Total Reflection X-ray Fluorescence Analysis of Plasma Elements in Autistic Children from India

Article *in* Biological Trace Element Research · March 2022

DOI: 10.1007/s12011-022-03199-2

8 authors, including:

Rajat Hegde
Karnataka Institute for DNA Research [KIDNAR]
25 PUBLICATIONS **10** CITATIONS
SEE PROFILE

Smita Hegde
Karnataka Institute for DNA Research Dharwad
18 PUBLICATIONS **8** CITATIONS
SEE PROFILE

Mahadevappa Y. Karidurаganavar
Karnatak University, Dharwad
110 PUBLICATIONS **2,639** CITATIONS
SEE PROFILE

Kusal K. Das
BLDE (Deemed to be University)
224 PUBLICATIONS **2,918** CITATIONS
SEE PROFILE

Biological Trace Element Research (2023) 201:644–654
https://doi.org/10.1007/s12011-022-03199-2

Total Reflection X-ray Fluorescence Analysis of Plasma Elements in Autistic Children from India

Rajat Hegde[1,2] · Smita Hegde[2,3] · Sujayendra Kulkarni[3,4] · Suyamindra S. Kulkarni[2] · Aditya Pandurangi[5] · Mahadevappa Y. Kariduraganavar[6] · Kusal K. Das[1] · Pramod B. Gai[2,7]

Received: 1 February 2022 / Accepted: 9 March 2022 / Published online: 25 March 2022

Abstract

Trace elements are essential for the human body's various physiological processes but if they are present in higher concentration, these elements turn to be toxic and cause adverse effect on physiological processes. Similarly, deficiency of these essential elements also affects physiological processes and leads to abnormal metabolic activities. There is a lot of interest in recent years to know the mystery behind the involvement of trace elements in the metabolic activities of autistic children suspecting that it may be a risk factor in the aetiology of autism. The present study aims to analyse the plasma trace elements in autistic children using the total reflection X-ray fluorescence (TXRF) technique. Plasma samples from 70 autistic children (mean age: 11.5 ± 3.1) were analysed with 70 age- and sex-matched healthy children as controls (mean age: 12 ± 2.5). TXRF analysis revealed the higher concentration of copper (1227.8 ± 17.8), chromium (7.1 ± 2.5), bromine (2695.1 ± 24) and arsenic (126.3 ± 10) and lower concentration of potassium (440.1 ± 25), iron (1039.6 ± 28), zinc (635.7 ± 21), selenium (52.3 ± 8.5), rubidium (1528.9 ± 28) and molybdenum (162,800.8 ± 14) elements in the plasma of autistic children in comparison to healthy controls. Findings of the first study from India suggest these altered concentrations in elements in autistic children over normal healthy children affect the physiological processes and metabolism. Further studies are needed to clarify the association between the altered element concentration and physiology of autism in the North Karnataka population in India.

Keywords Autism · Total reflection X-ray fluorescence (TXRF) · Element analysis · Plasma · North Karnataka · India

✉ Pramod B. Gai
pramodbgai@gmail.com

Rajat Hegde
rajat.hegde007@gmail.com

1 Laboratory of Vascular Physiology and Medicine, Department of Physiology, Shri B.M Medical College, Hospital and Research Centre, BLDE (Deemed To Be University), Vijayapura 586101, India

2 Karnataka Institute for DNA Research (KIDNAR), Dharwad 580003, India

3 Human Genetics Laboratory, Department of Anatomy, Shri B.M Medical College, Hospital and Research Centre, BLDE (Deemed To Be University), Vijayapura 586101, India

4 Division of Human Genetics (Central Research Lab), S. Nijaliangappa Medical College, HSK Hospital and Research Center, Bagalkot 587102, India

5 Department of Psychiatry, Dharwad Institute of Mental Health and Neurosciences, Dharwad 580008, India

6 Department of Chemistry, Karnatak University, Dharwad 580003, India

7 Karnatak University Dharwad, Dharwad 580003, India

Introduction

Autism or autism spectrum disorder (ASD) [MIM 299850] is a neurodevelopmental disorder that usually appears before the age of 3 and is characterised by the altered social response, communication (verbal and/or non-verbal) and repetitive behaviour (https://www.psychiatry.org/psychiatrists/practice/dsm; https://www.Who.int/classifications/icd/icd/onlineversions/en/). Genetic factors might be largely responsible for the occurrence of ASD that alone or in a combination with specific environmental factors trigger the development of ASD [1]. The human body requires a sufficient supply of essential elements like iron, copper, zinc, cobalt, manganese and selenium, for proper functioning, especially for brain function. These elements play an important role in regulating the immune system and antioxidant system [2]. Neurochemical and neurophysiological evidence indicates that trace elements remarkably affect the functionaing of neurotransmitters [3]. An altered profile of trace elements has been observed among different medical

conditions including neurological disorders and psychological disorders [2].

Very little is known about how trace elements contribute to the molecular mechanism of autism. Several studies suggest that dysfunction in excitatory and inhibitory synapses is a causative factor for autism like symptoms and trace elements influence synaptic functions in autism [2, 3]. Particularly, zinc is required for scaffolding of ProSAP/Shank proteins related to excitatory synapses. It is, thus, expected that altered zinc profile to be associated with different brain diseases and disorders. Higher zinc values might contribute to epileptogenesis [2], whereas lower zinc levels have been implicated in depression and ASD [4, 5]. Magnesium is a regulatory cation that modulates gamma-aminobutyric acid (GABA) signalling; thus, an altered magnesium profile might contribute to ASD. Iron is an essential element that plays important role in regulating brain function. Several studies have recorded that impaired iron haemostasis in neurodegenerative disorders [6].

Different habits in dietary consumption and environmental circumstances cause changes in these necessary nutrients. Several researches consider various biomarkers for autism diagnosis, but it is still difficult to explain the link between ASD and trace elements because of inconsistencies across studies. The purpose of this study is to examine the trace elements in the blood plasma of 70 autistic children and compare them to unaffected healthy children using total reflection X-ray fluorescence (TXRF) technology, as well as to look into the relationship between trace element concentration in male and female children and autism severity.

Materials and Methods

Participants

In total, 1870 mentally ill children below 18 years of age were diagnosed using DSM-V (https://www.psychiatry.org/psychiatrists/practice/dsm) and ICD-10 (https://www.Who.int/classifications/icd/icd/onlineversions/en/) criteria from the North Karnataka region of India. A total of 150 autistic children were identified and 70 autistic children ($N_{male} = 50$ and $N_{female} = 20$, mean age $= 11.5 \pm 3.1$) included in the study as a case group. A control group was selected, which included 70 age- and gender-matched healthy children. These control group children were unrelated to the cases. Consent was taken from parents/guardians and ethical approval for the study was obtained from the Institutional ethical committee of Shri B.M Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura (Ref No: BLDE (DU) IEC/337–2018-19). If the children were suffering from any progressive neurological disorder, liver or kidney disease, anaemia or under any treatment or medication were excluded from the study. The severity of autism was measured using Childhood Autism Rating Scale (CARS). Total scores can range from a low of 15 to a high of 60; scores below 30 indicate that the individual is in the non-autistic range, scores between 30 and 36.5 indicate mild to moderate autism, and scores from 37 to 60 indicate severe autism [7].

Sample Collection

Two-millilitre peripheral blood samples were collected in EDTA vacutainer and plasma was separated immediately by centrifuging at 10,000 rpm for 10 min and separated plasma was stored in sterile microcentrifuge tube at − 80 °C until further analysis.

