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Zinc Deficiency in Autism: A Controlled Study

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Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised by impaired socialisation and restricted and repetitive patterns of behaviour. Zinc deficiency has previously been reported in patients with ASD. A retrospective controlled trial of serum zinc levels in patients with ASD vs. non-ASD controls was undertaken to explore the potential presence of zinc deficiency in the ASD population. 72 patients with ASD were compared with 234 non-ASD controls. Serum zinc levels were compared between groups and correlations analysed for age, sex, supplement use and diet. Serum chromium and manganese levels were also compared between ASD and control groups to assess general micronutrient status. Further analysis was undertaken in the ASD group investigating potential correlations between serum zinc levels and immune function. 86% of patients with ASD were found to be zinc deficient versus 24% of the non-ASD control group. There was a mean difference of serum zinc levels between the ASD and non-ASD groups of 1·75 µmol/l (P<0·001, CI 1·2-2·1). There was no effect of age or sex on serum zinc levels in either the ASD or control groups. There was no significant difference in chromium or manganese levels between the ASD and control group. These results suggest zinc deficiency is likely to be common in ASD patients and is a potentially modifiable environmental factor associated with the condition. Zinc's potential role in the aetio-pathogenesis and disease evolution is discussed, and the need to consider zinc status in patients with ASD is highlighted.

Keywords: Autism; Zinc; Lymphocytes; Neurodevelopment

Abbreviations: AP-1: Activator Protein 1; ASD: Autism Spectrum Disorder; CDC: Centre for Disease Control; CNS: Central Nervous System; EED: Environmental Enteric Dysfunction; ESR: Erythrocyte Sedimentation Rate; GABA: Gamma-Aminobutyric Acid; NFAT: Nuclear Factor of Activated T-Cells; NMDA: N-Methyl-D-Aspartate; SEM: Standard Error of Mean; VOCs: Volatile Organic Chemicals

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Introduction

There is increasing efforts to understand the etio-pathogenesis, pathologies and evolution of Autism Spectrum Disorder in a concerted effort by the scientific community to provide prevention, harm-reduction and treatment modalities for what can be a debilitating neurological disease. Within these efforts there are several insights worth considering from the body of scientific investigations [1-3].

1. ASD is a multi-factorial disease.
2. There is significant variation in outcome.
3. The condition is highly heterogeneous.

Whilst it is expected that there are multiple factors leading to the development of ASD, there remains the possibility of identifying independent risk factors, and preventing the disease, reducing the severity and/or reducing the burden of disease. Exploring co-factor metabolism and micronutrient deficiency has previously proved successful in reducing rates of other developmental disorders, with notable examples being the identification of maternal iodine deficiency as the major cause of congenital hypothyroidism and folate supplementation as a preventative intervention for Neural Tube Defects. It is worth noting that prior to the introduction of prenatal folate supplementation and folic acid fortification of foods, heritability for Neural Tube Defects was around 70% [4]. Exploring general health parameters in

patients with ASD, such as nutrient status is also useful in the attempt to reduce the burden of co-morbid illness.

Epidemiology of autism

Recent evidence suggests a high level of disability in affected individuals, with 60-75% achieving poor or very poor outcomes in adulthood [5]. Case detection rates continue to increase. The Centre for Disease Control (CDC) reported a prevalence of 1 in 110 eight year olds in 2006 (Prevalence of autism spectrum disorders - Autism and Developmental Disabilities Monitoring Network, USA, 2006. 2009), a rate of 1 in 88 in 2008(Prevalence of autism spectrum disorders--Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. 2012) and a rate of 1 in 68 in 2010 (Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, USA, 2010. 2014). Early twin studies suggested a predominant genetic component; however, these studies had weak power [6,7]. Indeed, both studies reported a 0% dizygotic twin rate. A more recent twin study published was well designed and of high statistical power. The study concluded: environmental factors have a strong causative role in autism [8].

Environmental factors in autism

Evidence for modifiable environmental risk factors in ASD is significant. Gardner et al. undertook a recent meta-analysis exploring prenatal and maternal risk factors in the development of autism. Whilst significance was found in several domains, the need for further, more extensive studies was apparent. Possible risk factors identified included advanced maternal and paternal age at birth, maternal gestational bleeding, gestational diabetes, being first born vs. third or later, maternal prenatal medication use, and maternal birth abroad [9].

