induced neuro-degeneration by disrupting mitochondrial function, and thereby deplete ATP, induce redox abnormality, and ultimately lead to cell death through necrotic mechanisms [31, 32]. Therefore, we concluded that environmental exposure to these toxic heavy metals may play a causal role in autism.

One of the most important mechanisms by which heavy metals can induce induction of cellular oxidative stress in the form of overproduction of free radicals or alteration of antioxidant defense mechanisms. Therefore, alteration in oxidative stress can be confirmed by the direct measurement of lipid peroxidation by-product malondialdehyde (MDA) [33]. The results of the current study showed an increased MDA levels in autistic children compared to the control group (Table 1).

This result was consistent with numerous studies who recorded that, autistic children suffered from oxidative stress with significant elevation in the levels of MDA. This elevation of MDA blood levels can be utilized as an indicator of tissue injury [34-36]. In addition, a growing amount of evidence has indicated that oxidative stress is a key stone in neuroinflammation in the pathophysiology of number of neuropsychiatry diseases [37,38] and especially in ASD [15,39,40].

In the present study, there is a significant increase in nitric oxide (NO) in autistic children compared to control (Table 1), this result was in agreement with that of **Sweeten et al** [41] who reported plasma levels of NO were significantly higher in the autistic subjects compared to their control. Aluminium-intoxicated rats show various markers or indicators of oxidative stress, and these can be expressed as elevated nitric oxide levels which in turn lead to the production and generation of reactive oxygen species [42, 43]. The increase in nitric oxide may be a result of the ability of aluminium to accelerate the expression of inducible nitric oxide synthase (iNOS) [44]. Although, nitric oxide has been known to exert neuroprotective effects at low to moderate concentration but nitric oxide changes to neurotoxic when the concentration increases [45].

Glutathione-S-transferases (GST) are multifunctional enzymes, involved in detoxification of toxic substances caused by oxidative stress products. Cellular GST efficiently scavenge toxic free radicals and are partly responsible for protection against lipid peroxidation due to aluminium exposure [46]. A few studies have suggested that children with autism have limited availability reduced glutathione storage capacity, resulting in decreased detoxification, increased oxidative stress and DNA damage, and chronic inflammatory responses [47-50].

According to the results of the present study, a significant decrease in GST activities was reported in the autistic children relative to the controls (Table 1). These changes can be related to decreasing in detoxification of toxic substances caused by oxidative stress products leading to remarkable elevation in aluminium level among autistic children.

Alabdali et al [51] were in agreement with our results as regard reduction of GST activity in the autistic group of their study in comparison to the control. Also, **Al-Yafee et al** [52] in his study on autistic children in Saudi Arabia found that GST activity was diminished compared to their control. On the contrary to our study, **Oshodi et al** [53] found that GST values of autistic and control population showed no statistically significant difference.

The results of the present study found a negative correlation between aluminium level and anti-oxidant status marker (GST) activity, Figure (1). Aluminium as a neurotoxic metal can induce oxidative damage as it binds to negatively charged phospholipid leading to easily attack to reactive oxygen species [44]. We hypothesized that aluminium may cause mal antioxidant status that induce oxidative stress.

Genetic susceptibility could play an important role in the response to environmental toxins. Therefore, detoxification genes (GST) polymorphism was studied in the present study. GSTM1 and GSTT1 enzymes, both are known to catalyse the detoxification of reactive oxygen and lipid peroxidation products [54].

In the present study the polymorphism of GST was studied in ASD children and controls relative to the two genotypes GSTM1 and GSTT1. Null GSTT1 and GSTM1 genotypes were significantly higher in autistic children compared to controls (32.9 % *vs* 6.7%). While 32.9% of autistic children had null GSTT1 and GSTM1, the other had GSTM1 allele (22.4%), GSTT1 allele (17.1%) and GSTT1and GSTM1 alleles (27.6%). Several studies have reported an association between the null allele and autism [55, 56]. On the other hand, **Hermawati** reported that GSTM1 null and GSTT1 null are not risk factors for ASD [57].

The results of this study also revealed that, aluminium levels were significantly higher in the autistic children with GSTT1 allele compared to the controls and to the autistic children with other types of GST alleles. Also, the aluminium levels of the autistic children with GSTM1 allele and those with Null (GSTM1 and GSTT1) allele were significantly higher compared to the controls (Table 4).

The polymorphism of GST revealed that there was no significant effect on the levels of aluminium between ASD children with the different GST active genotypes. But aluminium seemed to be the highest (in the children with GSTT1 in the examined groups. However, we find only one previous literature supporting these hypotheses. **Mandic-Maravic et al** [1] reported that GSTT1 active increased the risk of ASD. Specifically, it is well established that GSTT1 enzyme is involved in bio-activation, rather than detoxification of several bifunctional alkylating agents, present in environmental pollution [58].

In population with GSTT1&GSTM1 null genotype, gene susceptibility seemed to have little role and the main role was for environmental exposures, as there was significantly elevation in aluminium level in ASD children GSTT1&GSTM1 null genotype compared to their controls.

Two studies have reported an association between the null allele and autism **(Buyske et al** [55] and **James et al** [56]**,** suggesting that GSTM1 contributes to the risk of oxidative stress and autism. In contrast to the results of this study which revealed that, oxidative biomarkers were significantly higher in the autistic children with null GSTT1&GSTM1 allele, active GSTM1allele and active GSTM1&GSTT1 allele compared to the controls, no difference in means of MDA and NO levels among different polymorphisms of autistic children (Table 4). Thus, there was no role for both GSTT1& GSTM1 in oxidative biomarkers (MDA& NO) among autistic children.

Polymorphisms within GST classes result in altering of GST enzyme activity, hence altering both the capacity for detoxification of different endogenous and exogenous compound, including oxidants, and in that way contributing to development of various neurological and mental disorders, along with ASD [59-60]. According to the present study results, a significant decrease in GST activities in null GSTM1&GSTT1 relative to the controls was observed that was associated with elevation of the aluminium level in this group (Table 3&5). These changes reflect the peroxidative conditions in blood that increase oxidative stress [61]. The GST function is to detoxify toxic compounds by binding them to inactivate electrophilic compounds [62].

So we concluded that null GSTM1& GSTT1 play a marked role in detoxification (GST enzyme activity) and not on the oxidative stress state (MDA&NO concentrations) among autistic children. Which leads to imbalance between oxidant and antioxidant mechanisms among those children and hence mal detoxification of the neurotoxic aluminum metal.

Limitations of the study:

The sample size was small, it was non-randomized sample and the control group was less in number than the cases.

Conclusion:

The results of this research indicate that autistic children have an increased aluminum levels in their hairs. Also, there was a decreased in the activity of GST enzyme among them. The reduction of GST enzyme activity usually associated with gene polymorphism Null heterozygous GSTM1&GSTT1 allele. The present study results showed that null GSTM1and GSTT1 genotype is the most common type in ASD. In addition, an increase in the frequency distribution of that allele was associated with an increase in serum biomarkers of oxidative stress among ASD children. Thus, we concluded that there is gene–environment interaction, as oxidative stress among ASD children changes due to both environmental (high aluminium level) and genetic factors (null heterozygous GST allele). This may be valuable in deciding to add antioxidant supplements to their daily diet to improve their antioxidant status and in term improving the management of children with ASD. Also, the present study results showed that although null GSTM1and GSTT1 heterozygous is the most common type in ASD, there was no difference in aluminium level with either GSTT1 or GSTM1 homozygous. Further studies with larger sample size are needed to describe other GST gene polymorphisms.

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Conflicts of interest
There are no conflicts of interest.

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