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Research Article

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Zinc Levels Assay in Children with Autism Spectrum Disorder by Quantum Magnetic Resonance Analyzer and Direct Colorimetry

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Abstract

Background: *Autism is a long-term disability and a developmental disorder in which many studies suggested an association with serum low levels of zinc. In addition, the zinc to copper (Zn/Cu) ratio can be a biomarker of ASD as it is abnormally low in individuals with autism.*

Aim:*The present study aimed to determine the levels of Zn in the bodies of children with autism spectrum disorder (ASD). The assay is done using new technology,the quantum magnetic resonance analysis (QMRA) method, and comparing its results with a reference testing laboratory method to determine the validity, sensitivity, and specificity of the new measurement method.*

Method: *The study was performed in a group of children (M=19; F=11; age range=3-15 years) with ASD (n=30) and a control group of typically developing children (n=30) matched in terms of sex and age. The main variables in this study were the body values of Zn measured with a QMRA-998 8th Generation device and in patients' sera by the reference direct colorimetric method.*

Statistical Analysis: *Results were compared across groups using descriptive statistics, Pearson and Spearman's correlation coefficients, Chi-Square significance, analysis of variance (ANOVA), and linear regression. In addition, a sensitivity and specificity cross-tabulation test was performed to evaluate the QMRA method in measuring Zn levels.*

Results: *Both methods showed lower means for Zn levels in the ASD group than in the control group with a significant correlation between the two methods when measuring Zn levels. With the QMRA method, the sensitivity was 84% and specificity was 87%.*

Conclusion: *It is suggested to test blood levels of Zn in all autistic children and give them a Zn supplement if needed. Non-invasive health measurement devices such as QMRA can be used as a screening or adjunct tool for the measurement of Zn levels in humans.*

Keywords: ASD,Autism, Zinc, Quantum Magnetic Resonance Analyzer, QMRA, Direct Colorimetry

Introduction

Autism is a long-term disability and a developmental disorder defined by the American Psychiatric Association in the Diagnostic and Statistical Manual of Mental Disorders (DSM V)[1]. Serum levels of Zinchave been associated with ASD, and the (Zinc/Copper) ratio can be a biomarker of ASDbeing abnormally low in ASD individuals [2, 3]. Thus, it is indispensable to avail simple, accurate, and reliable alternative analytical methods that conveniently and promptly assess traceelement levels, especially, as screening quick tools in schools or rural and remote locations [4].The normal range of serum Zn is 60-90 µg/dl between the ages of 1-12 months, 80-110 µg/dl between the ages of 1-10 years, and 90-120 µg/dl between the ages of 10-15 years[4]. The normal Zn/Cu ratio is approximately 1:1and its lower limit is 1.4[5].

One of the non-invasive diagnostic tools available now is the Quantum Magnetic Resonance Analyzer (QMRA), which can display the coefficient values of trace elements in the human body. It should not be confused with other similar technologies like Magnetic Resonance Imaging (MRI), Nuclear Magnetic Resonance Imaging (NMR), Quantitative Magnetic Resonance

Imaging (QMR), or Quantum Magnetic Resonance Spectrometry (QRS) even though the latter shares with it the functional principle. This invention gained world recognition as early as 1989 when Professor Dr. Masaru Emoto of Yokohama, Japan received exclusive rights to market the first version of Magnetic Resonance Analyzer, which was alleged to be able to detect the magnetic field around a human hair, for example, and diagnose almost any disease as described in the Ronald Weinstock of the USA patent[6, 7]. Emoto renamed it the "Vibration-o-Meter" and became an operator himself [8].Up until now, very few scientific studies applied QMRA in research and almost none have been published stating that this tool is equivalent to the reference methods. Accordingly, the sensitivity and specificity of the QMRA tool should be confirmed by comparing it with a reference serum test.

The purpose of this study was to compare two methods of Zn levels' measurement in the body; namely, Quantum Magnetic Resonance Analyzing (QMRA) compared to the reference laboratory direct colorimetry method. The result of such a comparison is expected to provide scientific evidence related to the accuracy of QMRA. If the claims for QMRA could be corroborated, such a powerful economical tool could be a more versatile analytical instrument, simple to use, and worthy of consideration. For instance, in the analysis of vitamins and trace elements, it can provide immediate results that will allow anyone to use or suggest individualized, effective protocols to achieve balance and optimal health. If it proves accurate and can acquire the touted multitude of information about the human body, such a simple instrument may help treatment guidelines.