Sample Preparation and Element Analysis

Minimal sample preparation was required for total reflection X-ray fluorescence for the analysis of elements in plasma. To 495µL of plasma and 5µL of internal standard (IS) gallium (Ga) solution (500 mg/L) were added to get concentration 5 mg. Sample was then vertexed slightly and 10µL of sample was deposited as a single drop over a quartz glass sample carrier. Samples were allowed to dry for 5 min. After drying, the sample carrier was inserted into the TXRF instrument (Bruker S2 Picofox TXRF, Bruker AXS GmbH, Germany) and analysis was carried with an integration time of 1000 s for each sample with 50-kV voltages and 698-µA Current. Each sample was prepared and analysed in triplicate.

Statistical Analysis

Collected data was revised, coded, tabulated and subjected to Statistical Package for Social Science (SPSS 15.0.1) analysis. Descriptive statistics was used to calculate the mean and standard deviation (± SD) for numerical data. Analytical statistics and Student's *t*-test were used to assess the statistical significance of the difference between two study group means, and ANOVA test was used to assess the statistical significance of the difference between more than two study group means. The chi-square test was used to examine the relationship between two qualitative variables. *p*-value of 0.05 was considered significant.

Results

Present study population included 70 autistic children (mean age: 11.5 ± 3.1, $n_{male} = 50$, $n_{female} = 20$) and 70 healthy age-matched children as control group (mean age: 12 ± 2.5, $n_{male} = 50$, $n_{female} = 20$). There was a significant difference

between these groups regarding age and sex. The demographic characters are illustrated in Table 1.

Results from statistical tests showed that, the children with ASD showed a higher concentration of copper, Cu (children with ASD 1227.8 µg/L, control group 811.9 µg/L); chromium, Cr (children with ASD 7.1 µg/L, control group 2.8 µg/L); bromine, Br (children with ASD 2695.1 µg/L, control group 2157.8 µg/L) and arsenic, As (children with ASD 126.3 µg/L, control group 28.6 µg/L).

While the element such as potassium, K (children with ASD 440.1 µg/L, control group 525.0 µg/L); iron, Fe (children with ASD 1039.6 µg/L, control group 2372.2 µg/L); zinc, Zn (children with ASD 635.7 µg/L, control group 1024.1 µg/L); selenium, Se (children with ASD 52.3 µg/L, control group 69.6 µg/L); rubidium, Rb (children with ASD 1528.9 µg/L, control group 1725.9 µg/L) and molybdenum, Mo (children with ASD 162,800.8 µg/L, control group 187,684.3 µg/L) showed lower concentration in ASD, compared to control group, manganese and rubidium showed statistically non-significant higher concentration in autism ($p > 0.05$) (Table 2, Fig. 1).

In males, elements like manganese, bromine and rubidium showed non-significant difference in their concentration (p-value 0.09, 0.08 and 0.4 respectively) compared to the control group (Fig. 2). In females, elements like potassium, manganese, chromium, rubidium and molybdenum showed statistically non-significant difference in concentration (p-value 0.8, 0.25, 0.09, 0.12 and 0.34 respectively) compared to respective control group (Fig. 3). There was no larger difference in concentration between the ASD male group and ASD female group and the difference among ASD males and ASD females was non-significant in all the elements except potassium (Table 3). Potassium, iron, zinc, manganese and selenium showed slightly higher concentration in males and copper, chromium, bromium, rubidium, molybdenum and arsenic showed higher concentration in females (Fig. 4).

The present study included 50 autistic male and 20 autistic female children. In 50 autistic male children, 34 (68%) children showed mild-moderate autism (CARS score 30–36.5) whereas 14 (32%) children showed severe autism (CARS score 37–60). In 20 autistic female children, 14 (70%) children showed mild-moderate autism and 6 (30%) children showed severe autism. Concentration of copper, chromium, bromine and arsenic was observed to be higher in ASD and this concentration increased as the severity of autism increased from mild to severe. Elements, such as potassium, iron, zinc, selenium, rubidium and molybdenum, showed lower concentration and this

Table 1 Demographic characteristics of autistic children

Demographic character	Autistic children	Healthy children (control)
No of children	70	70
Mean age	11.5 ± 3.1	12 ± 2.5
Ethnic origin	Indian	Indian
Average age of father at child's birth	39 ± 2.3	30 ± 2.7
Average age of mother at child's birth	34 ± 2.1	29 ± 1.8
Consanguineous marriage		
• Yes	48	11
• No	22	57
Prenatal factors		
• Preeclampsia	16	03
• Maternal hyperthyroidism	04	04
• Hypertension	03	01
• Gestational problem	07	00
Postnatal factor		
• Labour complication	12	06
• Forceps-mediated delivery	08	01
• Birth asphyxia	10	02
• Feeding problem	04	02
• Delayed crying	09	01
Intelligent quotient (IQ)	30 ± 10	60 ± 5
Severity		
• Mild to moderate	48	00
• Severe	22	00
Comorbid condition	None	none
Family history of the neurodevelopmental condition		
• Autism	00	00
• ADHD	01	00
• Intellectual disability	10	02
• Learning disability	03	00
• Speech problem	08	01

Table 2 Plasma element levels (µg/L) in children with ASD and controls

Element (µg/L)	ASD Mean ± SD	Control Mean ± SD	p-value
Potassium, K	440.1 ± 25	525.0 ± 20	0.005*
Iron, Fe	1039.6 ± 28	2372.2 ± 35	0.001*
Zinc, Zn	635.7 ± 21	1024.1 ± 15	0.001*
Copper, Cu	1227.8 ± 17.8	811.9 ± 14.5	0.001*
Manganese, Mn	95.5 ± 14	117.6 ± 12	0.7**
Selenium, Se	52.3 ± 8.5	69.6 ± 10	0.001*
Chromium, Cr	7.1 ± 2.5	2.8 ± 1.1	0.001*
Bromine, Br	2695.1 ± 24	2157.8 ± 18.4	0.018*
Rubidium, Rb	1528.9 ± 28	1725.9 ± 34.6	0.114**
Molybdenum, Mo	162,800.8 ± 14	187,684.3 ± 17.2	0.001*
Arsenic, As	126.3 ± 10	28.6 ± 5.9	0.001*

Data presented as Mean ± SD; *significant difference as compared to the control values at $p < 0.05$; **non-significant difference as compared to the control values at $p > 0.05$