Xenobiotic exposure has recently been reviewed in detail and a strong correlation was found for autism risk and air pollution, pesticides and volatile organic chemicals (VOCs). Air pollution is worthy of considerable note, given the increasing evidence for the effects of air pollution on cognitive impairment and neurobehaviour; immunotoxicity; neurotoxicity; autonomic effects and autism specifically [10-29].

Zinc: Zinc is the second most abundant metal in the human body (second only to iron). It is essential for cellular life. Closely associated with DNA, zinc is a rate-limiting co-factor in hundreds of enzymatic processes including the polymerases underpinning protein synthesis generally [30]. At this basic level zinc is involved in gene expression and epigenetic mechanisms, and as such zinc deficiency can manifest across a diverse range of body processes, depending on individual genetic factors.

Beyond the biochemical functions of zinc (not limited to co-factor metabolism), zinc has a biophysical role throughout the body. Zinc-finger motifs are proteins, often configuring cell receptors, where zinc has a crucial role in allowing functional folding to occur, thus permitting the receptor function [31].

Zinc has also gained increasing attention for its role in cell-signaling [32,33]. Zinc has been demonstrated to provide immune and nervous system signaling, inducing T cell proliferation and activation, as well as mediating NMDA and GABA receptors centrally [34,35].

Zinc is predominately found in meat, fish, dairy, nuts and grains. Absorption is dependent on the digestion of proteins to release the mainly protein bound zinc, and the passage of zinc to the jejenum without microbial uptake or binding with inhibitors. The absorbed zinc transiently increases plasma zinc levels prior its incorporation into the extravascular space with the greatest quantity of zinc being present in bone and skeletal muscle with high concentrations being found in brain, testes, skin, kidney, liver and placenta [36,37]. Whilst zinc is considered a non-stored essential element, the extravascular pool serves as a form of zinc storage with the majority of zinc being bound to proteins such as albumin. During times of shortage a reduction in faecal loss occurs, followed by release of zinc from bone, skeletal muscle, and other organs through a shifting pool homeostatic mechanism. Bone appears to have greater resilience to zinc deficiency, and failure of bone growth is typically a late feature of marked zinc deficiency [36]. The most recent national nutritional survey calculated average zinc levels in 4 to 18 years of age to be 14.7 μmol/l [38].

Zinc deficiency in early life: There have been limited human trials with prenatal zinc supplementation, the majority of studies beginning zinc supplementation during gestation. Prenatal zinc supplementation has been shown to improve autonomic function in children versus controls, and this improvement was noticeable during gestation [39-41]. Maternal zinc deficiency has been shown to increase obstetric complications and zinc deficiency may be involved in the increased obstetric risk associated with higher maternal age in a low socioeconomic group [42,43]. A recent meta-analysis of zinc supplementation given during gestation demonstrated a statistically significant reduction in preterm labour [44]. The meta-analysis included only one study where supplementation began prenatally. A recent Cochrane review of zinc supplementation on mental and motor function in children also found a low number of high-power studies and substantial study differences [45]. Of the 12 studies included, none supplemented zinc prenatally or maternally, and only two studies followed up beyond 13 months of age [46,47].

Zinc and immune function: The role of zinc and the effect of zinc deficiency on the immune system has been investigated in several reviews [48-52]. It has been suggested that under chronic zinc deficiency conditions the adaptive immune system is less efficient and dependence on the innate immune system occurs, despite the innate immune system also suffering impairment under zinc deficiency conditions [49-52]. Neutrophils appear sensitive to zinc deficiency with impaired production, recruitment and phagocytic function. The cascade to adaptive immunity is also sensitive to zinc, with zinc deficiency causing impaired T-cell and B-cell production, reduced cytokine signaling and ultimately impaired function.

With the general shift to a Th2 response, impaired phagocytosis, reduced antibody production and the reduced cell-mediated messaging that occurs with zinc deficiency, it is of little surprise

that zinc supplementation is utilized as a first line augmentation in the management of diarrheal illnesses and respiratory infections worldwide **(Table 1)**. Zinc has been reported to be anti-inflammatory, and zinc deficiency to increase pro-inflammatory cytokines (Bonaventura *et al.* 2014) and to increase central nervous system (CNS) inflammasome activity [53].