To date, there is no FDA approval for the device[9]. In a Google Scholar recent search, the total studies using QMRA in scientific research that showed up were few and published only in Indonesia and Nigeria. First, in Indonesia, five of them used it as an adjunctive diagnostic tool in the medical field and relied completely on its results as a proven method[10-14].In Nigeria, there were other studies using it in the same manner[15-23]. The only studies that questioned the use of QMRA by actually comparing it to reference laboratory methods were two studies[24, 25].

Subjects & Methods

This present study is a comparative cross-sectional study. The study was carried out in a Center for Children with Special Needs affiliated with a university in Cairo and a Private Pediatric Clinic in Giza; Egypt in the period between January 2019 and September 2021.

Subjects

The study enrolled 60 children;a study group diagnosed as per DSM-V criteria [1] with ASD (n=30) and a controlgroup of typically developing(TD) children (n=30), matched in terms of sex and age(3-15 years).Included groups were well-hydrated and receiving adequate daily intake of water and not on any supplements for the last two months. Excluded were those with chromosomal or neurological disorders other than autism and those on medications that affect serum Zn levels.

Methods

History, Clinical and Psychometric Examination

All children in this study underwent history-taking, anthropometric measurements, and systemic clinical examination. The ASD group had a psychometric assessment includingIntelligence quotient (IQ) by Stanford−Binet Intelligence Scale for Children, diagnosis of autism according to DSM-V,and assessment of the severity of autistic symptoms using the Childhood Autism Rating Scales (CARS)[1,26, 27].

laboratory Investigation

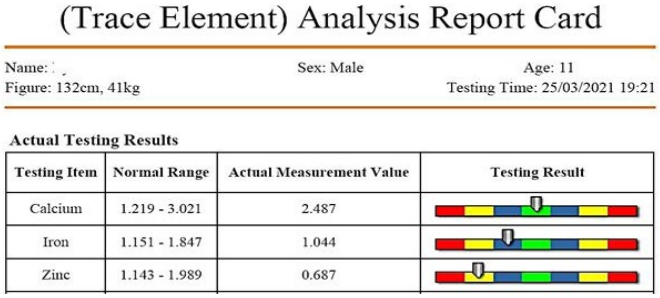
i. The Zn Serum Level by Direct Colorimetric Method with Ready-to-use Kit (Reference Method):With5-Brom-PAPS[28]. The normal range for all children was set at 64-110µg/dl.

*ii. The Zn Body Content by Proposed Quantum Magnetic Resonance Analyzer (QMRA) (Validation Method):*The QMRA tool used in this research was the Korean-design QMRA Model Number: QMR-998/2021.1 manufactured by Guangzhou Zhenyuesheng Electric Co. Ltd; PRC in 2021 (Fig. 1). In this study, the QMRA values used were the electromagnetic coefficients of Zn levels, which were not, unfortunately, explained in the manual (Table 1; Fig. 1 & 2).

Figure 1: The QMR-998 Model Device (Photos taken from the original setup used in the study).

Table 1: QMRA Reference Standards for Zn Levels (From the Machine's Manual).

Reference Standard	Normal(-)	Mildly Abnormal(+)	Moderately Abnormal(++)	Severely Abnormal(+++)
Zinc	1.143-1.989	0.945-1.143	0.532-0.945	<0.532



(Trace Element) Analysis Report Card

Name: Sex: Male Age: 11

Figure: 132cm, 41kg Testing Time: 25/03/2021 19:21

Actual Testing Results

Testing Item	Normal Range	Actual Measurement Value	Testing Result
Calcium	1.219 - 3.021	2.487	
Iron	1.151 - 1.847	1.044	
Zinc	1.143 - 1.989	0.687	

Figure 2: Display of the QMRA analysis results related to trace Elements level (Screenshot from an actual participant test).

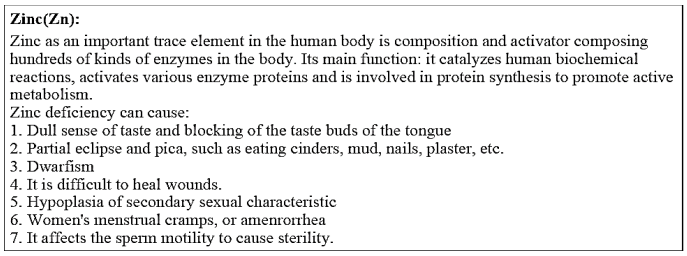


Figure 3: Parameter Descriptions and Recommendations in QMRA Report (Screenshot from a QMRA test).