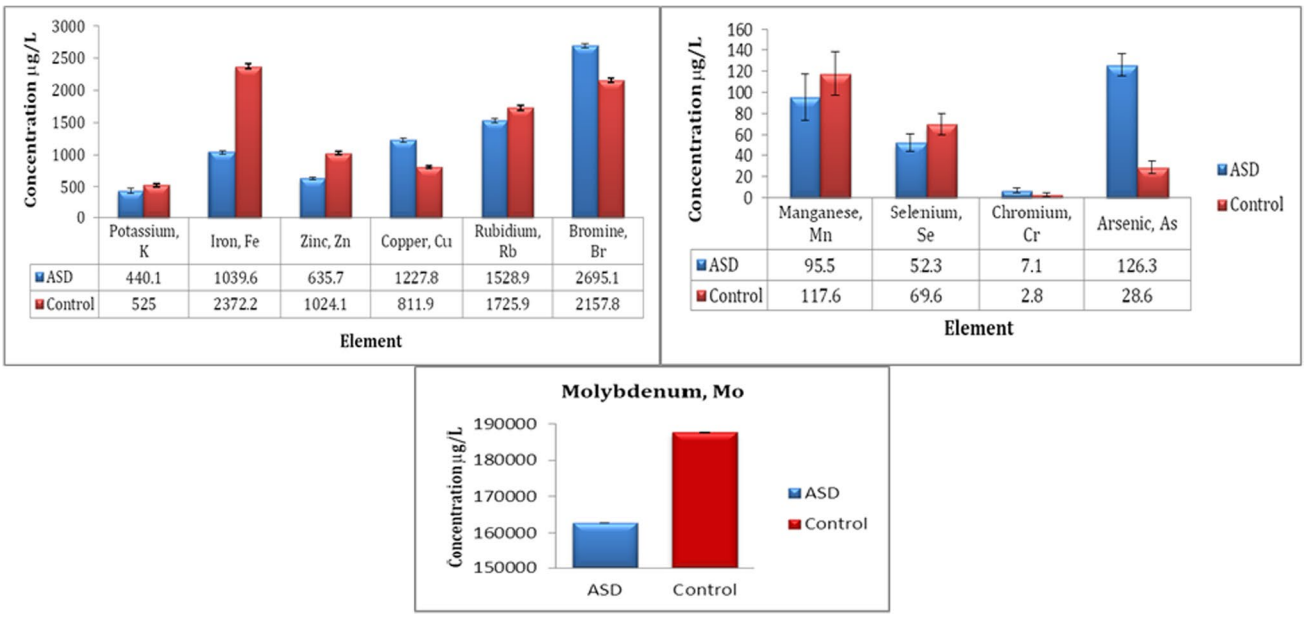

Fig. 1 Plasma element levels (µg/L) in children with ASD and controls

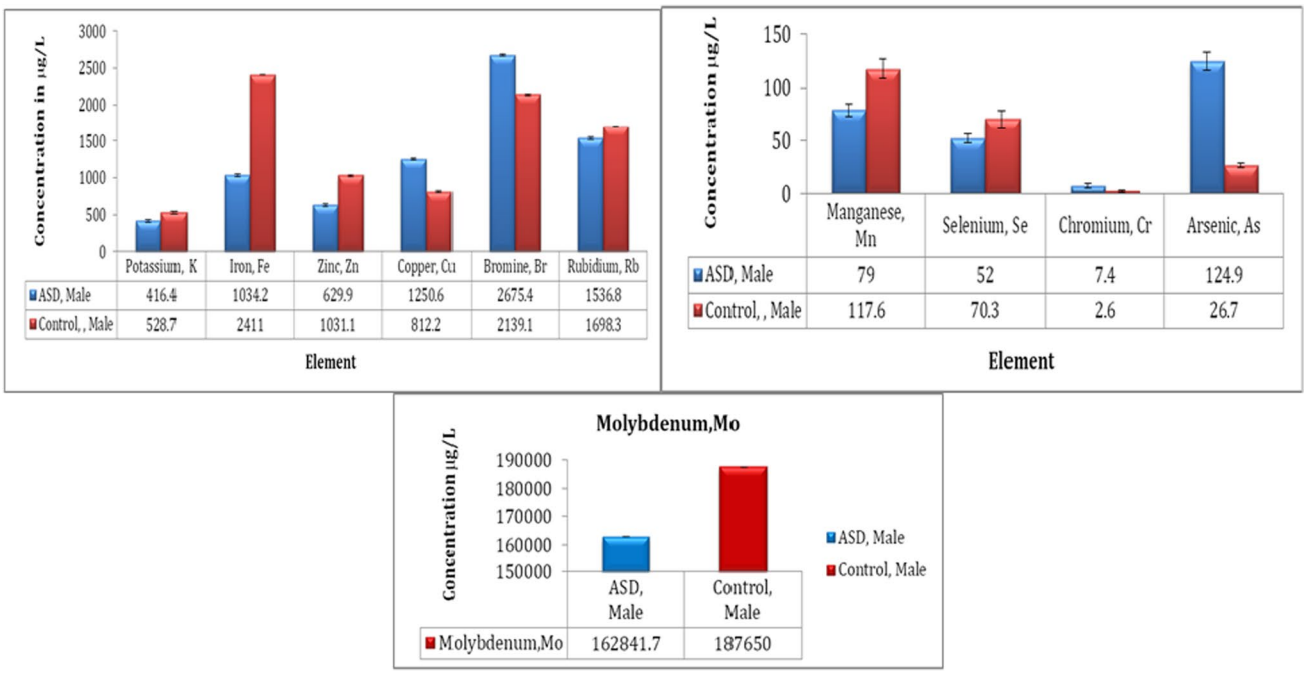

Fig. 2 Distribution of plasma element levels (µg/L) in male children with ASD and control group

plasma concentration decreased as the severity of autism increased from mild to severe (Table 4) (Figs. 5, 6 and 7). Manganese and rubidium showed non-significant difference among both male and female ASD groups.

Discussion

Autism [MIM 299850] is a complex neurological condition that is characterised by abnormal social interaction,

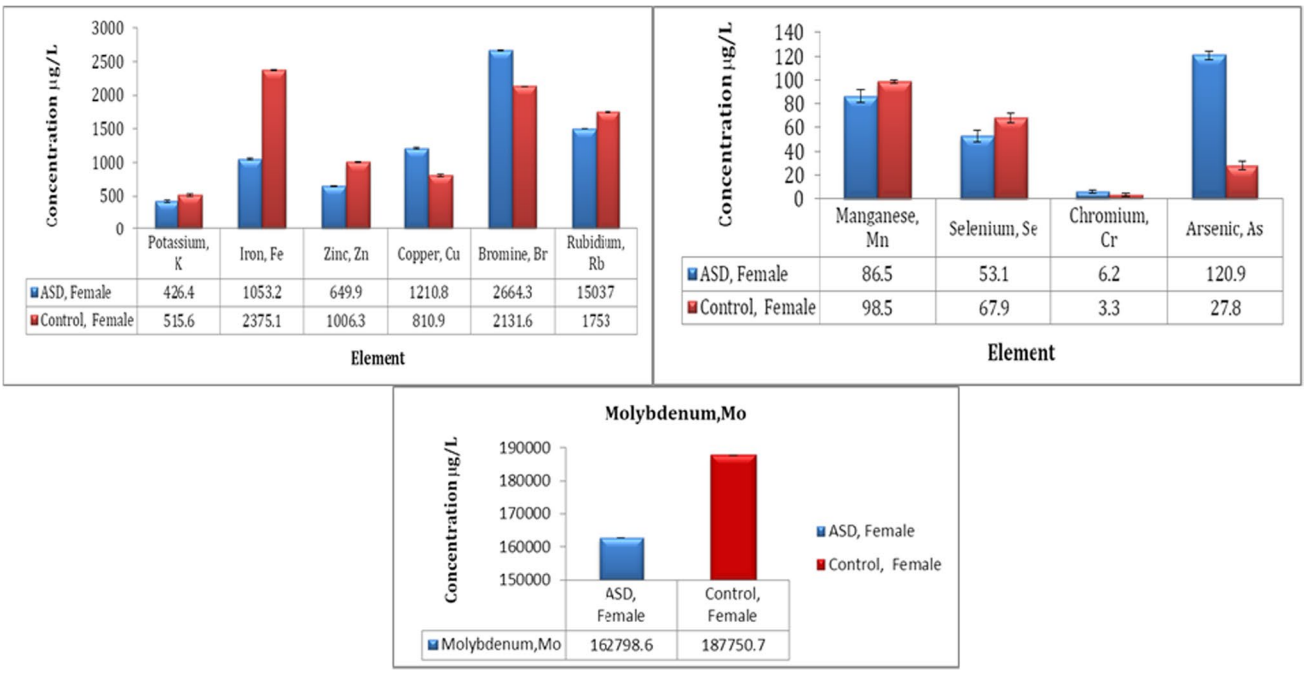

Fig. 3 Distribution of plasma element levels (µg/L) in female children with ASD and control group