Zinc and the Nervous System: Zinc and the nervous system has been reviewed recently [54,55]. It is useful, given zinc's many functions, to consider the three broad categories: structure, cell-signaling and enzymatic co-factors. Zinc is a required co-factor for DNA and RNA polymerases, histone catalyses and DNA ligase. As such, zinc is involved in most aspects of protein synthesis within the CNS, and is an independent factor involved in gene expression [56]. More recently zinc has been identified as a key component in structural proteins such as zinc-finger motifs [54]. These ubiquitous proteins often form the structure of receptors such as the oestrogen, thyroid hormone and glucocorticoid receptors in the brain [57]. The presence of zinc within these proteins allows folding and the formation of the functional structure of such receptors [58]. The effects of zinc deficiency on zinc-finger motifs, and whether zinc is liberated from such proteins under chronic zinc deficiency, remain unknown.

10-20% of CNS zinc is considered free, and is largely present pre-synaptically, and more often in the glutamenergic neurons. The release of zinc has been shown to modulate post-synaptic receptors including NMDA, GABA and voltage-gated calcium channel receptors. Zinc is essential to normal brain development.

Zinc has been implicated in olfactory, cerebellum and hippocampal development, and even mild zinc deficiency has been shown to affect memory and learning [59-62]. It has also been demonstrated that transient gestational zinc deficiency can affect memory and learning that persists into adulthood [63].

Zinc and Autism: Zinc has been examined in autism **(Table 2).** The lack of population based longitudinal studies hampers the generalisations to the aetio-pathogenesis of ASD. Taken together the studies conducted do though suggest zinc deficiency may be common in ASD.

Methods

Serum zinc levels were identified in 72 unique individual patients attending neurodevelopmental outpatient clinics. The inclusion criteria for cases in the study was male and female patients under 16 with a confirmed diagnosis of ASD without a previous confirmed diagnosis of zinc deficiency or evidence of treatment with zinc replacement therapy. The notes of the patients were individually scrutinized to ensure only those with a confirmed diagnosis of ASD were included in the study. The files were also analyzed to ascertain whether a previous diagnosis of zinc deficiency had been made and whether the patient was or had been on zinc replacement therapy – such individuals were excluded from analysis.

Case files were further analyzed for immune related parameters i.e., differential white cell count, ESR and platelets, to ascertain if zinc status influenced immune function in ASD patients. Plasma manganese and chromium levels were also recorded as a method of recording general nutrient status. A control group was extracted from general outpatient clinics. Following exclusion of those over 16 years of age, the control group consisted of 234 individual patients analyzed in the same time period as the ASD-cohort. These results were analyzed in SPSS.

Table 1 Summary of previous studies exploring a relationship between zinc and immune function.

Immune Cell	Effect of zinc deficiency	Study
Neutrophil Granulocytes	Impaired recruitment	60
Neutrophil Granulocytes	Impaired chemotaxis	61
Neutrophil Granulocytes	Reduced total number	55,62
Neutrophil	Impaired Phagocytosis	63
Monocytes	Altered cytokine production (TNF-alpha & IL-6)	74
B-lymphocytes	Reduced total count	68
B-lymphocytes	Impaired antibody production	69,70
T-cell	Impaired development (in thymus)	71
T-cell	Impaired peripheral function	72
T Cell	Altered cytokine signalling	40,67,73
T-Cell	Altered LPS activation pathway	38
CD-8	Impaired proliferation	72
Natural Killer Cell	Reduced Activity	63,66
Natural Killer Cell	Reduced total count	64,67

Measurement of serum zinc levels were completed via the standardized Gas Chromatography Mass Spectrometry methodology via a routine clinical laboratory.

Statistical Analysis

Differences between the case and control groups were analyzed using a standard homoscedastic unpaired 2-tailed T-Tests, unless there was statistically significant evidence that the standard deviations were different: This was determined using Levene's Test. If evidence of hereroscedasticity was found, then Welch's T-Test assuming unequal variances was used to better estimate p-values and confidence intervals. Parametric analysis was used over non-parametric analysis as the sample-size of the control and experimental groups make it reasonable to expect that the standard error of the mean-difference between groups should be normally distributed. Additionally, a multivariable analysis was conducted to investigate the effect of age and sex on zinc status. This was conducted using a Factorial ANOVA as the data did not meet the criteria for an ANCOVA analysis.

A hypothesis generating investigation of the relationship between zinc status and white-cell count was undertaken in the case group. Correlations were analysed across the entire ASD case group and in sub-groups determvned by nutritional status. These were analysed both parametrically and non-parametrically using Pearson's and Spearman's correlations respectively as it is unclear in this instance, due to smaller sample sizes of sub-groups, whether the conditions for parametric analysis were met.

Possible confounding due to age, sex, dietary factors and nutritional supplementation in the case group was also undertaken to see if these impacted zinc levels or white blood cell counts.