Statistical Analysis

Data were collected, coded, and then fed to the computer where it was analyzed, and tabulated using the Statistical Package for Social Science (SPSS version 25). For statistical assessment of the differences between the groups according to levels of Zn in the serum and by QMRA device as well as the comparison between the two methods, the collected data were statistically managed by calculating the Test of Independence or Pearson Chi-Square (χ^2), Analysis of Variance (ANOVA), Linear Regression,and Pearson's correlation coefficients. The sensitivity and specificity of QMRA were calculated by cross-tabulation 2x2. The accepted level of significance, *p*-value, was set at ≤0.05. Finally, the results were represented in tabular and diagrammatic forms.

Ethical Aspects

Researchers declare that informed written consent from parents and/or verbal assent from all individual participants involved in the studywas obtained before enrollment in the study. Confidentiality was maintained. The authors declare that they have no conflicts of interest. All procedures performed in studies involving human participants were by the ethical standards of the institutional (Faculty of Postgraduate Childhood Studies and the Ain Shams University) and the national research committees. The Scientific Ethics Committee of the faculty approved the study protocol. This research does not contain any studies involving animals performed by any of the authors.

Results

Anthropometry

Anthropometric means between study groups showed statistically significant differences only with head circumferences (F=6.42; p=0.01) (Table 2). However, generally, the ASD group subjects demonstrated taller, heavier, and higher BMIs than the control group subjects. By way of correlation, the head circumference tended to increase in the ASD group, and, generally, with age (ρ=0.67, p=0.00), height (ρ=0.72, p=0.00), weight (ρ=0.74, p=0.00) and BMI (ρ=0.47, p=0.00). When measured by QMRA, it also correlated inversely with Zn levels (Table 3).

Table 2: Head Circumference Descriptive Statistics and Differences between Groups.

Descriptive Statistics	ASD		Controls		ANOVA	
	Mean	Std. Dev.	Mean	Std. Dev.	*F*	Sig.
Head Circumference	54.27	4.54	51.9	2.35	6.42	0.01

Table 3: Head Circumference Significant Correlations with Other Anthropometrics and Zn Levels.

Variables	Correlations	Age	Height	Weight	BMI	Serum Zn	QMRA Zn
Head Circumference	*Pearson*	0.67	0.72	0.74	0.47	-0.08	-0.21
	Sig.	0.00	0.00	0.00	0.00	0.34	0.05

Clinical Findings

Through the history, general, and systemic clinical examination, the ASD group showed statistically significant differences from the control group. They had more GIT and respiratory abnormalities frequencies such as diarrhea, and pica (χ^2=13.58; p-value=0.00) and higher frequencies of upper and lower recurrent chest infections (χ^2=15.36; p-value=0.00) (Table 4).

Statistically significant differences between both methods measuring Zn levels and clinical findings were found in the whole study sample. These were evident with the gastrointestinal system (serum χ^2=10.62, p-value=0.00; QMRA χ^2=5.66, p-value=0.02) and the respiratory system (serum χ^2=18.22, p-value=0.00; QMRA χ^2=10.09, p-value=0.00) (Table 5). These findings concur with the differences between groups shown above, probably due to low Zn levels.

Table 4: Significant Clinical Findings Distribution of Study Groups.

Findings	ASD	Control	χ^2	p-value
Constipation	2	6	13.58	0.00
Diarrhea	19	6		
Pica	6	0		
Recurrent Infections	17	6	15.36	0.00
Wheezes	5	6		

Table 5: Significant Clinical Findings Distribution as regards Zn Levels by both Methods.

Method	Serum Zn		QMRA Zn	
System	χ^2	*p-value*	χ^2	*p-value*
GIT	10.62	0.00	5.66	0.02
Resp.	18.22	0.00	10.09	0.00

Cognitive Abilities and CARS Scores

Cognitive abilities (IQ) and CARS scores frequencies and descriptive statistics of the ASD groupwereanalyzed. The results of IQ-score in the ASD group showed most patients in the extremely low range. ASD group mean score was 54.97±19.24 (extremely to the very low range) (Table 6). The autism severity test CARS score results showed the highest frequency in the ASD group was in the severe range. The mean was 37.5±3.5 (severe range) (Table 7). There was a highly significant negative correlation between IQ and CARS scores within the ASD group (ρ=-0.485, p=0.01). The higher the CARS, the lower the IQ score. However, correlation there was no statistically significant relationship between the Zn levels in the ASD group with either IQ- or CARS score.