Table 3 Sex-wise distribution of plasma element levels (µg/L) in children with ASD and control

Element µg/L	Male		*p*-value	Female		*p*-value	Male v/s female *p*-value
	ASD $n=50$	Control $n=50$		ASD $n=20$	Control $n=20$		
Potassium, K	416.4 ± 13.6	528.7 ± 15.9	0.013*	426.4 ± 21.0	515.6 ± 15.6	0.8**	0 .025*
Iron, Fe	1034.2 ± 16.5	2411 ± 2.2S	0.001*	1053.2 ± 8.0	2375.1 ± 8.8	0.008*	0.63**
Zinc, Zn	629.9 ± 14.8	1031.1 ± 11.3	0.001*	649.9 ± 6	1006.3 ± 6.2	0.001*	0.55**
Copper, Cu	1250.6 ± 10.6	812.2 ± 14.2	0.001*	1210.8 ± 12.7	810.9 ± 17.1	0.005*	0.98**
Manganese, Mn	79 ± 5.8	117.6 ± 9.0	0.09**	86.5 ± 5.1	98.5 ± 1.3	0.25**	0.10**
Selenium, Se	52 ± 3.8	70.3 ± 8.3	0.001*	53.1 ± 5.0	67.9 ± 4.3	0.03*	0.83**
Chromium, Cr	7.4 ± 2.1	2.6 ± 1.0	0.001*	6.2 ± 1.4	3.3 ± 1.1	0.09**	0.49**
Bromine, Br	2675.4 ± 13.2	2139.1 ± 10.3	0.08**	2664.3 ± 8.2	2131.6 ± 3.2	0.03*	0.14**
Rubidium, Rb	1536.8 ± 14.5	1698.3 ± 6.3	0.4**	1503.7 ± 3.0	1753.0 ± 10.0	0.12**	0.35**
Molybdenum,Mo	162,841.7 ± 30	187,650 ± 16	0.001*	162,798.6 ± 45	187,750.7 ± 15.6	0.34**	0.20**
Arsenic, As	124.9 ± 8.9	26.7 ± 2.3	0.001*	120.9 ± 3.5	27.8 ± 3.7	0.001*	0.72**

Data presented as Mean ± SD; *significant difference as compared to the control values at $p<0.05$; **non-significant difference as compared to the control values at $p>0.05$

verbal and non-verbal communication and impaired behaviours (https://www.psychiatry.org/psychiatrists/practice/dsm; https://www.Who.int/classifications/icd/icd/onlineversions/en/). There is a lot of interest in recent days to know about the involvement of elements in the metabolism in autistic children in order to see whether these elements are risk factor in the aetiology of autism. Certain essential trace elements are required for the bodies' various physiological processes but if they are present in higher concentration, these trace elements result in toxicity and cause adverse effect on physiological processes. Similarly, deficiency of these essential elements also affects physiological processes and leads to abnormal metabolic activities [2]. So it is very important to determine the trace elemental concentration in the neurological condition in affected individuals to monitor and assess their impact on health. Many studies recently have revealed that autistic children have multiple elemental variations in their body

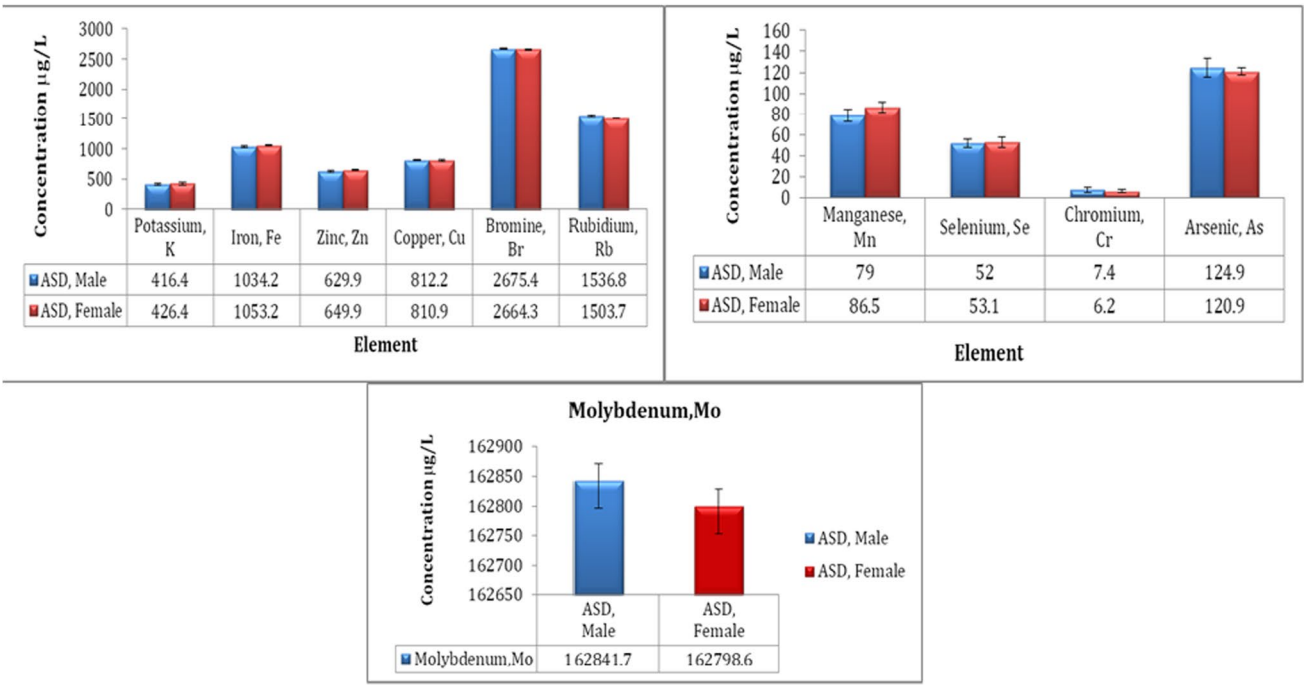

Fig. 4 Distribution of plasma element levels (µg/L) in male children with ASD and female children with ASD

Table 4 Plasma element levels (µg/L) in children with mild-moderate ASD and children with severe ASD