Results

After applying exclusion criteria there were 72 ASD cases and 234 controls. In the ASD group mean age was 7.0 yrs. of age (range 2-16) and in the control group was 10.1 yrs. of age (range 2-16). Male to female ratio in the ASD group was 3.8 and in the control group was 1.3. The ASD-cohort had a mean plasma zinc level of 10.01 µmol/L (SD 1.52 µmol/L) and the case controls had a mean plasma zinc level of 11.76 µmol/L (SD 2.14 µmol/L). The ASD-cohort had a significantly reduced plasma zinc level than controls (Mean Difference = 1.75 µmol/L, $P<0.0001$ CI 1.2 to 2.3). The results withstood correction for age and sex. There were no significant differences between ASD and controls in relation to Manganese or Chromium **(Table 3).**

Box whisker plots show the distributions of zinc, chromium and manganese in the ASD-group in comparison to controls **(Figure 1).** Mean lymphocyte count in the ASD cohort was 3.68×10^{-9}/L (SD 1.6). There was a significant correlation between total lymphocyte count and plasma zinc levels when zinc was over 10.5 umol/l in the ASD cohort ($P<0.04$). When zinc fell below 10.5 µmol/L there was no direct correlation with lymphocyte count, although lymphocyte count was lower generally at a mean of 3.23×10^{-9}/L (SD 1.8).

Confounders

Factorial ANOVA was used to determine whether Age or Sex had an effect on mineral status. There are no statistically significant interaction between case/control group and age; case/control and sex; and case/control, age and sex for zinc **(Table 4).**

Of the 72 patients in the ASD-cohort, 41 patients had reliable medical records of whether the patient was following a gluten-free diet or a gluten-containing diet. Of these, 22 patients were at the time of blood analysis maintaining a gluten-free diet, 19 were not. Zinc did not differ significantly between the two groups (gluten-free diet = 9.96 µmol/l (SEM 0.36), not gluten-free diet = 9.74 µmol/l (SEM 0.36)). Lymphocyte count was higher in the gluten-free group versus the not gluten free group (4.68×10^{-9}/L, SEM 0.54 vs. 3.02×10^{-9}/L, SEM 0.37).

Of the 72 patients in the ASD-group, 41 patients had reliable record of presence or absence of a dairy-free diet. 24 patients were on a dairy-free diet at blood analysis. Zinc did not differ significantly between the dairy-free and not dairy free groups (9.75 µmol/l

Table 2 A summary of previous studies exploring a relationship between zinc and autism.

Sample	Number	Results	Comments	Study
Hair and nail	95 (50 controls)	Low Zinc correlated with Low Functioning Autism. Zinc levels in hair and nails correlated with CARS scores.	Authors noted higher variability of zinc levels in ASD vs. controls.	86
Serum	37 (patients with Phelan-McDermit Syndrome)	Zinc deficiency correlated with attention deficit and hyperactivity and seizures	Authors also undertook transgenic work demonstrating alteration of ProSAP/Shank levels, and alterations in cerebellum and hippocampal volumes.	87
Plasma	79 ASD & 18 controls	No correlation identified	Limited details on controls, and samples were not fasting.	88
Hair and Urine	25 ASD & 25 controls	Zinc hair levels were significantly lower in the ASD group.	Limited participant numbers.	89
DNA-samples	761 low verbal autism ASD	*ZNF804A* SNP was associated with Low Verbal Autism.	Analysis of zinc associated transporter. No zinc analysis was conducted	90
Serum	230 ASD	20.4% deficient 50.8% in lower ten percent of mean normal values	Study lacked control group.	91
Hair	1967 ASD	50% under 3yrs had zinc levels below 2SD versus 30% of all ASD children analysed	High numbers, and age correlation significant. Lack of controls.	92
Plasma	102 ASD & 18 controls	Zinc deficiency correlated with hyperactivity and fine motor skills impairment.	Limited controls.	93
Hair	44 ASD	50% were deficient for zinc. Zinc negatively correlated with fear and nervousness and verbal communication.	Low participant numbers and lack of controls.	94
Serum	60 ASD vs. 60 Controls	Zinc levels were significantly lower in the ASD group.	Zinc: Copper ratio was associated with symptom severity on CARS	95

Table 3 Means levels of plasma elements, difference and significance in ASD Group and Non-ASD Control Group.