Table 6: IQ Scores Distribution in the ASD Group

Parameters	Frequency	Percent
Extremely Low (≤69)	22	73%
Very Low (70–79)	4	13%
Low Average (80–89)	2	7%
Average (90–109)	2	7%
High Average (110–119)	0	0%
Very High (120–129)	0	0%
Total	**30**	**100%**
Mean ± Std. Dev	***54.97***	***19.236***
Minimum - Maximum	**9**	**90**

Table 7: CARS Scores Distribution in the ASD Group.

Parameters	Frequency	Percent
No ASD (<30)	0	0%
Mild to Moderate (30-36.5)	10	33%
Severe (37-60)	20	67%
Total	**30**	**100%**
Mean ± Std. Dev	***37.50***	***3.50***
Minimum - Maximum	***30.00***	***44.00***

Measurement of Zn by Both Methods

The values of Zn of both groups of children were measured by both traditional *(Direct Colorimetric Method)* and QMRA *(Validation)* methods (Table 8; Figures 4-6).

Table 8: Descriptive Statistics, ANOVA, and Pearson's Correlation of Zn levels by both Methods.

Descriptive Statistics	ASD		Controls		ANOVA		Correlation	
	Mean	Std. Dev.	Mean	Std. Dev.	*F*	Sig.	*ρ*	Sig.
Serum Zn Value (µg/dl)	59.51	11.17	63.77	10.92	2.23	0.14	0.43	0.00
QMRA Zn Coefficient	0.87	0.18	1.31	0.33	39.57	0.00		

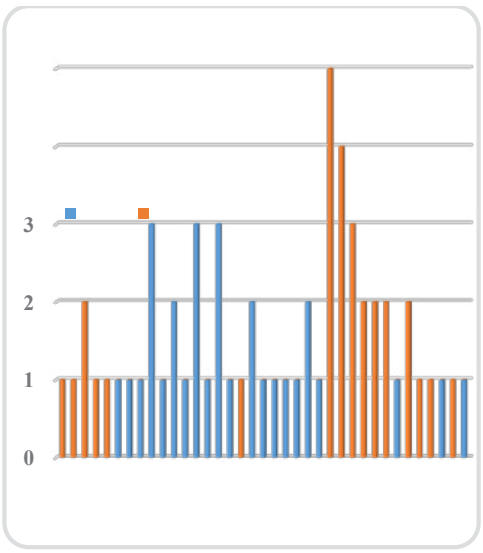


Figure 4: Zn Level Distribution in both Study Groups by Direct Colorimetry.

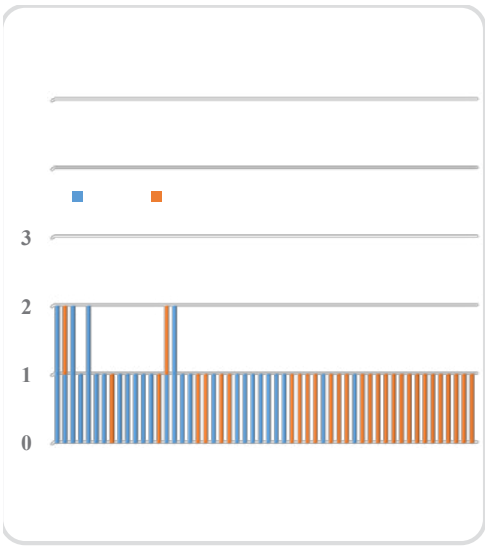


Figure 5: Zn Level Distribution in both Study Groups by QMRA.

Figures 4&5 show a higher association between high Zn serum and QMRA levels with the control group than with the ASD group indicating lower levels.