Element µg/L	Sex	Control	Mild to moderate autism (score 30–36.5)	Severe autism (score 37–60)	*p*-value
Potassium, K	Male	528.7 ± 15.9	426.5 ± 3.5	406.8 ± 2.1	0.006*
	Female	515.6 ± 15.6	439.6 ± 5.0	412.1 ± 2.0	0.07**
Iron, Fe	Male	2411 ± 2.2S	1045.5 ± 3.7	1021.5 ± 2.0	0.001*
	Female	2375.1 ± 8.8	1060.2 ± 1.0	1044.0 ± 0.8	0.004*
Zinc, Zn	Male	1031.1 ± 11.3	635.8 ± 5.0	619.0 ± 2.0	0.001*
	Female	1006.3 ± 6.2	654.4 ± 1.5	644.4 ± 0.5	0.001*
Copper, Cu	Male	804.2 ± 2.1	819.2 ± 3.6	1250.6 ± 10.6	0.001*
	Female	823.0 ± 3.1	824.2 ± 1.1	1210.8 ± 12.7	0.001*
Manganese, Mn	Male	117.6 ± 9.0	83.2 ± 1.5	76.6 ± 1.2	0.062**
	Female	98.5 ± 1.3	89.2 ± 1.1	82.5 ± 0.6	0.132**
Selenium, Se	Male	70.3 ± 8.3	52.3 ± 2	50.5 ± 0.8	0.001*
	Female	67.9 ± 4.3	56.1 ± 2.0	49.1 ± 0.7	0.001*
Chromium, Cr	Male	2.6 ± 1.0	6.5 ± 1.8	7.8 ± 1.2	0.001*
	Female	3.3 ± 1.1	5.6 ± 1.0	7.0 ± 0.4	0.001*
Bromine, Br	Male	2139.1 ± 10.3	2668.4 ± 3.0	2685.8 ± 1.2	0.416**
	Female	2131.6 ± 3.2	2660.6 ± 0.9	2670.6 ± 1.2	0.001*
Rubidium, Rb	Male	1698.3 ± 6.3	1545.0 ± 2.8	1547.0 ± 2	0 .498**
	Female	1753.0 ± 10.0	1504.7 ± 0.6	1505.7 ± 0.2	0.296**
Molybdenum, Mo	Male	187,650 ± 16.0	162,832.9 ± 3.5	162,866.0 ± 2.9	0.04*
	Female	187,750.7 ± 15.6	162,805.6 ± 10	162,830.6 ± 15	0.042*
Arsenic, As	Male	26.7 ± 2.3	118.9 ± 3.5	127.0 ± 3.8	0.001*
	Female	27.8 ± 3.7	118.0 ± 1.5	122.1 ± 0.5	0.001*

Data presented as Mean ± SD; *significant difference as compared to the control values at $p < 0.05$; **insignificant difference as compared to the control values at $p < 0.05$

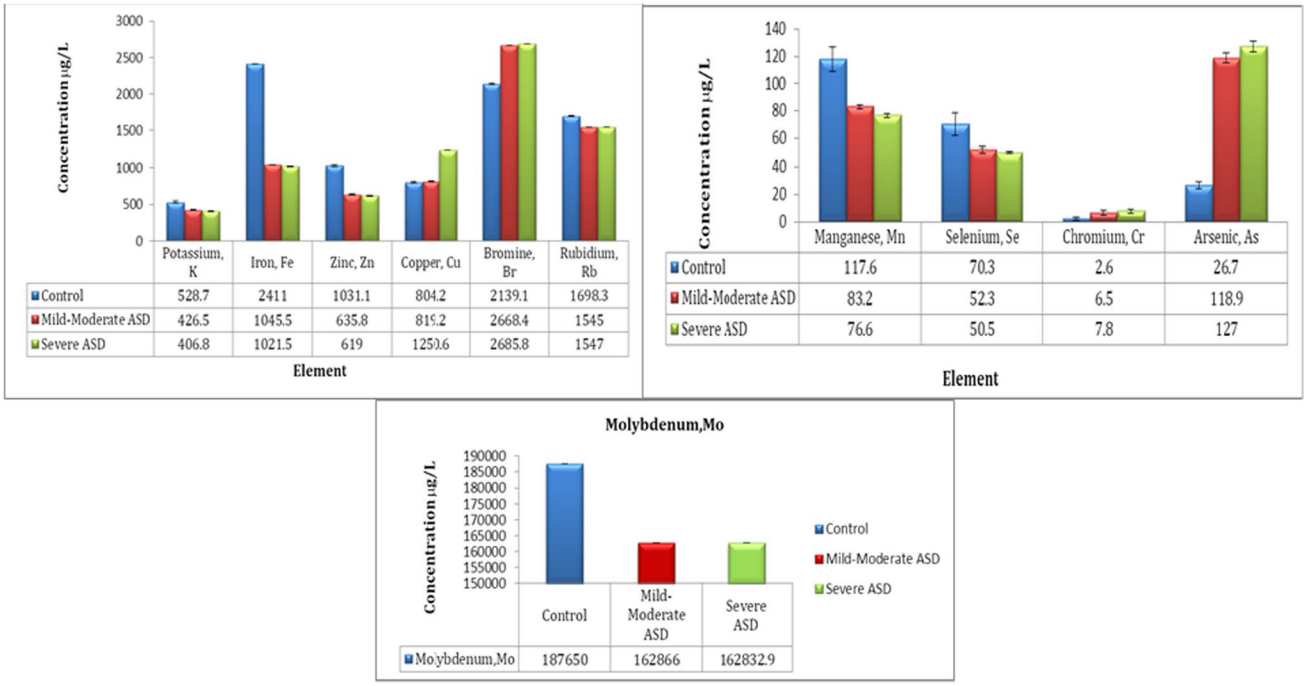

Fig. 5 Plasma element levels (µg/L) in male children with mild-moderate ASD and male children

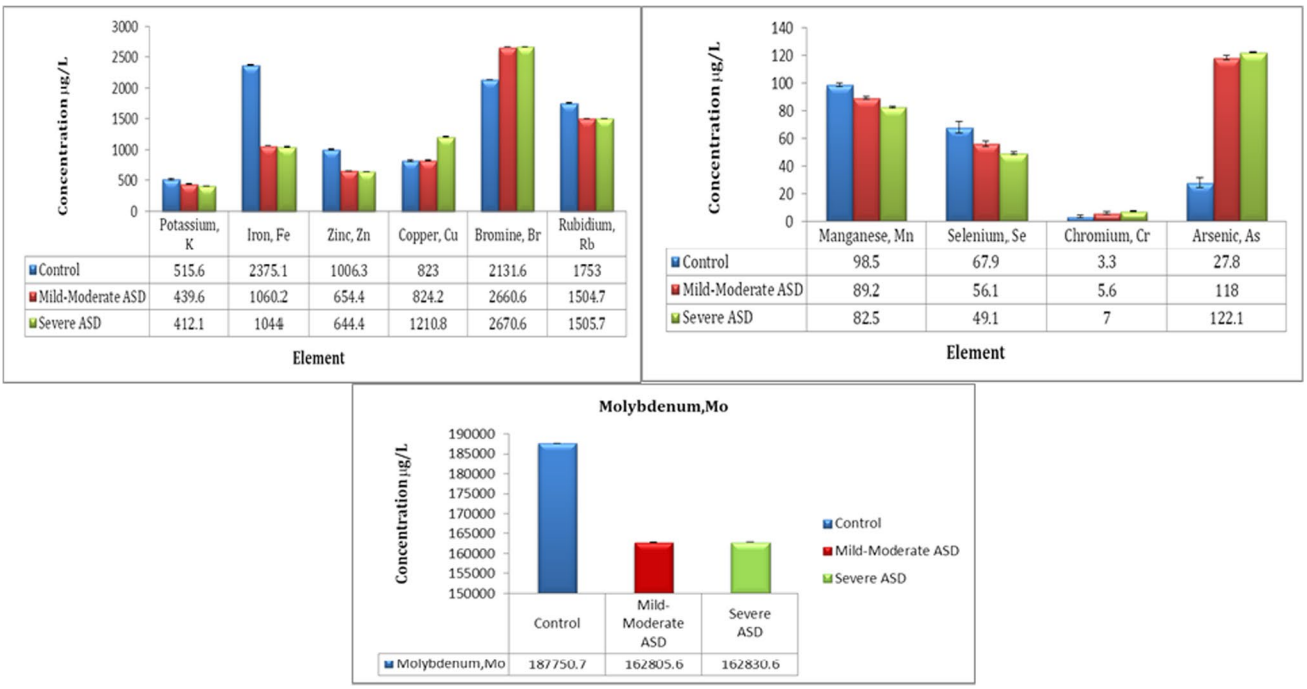

Fig. 6 Plasma element levels (µg/L) in female children with mild-moderate ASD and female children with severe ASD

which leads to abnormal metabolism [1, 2]. In the present study, we have analysed the trace elements in plasma of 70 autistic children including 50 male and 20 female children with a mean age of 11.5 ± 3.1 using the total reflection X-ray fluorescence (TXRF) technique.