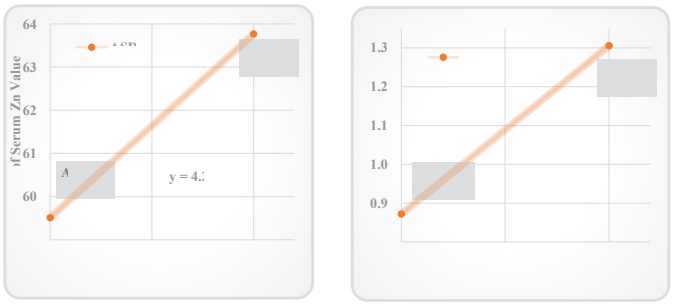


Figure 6: Comparison between both Methods Means for Measuring Zn in both Study Groups

Even though the highest values were reserved for the ASD group, many controls also registered high values.

Prediction of Zn Values by QMRA to Colorimetry

The linear regression analysis demonstrated a model and coefficients for Zn level by QMRA in relation to the Colorimetry measurement of Zn (Table 9). The curve depicting this prediction relation showed converging of values by both methods towards the trendline and expressing the linear equation **(Zn_{Serum} =13.924* Zn_{QMRA} + 46.478 (µg/dl); R^2=0.1832) (Fig. 7).**

Table 9: Linear Regression Model and Coefficients for QMRA to Serum ZnLevels.

Model Summary		**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
Serum Zn*QMRA Zn levels		**0.428**	**0.183**	**0.169**	**10.175**
ANOVA	Sum of Squares	df	Mean Square	*F*	Sig.
Zn Regression	1347.03	1	1347.03	13.011	0.001
Coefficients for QMRA Levels	Unstandardized Coefficients	Std. Error	Standardized Coefficients	*t*	Sig.
*QMRA Zn Coefficient**	13.924	3.86	0.428	3.607	0.001
Zn (Constant)	46.478	4.404		10.555	0
**The independent variablesare QMRA Coefficients.*					

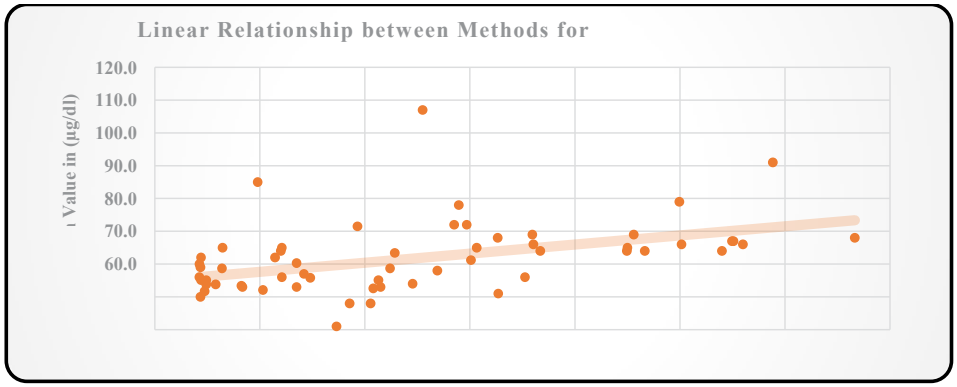


Figure 7: Linear Regression Analysis for both Methods in measuring Zn.

QMRA Specificity and Sensitivity

Cross-tabulation (two-by-two) of the measurements of serum vs. QMRA Zn levels was performed to find out the sensitivity and specificity as well as the true and false positive and negative readings of QMRA (Table 10).

Table 10: Sensitivity, Specificity, PPN, and NPV Values for QMRA in Reference to Direct Colorimetric Method.

QMRA Zn Sensitivity & Specificity			**QMRA Zn**			
			Positive	**Negative**	**PPV**	**NPV**
Zn Levels	*Positive*	Count	31 (TP)	3 (FP)	91%	77%
		%	83.80%	13.00%		
	Negative	Count	6 (FN)	20 (TN)		
		%	16.20%	87.00%		
	Sensitivity			Specificity		
	84%	87.00%				

TP= true positive, ***FP***= false positive, ***FN***= false negative, ***TN***= true negative, ***Sensitivity*** = (true positive) / (true positive + false negative) = Probability of being test positive when disease present. ***Specificity*** = (true negative) / (true negative + false positive) = Probability of being test negative when disease absent. ***Positive Predictive Value (PPV)*** = (true positive) / (true positive + false positive) = Probability (patient having disease when test is positive) and ***Negative Predictive Value (NPV)***= (true negative) / (false negative + true negative) = Probability (patient not having disease when test is negative).