Potassium is one of the most important elements for the functioning of the human body. It helps in the regulation of fluid balance, muscle contractions and nerve signals. Potassium allows brain cells to communicate with each other and also with cells that are farther away. Potassium deficiency

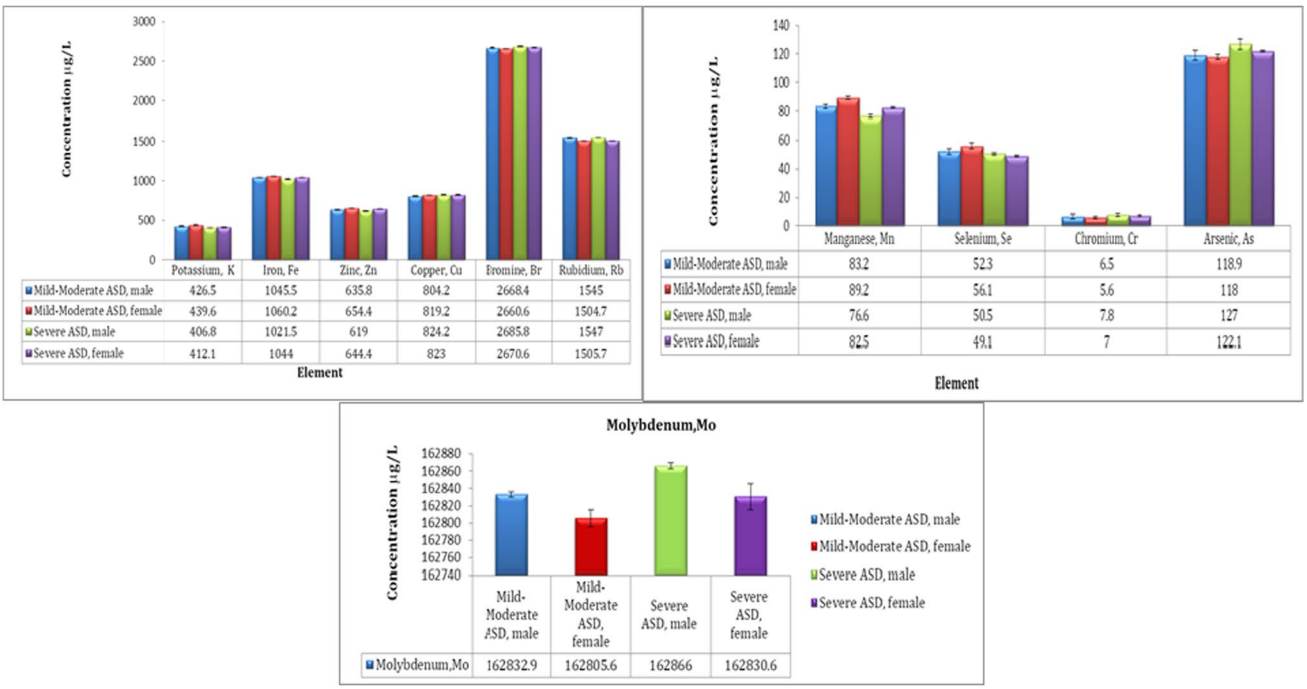

Fig. 7 Plasma element levels (µg/L) in male children with mild-moderate and severe ASD and female children with mild-moderate and severe ASD

in the blood disrupts the signals that help to maintain the proper functioning of the brain [8]. About 20% of the individuals with mental disorders have potassium deficiency [9]. The present study showed a significant decrease in the plasma concentration in autistic children (440.1 ± 25 vs 525.0 ± 20, p-value 0.005). Male autistic children showed significantly low concentration of potassium in comparison to control male children but female autistic children showed non-significant low concentration (Table 3). Plasma concentration of potassium was found to be significantly decreasing in both male and female children as severity increased from mild-moderate to severe (Table 4). A similar result was recorded in a previous study done in 2016 [10] but Wecker L et al. [11] recorded a high concentration of potassium in hairs of autistic children.

The human body requires *iron* as a trace metal to regulate a variety of metabolic activities, including the electron transport chain and oxygen metabolism [12, 13]. Our cells absorb iron from our food and transfer it in the form of ferritin. Maintaining homeostasis necessitates proper iron metabolism. Iron insufficiency can lead to a variety of problems, the most common of which is iron deficiency anaemia. Iron deficiency has been linked to ASD symptoms, and is notably connected with the severity of emotional and behavioural issues, as well as developmental delay in autism [2, 14]. The current study supports that idea by finding that autistic children have considerably lower iron levels than the control group (1039.6 28 vs 2372.2 35, p-value 0.001). In comparison to the respective control groups, severe autistic children from both the male and female groups had considerably lower iron concentrations (Table 4). In comparison to ASD females, ASD males had a lower concentration (Table 2).

Zinc is a very essential element for spermatogenesis and maturation, genomic integrity of sperm and normal functioning of neurotransmitters. However, data on serum Zn levels in ASD are more contradictory. In particular, certain studies have demonstrated the absence of significant changes or a significant increase in serum Zn levels in ASD patients as compared to the controls [15, 16]. Several studies have showed lower concentration of zinc from plasma samples of children with ASD [17, 18]. Our study supports these findings by recording a significantly lower concentration of zinc in plasma of autistic children (635.7 ± 21 vs 1024.1 ± 15, p-value 0.001) compared to healthy control group. Both the sexes showed significantly very low concentration with respect to their control group (Table 2). Zinc is involved in the gut-brain interaction, and many ASD patients also have gastrointestinal symptoms [19, 20]. There are considerable evidences for an association between zinc deficiency and ASD [17, 21]. Zinc levels may also be correlated to the severity of ASD [22]. Our study records a significantly low concentration of plasma zinc in mild-moderate autism to severe autism and this zinc concentration decreases gradually with increase of ASD severity.

Copper is an essential element that plays a significant role in cellular functioning and required for the normal development and functioning of the brain. As a cofactor of several enzymes and/or as a structural component, copper is involved in many physiological pathways in the brain. Our findings showed a significantly increased concentration of copper in autistic children compare to healthy age-matched controls (1227.8 ± 17.8 vs 811.9 ± 14.5, *p*-value 0.001). Both male and female groups also recorded increased concentration of copper. A previous study by Qin Y yan et al. (2018) results supports our study results and a study of 79 autistic individuals found a similar pattern, in which autistic and pervasive developmental disorder-not otherwise specified (PDD-NOS) patients had significantly higher plasma levels of copper [16]. But a study by A.V. Skalny et al. [10] showed no significant difference between the autistic and control group. It is supposed that altered copper metabolism in autism may be related to impaired metallothionein system functioning and activation of free radical oxidation [23]

Copper and zinc play competing roles physiologically, such that an increase in copper leads to zinc deficiency [20]. Our findings support this by recording significantly higher copper concentration in autistic children and a significantly low concentration of zinc (Table 2).