Discussion

Defined by APA in the (DSM V), ASD is a neurodevelopmental disorder with a group of long-term disabilities[1]. Many studies suggested an association between serum levels of Zn and ASD, and that the Zinc to copper (Zn/Cu) ratio, being low, can be a biomarker of ASD[2, 3]. Thus, it is indispensable to find simple, accurate, selective, and reliable alternative analytical methods that can conveniently, and promptly assess trace elements' levelsin the body[4].The present study aimedto determine the levels of Zn in ASD children by two methods and to compare their results. The assay was done using a new technology (QMRA) method and its results were compared with a reference laboratory method (direct colorimetry) to determine the sensitivity and specificity of the new measurement method.

The study was performed on a group of children (n=30; M=19; F=11; age range=3-15) with ASD and a control group of typically developing (TD) children (n=30) matched in terms of sex and age in a university center for children with special needs in Cairo, and a private clinic in Giza from Jan 2019 through September 2021. The measurement of Zn was performed with a QMRA-998 8th Generation device and in patients' sera by the reference direct colorimetric method.

As far as specific anthropometric measures are concerned, body composition and anthropometric measures of both groups revealed that the ASD group demonstrated more height (ASD=132.57±22.45; Controls=125.67±19.75 cm), height for age percentile, weight (ASD=37.20±18.32; Controls=31.47±14.61 Kg), weight for age percentile, head circumferences (ASD=54.27±4.54; Controls=51.90±2.35 cm) and BMIs (ASD=19.87±4.71; Controls=18.82±3.41 Kg/m2) than with the control group subjects. Specifically, results showed statistically significant differences in head circumferences (ANOVA: F=6.42; p=0.01) between children with and without ASD. The head circumference tended to increase with gender (female), age (ρ=0.67, p=0.00), height (ρ=0.72, p=0.00), weight (ρ=0.74, p=0.00) and BMI (ρ=0.47, p=0.00). The results agree with those of other authors who found a higher prevalence of bigger head circumferences tallerandheavier with greater BMIs in ASD than in TD children [29-34]. The head circumference significantly increased in females and/or older children, suggesting the relative overgrowth of the brain in this sample with autism[29-32, 35-37]. The higher tendency to higher BMI is probably due to a sedentary lifestyle, food selectivity, and restrictive diets in people with ASD that may affect their growth with a probable decrease in muscle mass and increase in subcutaneous fat thickness [32, 38]. Therefore, increased fat composition in autistic children with decreased muscle mass necessitates tailoring of specially designed food supplementation programs to ameliorate the severity of autism symptoms [32].

Complete history taking, medical examinationof major relevant body systems, and neuropsychiatric examination were done on all subjects from both groups. Generally, in both groups, neck, cardiovascular and abdominal examinations were normal but many of the ASD patients showed neuromuscular and language symptoms, stereotypies, and hyperkinesia. These findings agree with findings of other studies in the literature[39-43]. In addition, the ASD group expressed higher frequencies of some clinical findings with statistically significant differences than the control groupin two domains: with GIT symptoms, e.g. diarrhea, and pica ($\chi2$=13.58; p-value=0.00), and with respiratory symptoms, e.g. upper and lower recurrent chest infections ($\chi2$=15.36; p-value=0.00) in agreement with other research[44-47]. In addition, in ASD, the range of co-morbid disorders, particularly those of gastrointestinal origins, greatly exceeded that of the general population [44].Similarly, theZn levels significantly correlated withgastrointestinal and respiratory systems' findings coinciding with the same differences between the two study groups. This is in agreement with literature correlatingzinc deficiency with chronic diarrhea due to lossand pica interfering with zinc absorption [48-50]. Subsequently, it confirms that autistic children, particularly those with GI disease, would have abnormal serumlevels of Zn [51]. As regardsthe recurrent chest infections exhibited in the ASD group, they may be related to low immunity due to low Zn.In such cases, the Zn level can be used as a biomedical marker relating to inflammation, immune dysfunctions, intestinal dysfunctions, and infections[45, 47].