Manganese (Mn) is an essential element that plays a fundamental role in brain development and its functioning. It acts as an activator of enzyme and as a component of metalloenzymes. They have a role to play in oxidative phosphorylation, fatty acids and cholesterol metabolism, mucopolysaccharide metabolism and the urea cycle [24]. Abnormal concentrations of manganese in the brain, especially in the basal ganglia, are associated with neurological disorders similar to Parkinson's disease. The present finding of decrease manganese concentration in plasma of autistic children (95.5 ± 14 vs 117.6 ± 12) was supported with previous data, in particular, a significant association between blood Mn and ASD [25, 26]. The observed decrease of Mn levels may be hypothetically associated with oxidative stress and an accompanying decrease in Mn-SOD activity, an Mn-dependent antioxidant enzyme widely distributed in brain structures [10]. The male ASD group shows low concentration compared to the female ASD group (Table 3).

Selenium is a vital metalloid for a variety of biological functions [10]. Selenium is required for neurobehavioural development in the foetus as well as cognitive performance in later life. It also plays a role in the central nervous system's various tasks, including motor performance, coordination, memory and cognition [27]. Selenium and selenium-dependent proteins are important for brain development and oxidative damage management, and dyshomeostasis in selenium has been linked to an increased risk of ASD [28]. There was a considerable drop in plasma selenium concentration in children with autism, according to existing studies [29, 30]. In comparison to a healthy age-matched control group, autistic children had significantly lower selenium concentrations (52.38.5 vs. 69.610, *p*-value 0.001). The concentration of selenium in males decreased significantly (*p*-value 0.001), but the difference was not significant in females (*p*-value 0.83). In the mild-moderate autism group and the severe autism group, there was a substantial steady drop in both male and female (Table 4).

Zn, Se and Mn shortage can affect metabolic processes and exacerbate heavy metal toxicity, impairing brain function and neural plasticity [31].

The production of glucose tolerance factor requires chromium. Chromium shortage results in impaired glucose tolerance, while intoxication causes renal failure, dermatitis and lung cancer [32]. In this study, we found that autistic children had a greater chromium concentration (7.1 ± 2.5 vs. 2.8 ± 1.1), and that male autistic children have a higher concentration than female autistic children (7.4 ± 2.1 vs. 6.2 ± 1.4). In both males and females with severe autism, the concentration of chromium was high. Previous research (https://www.Who.int/classifications/icd/icd/onlineversions/en/) [33] backed up this conclusion. Other investigations found reduced chromium levels in autistic children's hair and serum [10, 34, 35].

Bromine is a naturally occurring element that can be found in a variety of chemicals such as insecticides, flame retardants and water treatment chemicals. Bromine toxicity is caused by a greater quantity of bromine in the body, which damages the membranes of neurons, affecting neuronal transmission over time. Bromism is the term for bromine toxicity. Bromism can also produce neurological, mental and psychotic symptoms, as well as convulsions, dermatological and gastrointestinal issues [36]. In this study, autistic children had a substantially higher concentration of bromine (2695.124 vs 2157.818.4, *p*-value 0.018), and there was no significant difference in concentration between the sexes (Table 3).

Rubidium is present in large concentrations in muscle tissue, red blood cells and viscera. Our study recorded lower concentration in autistic children compared to age-matched healthy children (1528.9 ± 28 vs 1725.9 ± 34.6). Interestingly female ASD children showed low concentration compare to male ASD children (Table 3).

Molybdenum is a trace element that is essential for life and it functions as a cofactor for sulphite oxidase, xanthine oxidase, aldehyde oxidase and mitochondrial amidoxime reducing component. Molybdenum deficiency and toxicity are rare but a varying concentration of molybdenum in the body is associated with reduced growth, histological changes in kidney and renal failure, reproductive abnormalities, bone deformities and anaemia [37]. Studies have shown that molybdenum toxicity appears to be a cause of some cases of autism. This is consistent with

toxic leukoencephalopathy caused by heavy metal toxicity. Our findings are contradictory to this because significantly low concentration of molybdenum is observed in autistic children compared to age-matched healthy children (162,800.8 ± 14 vs 187,684.3 ± 17.2 *p*-value 0.001). Male autistic children showed a significant difference with healthy male children (162,841.7 ± 30 vs 187,650 ± 16, *p*-value 0.001). Female autistic children showed a non-significant decrease compare to healthy female children (162,798.6 ± 45 vs 187,750.7 ± 15.6, *p*-value 0.34).

Arsenic is a human developmental neurotoxicant. Arsenic appears to have toxic effects on neurotransmitters involved in cell-to-cell signalling within the brain. A study of rats demonstrated that arsenic induces regional increases in levels of dopamine, serotonin and their metabolites and also induces a decrease in norepinephrine levels in discrete brain regions [38]. Autistic children from our study group showed a significantly very high concentration of arsenic in the plasma compared to age-matched healthy children (126.3 ± 10 vs 28.6 ± 5.9 *p*-value 0.001). Male autistic children showed high concentration compare to female autistic children (124.9 ± 8.9 vs 120.9 ± 3.5). Mild to moderate autistic children from both male and female groups showed similar arsenic concentration (118.9 ± 3.5 vs 118.0 ± 1.5). Similar results were recorded in a previous study carried in 28 autistic children from Italy [39].

Conclusion

For the first time present study investigated the concentrations of trace elements in blood plasma of children with ASD and unaffected children from the North Karnataka population from India. These altered concentrations in trace elements in autistic children compared to normal healthy children affect the physiological processes and metabolism. Further studies are needed to clarify the association between the altered trace elemental concentration and physiology of autism in the North Karnataka population in India.

Acknowledgements We thank all autistic children and their parents/guardians for agreeing to participate in the study. We also thank all the special schools for participating in our study. We sincerely thank Director, University Science Instrumentation Centre (USIC), Karnatak University Dharwad, for providing total reflection X-ray fluorescence (TXRF) instrument facility. We also thank Ms Renuka Niralagi, DST PURSE phase-II programme, Karnataka University Dharwad, for her help during sample analysis. We thank Karnataka Institute for DNA Research (KIDNAR), Dharwad and Shri B.M Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura, for their constant support throughout the research.

Funding This study was supported by Grant-in-Aid for research from the Department of Higher Education, Govt. of Karnataka, India (Grant No: Department of Higher Education, ED 15 UKV 2018, Bangalore, date:12–13-2018).

Declarations

Statement of Ethics Ethical approval for the study was taken from the Institutional Ethical Committee of Shri B.M Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura (Ref No: BLDE (DU) IEC/337–2018-19). Informed consent was obtained from parents/guardians before the collection of blood samples.

Conflict of Interest The authors declare no competing interests.