The cognitive abilities of the ASD group were assessed by the Intelligence quotient (IQ)using the Arabic forms of the Stanford−Binet Intelligence Scale for Children [26]. The IQ score results showed that 73% were in the Extremely Low (≤69), 13% in the Very Low (70–79), and 7% in both the Low Average (80–89) and Average (90–109), which did not fully agree with the percentages according to CDC[52]. The CDC reports that only 31% of children with ASD have an intellectual disability (IQ <70), 25% are in the borderline range (IQ 71–85), and 44% have IQ scores in the average- to above-average range (i.e., IQ >85). In our ASD group, the mean score was 54.97±19.24 (extremely- to the very-low range), min=9, max=90. In addition, the autism severity test (CARS) score results showed the highest frequency in the ASD group to be in the Severe (37-40) range (67%) followed by the Mild to Moderate (30-36.5) range (33%). The mean was 37.5±3.5 (severe range). There was a highly significant negative correlation between IQ and CARS scores within the ASD group (ρ=-0.485, p=0.01) indicating that the lower IQ, the higher the CARS score or vice versa[53].

At this point, the correlation of the Zn levels showed significance with IQ-score but not with CARS scores (ρ=-0.41, p=0.03). Hypothesizing that low Zn is responsible, this result should confirm that zinc is essential for the normal development and function of biological systems including the CNS and that it is beneficial

to intelligence[54]. Zinc deficiency may lead to deficits in children's neuropsychological functioning, activity, or motor development, and thus, interfere with cognitive performance[55]. Being an essential catalytic or structural element of many proteins and important for neural activity and the control of neuronal death, zinc may be an important trace element for the etiology and symptoms of subgroups of ASD[56]. However, since the results did not confirm a correlation with CARS score, they did not match the results of other studies reporting a significant negative association between the Zn/Cu ratio and CARS scores [2].

The normal ranges mentioned in the literature for serum Zn is 60-90 µg/dl between the ages of 1-12 months, 80-110 µg/dl between the ages of 1-10 years, and 90-120 µg/dl between the ages of 10-15 years[5, 4]. However, the value we employed in the study was 63.8-110 µg/dL for all children of all age groups as per the laboratory method reference range provided by the manufacturers. As for QMRA, the values used were the coefficients of Zn levels of which the normal value range is 1.143-1.989. A coefficient value for Zn >1.989 is considered to show an increase in its levels and a coefficient <1.143 is considered to show a decrease in its levels. Unfortunately, the coefficient values of the measurement of Zn levels using electromagnetic waves by the QMRA tool have not been explained in the manual.

Disturbanceof the Zn/Cu ratiooccurs mainly because of differences in the level of Zn, which may be attributed to dietary deficiencies[57-59]. The etiological mechanism of disturbed Zn/Cu metabolism may be due to reduced activity of the detoxification mechanism of metallothionein and/or Cu/Zn SOD1, resulting in excessive oxidative stress and damage to brain cells[60, 61]. Conversely, it may be due toreduced levels of Zn as a result of increased oxidative stress, which increases the activity of the metallothionein system and its binding to Zn and Cu, leading to their reduced availability for activity in other enzymatic pathways[4]. These disturbances, however, are not specific to ASD. They were also reported with other developmental and behavioral disorders such as ADHD [62-64]. In the present study, we did not examine other cases than ASD; therefore, it may be reasonable to extend the study to include other neurodevelopmental disorder cases. Moreover, regardless of the mechanism of the origin of Zn/Cu metabolic disturbances, considering the results of the present study, it would be reasonable to add Zn to the food in all those children with ASD who have reduced levels of Zn or low Zn/Cu ratio in the plasmaas reports indicate the positive effect of treatment with Zn in certain children with ASD[65, 66].

Measuring Zn by the two methods, both methods showed lower means for Zn levels in the ASD group (serum=59.51±11.17 µg/dL; QMRA Coefficient=0.87±0.18) than in the control group (serum=63.77±10.92 µg/dL; QMRA Coefficient=1.31±0.33) where the QMRA method was even more sensitive to detection of the differences between groups (ANOVA F=39.57; p=0.00). This was in agreement with other authors as the results of our study revealed lower serum Zn levels in the ASD group than normal reference values as well as when compared to another group of typically developing children [2, 4, 5]. The correlation of both measurement methods as regards Zn levels in the whole study population demonstrated high significance (ρ=0.43; p=0.00). The linear regression predictions of the relationship between serum Zn levels observed readings against QMRA coefficients revealed that Zn regression was significant by the ANOVA (F=13.011; p=0.001), and coefficient (t=3.607; p=0.001). The regression curve for Zn showed a converging of values by both methods towards the trendline with the linear equation (ZnSerum =13.924* ZnQMRA + 46.478 µg/dl; R2=0.1832).In addition, the evaluation of the QMRA method sensitivity was 84% while specificity was 87%. Moreover, the ability of QMRA to detect patients having abnormal Zn values when the test is positive or Positive Predictive Value (PPV) is (91%) and its ability to detect not having abnormal Zn values when the test is negative or Negative Predictive Value (NPV) is (77%).