References

1. Pfaender S, Grabrucker AM (2014) Characterization of biometal profiles in neurological disorders. Metallomics 6(5):960–977
2. Saghazadeh A, Ahangari N, Hendi K, Saleh F, Rezaei N (2017) Status of essential elements in autism spectrum disorder: systematic review and meta-analysis. Rev Neurosci 28(7):783–809
3. Saldanha Tschinkel PF, Bjørklund G, Conón LZZ, Chirumbolo S, Nascimento VA (2018) Plasma concentrations of the trace elements copper, zinc and selenium in Brazilian children with autism spectrum disorder. Biomed Pharmacother [Internet] 106(June):605–609
4. Swardfager W, Herrmann N, Mazereeuw G, Goldberger K, Harimoto T, Lanctôt KL (2013) Zinc in depression: a meta-analysis. Biol Psychiatry 74(12):872–878
5. Grabrucker S, Jannetti L, Eckert M, Gaub S, Chhabra R, Pfaender S et al (2014) Zinc deficiency dysregulates the synaptic ProSAP/Shank scaffold and might contribute to autism spectrum disorders. Brain 137(1):137–152
6. Grissom M (2011) Childhood Autism Rating Scales. In: Kreutzer JS, DeLuca J, Caplan B (eds) Encyclopedia of clinical neuropsychology. Springer, New York
7. Spence SJ (2004) The genetics of autism. Semin Pediatric Neurol 11(3):196–204
8. Inshasi JS, Jose VP, Van Der Merwe CA, Gledhill RF (1999) Dysfunction of sensory nerves during attacks of hypokalemic periodic paralysis. Neuromuscul Disord 9(4):227–231
9. Lam MHbun, Chau SWho, Wing Ykwok (2009) High prevalence of hypokalemia in acute psychiatric inpatients. Gen Hosp Psychiatry [Internet]. 31(3):262–5
10. Skalny AV, Simashkova NV, Klyushnik TP, Grabeklis AR, Radysh IV, Skalnaya MG, Nikonorov AA, Tinkov AA (2016) Assessment of serum trace elements and electrolytes in children with childhood and atypical autism. J Trace Elem Med Biol S0946672X16301468
11. Wecker L, Miller SB, Cochran SR, Dugger DL, Johnson WD (1985) Trace element concentrations in hair from autistic children. J Intellect Disabil Res 29(1):15–22
12. Lieu PT, Heiskala M, Peterson PA, Yang Y (2001) The roles of iron in health and disease. Mol Aspects Med 22(1–2):1–87
13. Theil EC (2003) Metal-binding proteins and trace element metabolism ferritin : at the crossroads of iron and oxygen metabolism. J Nutr 133(5):1549–1553
14. McCann JC, Ames BN (2007) An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 85(4):931–945
15. Russo AJ, Bazin AP, Bigega R et al (2012) Plasma copper and zinc concentration in individuals with autism correlate with selected symptom severity. Nutr Metab Insights 5:41–47
16. Vergani L, Cristina L, Paola R, Luisa AM, Shyti G, Edvige V, Giuseppe M, Elena G, Laura C, Adriana V (2011) Metals,

metallothioneins and oxidative stress in blood of autistic children. 5(1):0–293. https://doi.org/10.1016/j.rasd.2010.04.010
17. Li SO, Wang JL, Bjørklund G, Zhao WN, Yin CH (2014) Serum copper and zinc levels in individuals with autism spectrum disorders. Neuro Rep 25(15):1216–1220
18. Russo AJ, Devito R (2011) Analysis of copper and zinc plasma concentration and the efficacy of zinc therapy in individuals with Asperger's syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS) and autism. Biomark Insights 6:127–133. https://doi.org/10.4137/BMI.S7286
19. Li H, Zhang J, Niswander L (2018) Zinc deficiency causes neural tube defects through attenuation of p53 ubiquitylation. Development 145(24):dev169797. https://doi.org/10.1242/dev.169797
20. Vela G, Stark P, Socha M, Sauer AK, Hagmeyer S, Grabrucker AM (2015) Zinc in gut-brain interaction in autism and neurological disorders. Neural Plast 2015:972791. https://doi.org/10.1155/2015/972791
21. Yasuda H, Tsutsui T (2013) Assessment of infantile mineral imbalances in autism spectrum disorders (ASDs). Int J Environ Res Public Health 10(11):6027–6043
22. Guo M, Li L, Zhang Q, Chen L, Dai Y, Liu L et al (2020) Vitamin and mineral status of children with autism spectrum disorder in Hainan Province of China: associations with symptoms. Nutr Neurosci 23(10):803–810
23. Faber S, Zinn GM, Kern JC, Skip Kingston HM (2009) The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. Biomarkers 14(3):171–180
24. Rehnberg GL, Hein JF, Carter SD, Linko RS, Laskey JW (1982) Chronic ingestion of mn3o4 by rats: tissue accumulation and distribution of manganese in two generations. J Toxicol Environ Health 9(2):175–188
25. Rahbar MH, Samms-Vaughan M, Dickerson AS, Loveland KA, Ardjomand-Hessabi M, Bressler J et al (2014) Blood manganese concentrations in Jamaican children with and without autism spectrum disorders. Environ Heal A Glob Access Sci Source 13(1):1–14
26. Rahbar MH, Samms-Vaughan M, Ma J et al (2015) Synergic effect of GSTP1 and blood manganese concentrations in autism spectrum disorder. Res Autism Spectr Disord 18:73–82
27. Pitts MW, Byrns CN, Ogawa-Wong AN, Kremer P, Berry MJ (2014) Selenoproteins in nervous system development and function. Biol Trace Elem Res 161(3):231–245. https://doi.org/10.1007/s12011-014-0060-2
28. Raymond LJ, Deth RC, Ralston NVC (2014) Potential role of selenoenzymes and antioxidant metabolism in relation to autism etiology and pathology. Autism Res Treat 2014:1–15
29. Yorbik O, Sayal A, Akay C, Akbiyik DI, Sohmen T (2002) Investigation of antioxidant enzymes in children with autistic disorder. Prostaglandins Leukot Essent Fatty Acids 67(5):341–343
30. Jory J, McGinnis WR (2008) Red-cell trace minerals in children with autism. Am J Biochem Biotechnol 4(2):101–104
31 Chapman L, Chan HM (2000) The influence of nutrition on methyl mercury intoxication. Environ Health Perspect. 108(Suppl 1):29–56
32. Cefalu WT, Hu FB (2004) Role of chromium in human health and in diabetes. Diabetes Care 27(11):2741–2751
33. Yorbik Ö, Kurt I, Haşimi A, Öztürk Ö (2010) Chromium, cadmium, and lead levels in urine of children with autism and typically developing controls. Biol Trace Elem Res 135(1–3):10–15
34. Adams JB, Holloway CE, George F, Quig D (2006) Analyses of toxic metals and essential minerals in the hair of Arizona children with autism and associated conditions, and their mothers. Biol Trace Elem Res 110(3):193–209
35. Skalny AV, Simashkova NV, Klyushnik TP, Grabeklis AR, Bjørklund G, Skalnaya MG et al (2017) Hair toxic and essential trace elements in children with autism spectrum disorder. Metab Brain Dis [Internet] 32(1):195–202
36. Olson KR (2003) Poisoning & drug overdose (4th ed.). Appleton & Lange, pp 140–141. ISBN 978–0–8385–8172–8
37. Novotny JA (2011) Molybdenum nutriture in humans. J Evid Based Complement Altern Med 16(3):164–168
38. Tolins M, Ruchirawat M, Landrigan P (2014) The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure. Ann Glob Heal [Internet] 80(4):303–314
39. Rossignol DA, Genuis SJ, Frye RE (2014) Environmental toxicants and autism spectrum disorders: a systematic review. Transl Psychiatry [Internet] 4(2):e360–e423