Finally, even though it is touted by the manufacturers as having been developed by a team of medical and computer experts based on the study of 100,000,000 clinical cases over many years with an alleged accuracy rate falling between 85- 95%, QMRA is still a disputed scientific invention becauseonly a few studies are published using QMRA scientifically [67, 68]. Castañeda et al. (2018) in Mexico and Muflih et al. (2019) in Indonesia were the only researchers that questioned the use of QMRA comparing it to reference measurement methods and demonstrated no concrete correlation between QMRA and these reference methods[24, 25]. However, as reported by QMRA proponents and manufacturers, trace elements measurement is only one function that QMRA is supposed to do among more than 40 other functions. In our present study, our results for measuring Zn by QMRA were satisfactory to a great extent and substantiate its use as an adjunctive diagnostic tool as was reported by other scientists in their studies in patients with disabilities and similar conditions as in our study [10,11, 12, 13, 14, 18, 19, 20, 22, 23, 69, 70].

The strengths of this study are that the results were obtained from a representative sample of pre-school- and school-age children. We controlled the group for such potential confounders as ethnicity, socioeconomic level, diet, chelating agents, and supplements. As far as assessment is concerned, no previous studies were published comparing QMRA in measuring trace elements with a reference method and only a few have used QMRA in scientific research, especially in the ASD population.

One of the limitations of the study is that not many of the subjects had been exclusively diagnosed with ASD without other neurodevelopmental disorders. The study did not aim at including other patients with other neurodevelopmental disorders in which disturbances of Zn levels may occur, e.g. ADHD. We also had a lower proportion of ASD girls than boys, so we were unable to obtain comprehensive results on differences between the two sexes. In addition, the study was comparative only for Zn measurements and no other trace elements or other clinical diagnoses.

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Conflicts of Interest
The authors declare no conflicts of interest.

Institutional Review Board Statement
The study was conducted according to the guidelines and approved by The Scientific Ethics Committee of the Department of Medical Studies for Children, the Faculty of Postgraduate Studies of Childhood, and the Ain Shams University.

Informed Consent Statement
The authors reviewed and agreed to the published version of the manuscript.

Data Availability Statement
The data that support the findings of this study are available on request from the corresponding author.

Discussion
Among the results, the significantly low plasma Zn in children with ASD compared to the control group of children stood out even though it was noted that both groups, generally, had a low Zn level that may have resulted from poor diet quality. The QMRA as a possible diagnostic non-invasive tool, one of whose functions is to measure body trace elements such as Zn accurately to 85-95% precision, may not have provided an exactsimilarity of the Zn levels of the study participants compared with the traditional laboratory (Direct Colorimetric) method. However, its correlation, sensitivity, and specificity tests were high in measuring Zn.

Recommendations
1. Addition of Zn to food in the case of its insufficiency, especially in cases of neurodevelopmental diseases such as ASD.
2. QMRA assay method is not recommended as the sole reference for measuring trace elements in the body until further more controlled studies are performed.
3. QMRA can be used as a screening or adjunct tool for measuring Zn levels in humans, especially in poor, remote, or rural location where it is difficult to have access to proper laboratory services.
4. More research is warranted to test the other functions of QMRA technology as a medical diagnostic and/or therapeutic tool.

List of Abbreviations
AAP American Academy of Pediatrics.
ADHD Attention Deficit Hyperactivity Disorder.
APA American Psychiatric Association
ASD Autism Spectrum Disorder.
BMI Body Mass Index
BMR Basal Metabolic Rate
CARS Childhood Autism Rating Scale.
CDC Centers for Disease Control and Prevention.
CNS Central Nervous System.
Cu Copper.
DSM-V Diagnostic and Statistical Manual of Mental Disorders—5th Edition.
IQ Intelligence Quotient.
MRI Magnetic Resonance Imaging.
QMR Quantitative Magnetic Resonance Analyzer; Analysis.
QMRA Quantum Magnetic Resonance Analyzer; Analysis.
QRA Quantum Resonance Analyzer; Analysis.
QRS Quantum Resonance Spectrometry.
SB5 Stanford–Binet scales—5th Edition.
TD Typically Developing.
Zn Zinc.

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