Variables	ASD group (n=72)	Control Group (N= 234)	Difference (umol/l)	Significance (Confidence Interval)
Zinc (serum levels as umol/l)	10.01	11.76	1.75	$P<0.001$ (CI: 1.2 – 2.2)
Chromium (serum levels as umol/l)	15.26	16.42	1.16	$P=0.20$ (CI, -0.6 – 2.9)
Manganese (serum levels as umol/l)	14.69	14.48	-0.21	$P=0.77$ (CI -1.6 – 1.2)

Table 4 Comparison of zinc status (and other plasma minerals and white cells) in ASD patients adhering to a gluten-free diet (n=22) and ASD patients not following a gluten-free diet (n=19), dairy-free diet (n=24) and not dairy-free diet (n=17), and those on supplements (n=18) and those not on supplementation (n=23). Standard deviations are in brackets. WCC: White Cell Count.

Variables	Gluten-free (n=22)	Not gluten-free (n=19)	Dairy-free (n=24)	Not dairy-free (n=17)	Supplements (n=18)	No supplements (n=23)
WCC ($\times 10^{-9}$/L)	8.89	8.14	8.63 (3.87)	8.42 (3.94)	9.13	8.09
	(3.74)	(3.80)			(3.87)	(3.65)
Lymphocyte Count ($\times 10^{-9}$/L)	4.68	3.02	4.34 (2.66)	3.31 (1.57)	4.59	3.38
	(2.55)	(1.63)			(2.49)	(2.05)
Zinc (umol/l)	9.91	9.74	9.75 (1.51)	9.95 (1.59)	9.95	9.75
	(1.61)	(1.45)			(1.74)	(1.38)
Manganese (umol/l)	17.12	12.37	17.11	11.79	17.72	12.96
	(6.55)	(3.83)	(6.60)	(2.59)	(7.56)	(3.29)
Chromium (umol/l)	15.40	13.84	15.42	13.62	14.87	14.54
	(3.63)	(3.80)	(3.60)	(3.79)	(2.72)	(4.44)

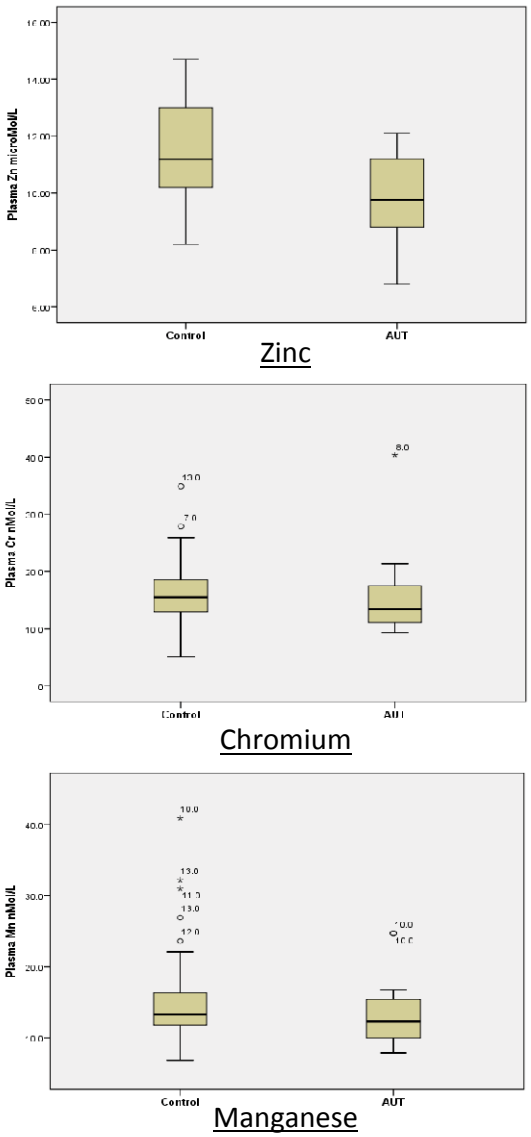

Figure 1 Box-whisker plots of serum zinc, chromium and manganese levels in ASD group (n=72) and control group (n=234).

SEM 0.31 vs. 9.95 µmol/l SEM 0.4). Again, lymphocyte count was higher in the dairy-free group than the not dairy-free group (4.34 × 10^{-9}/L, SEM 0.34 vs. 3.31 × 10^{-9}/L, SEM 0.38).

Of the 72 patients in the ASD-cohort, 41 had reliable record of supplement usage. 23 were taking nutritional supplementation. Zinc levels did not differ greatly between the supplemented and non-supplemented groups (9.95 µmol/l SEM 0.42 vs. 9.75 µmol/l SEM 0.29). Plasma manganese was significantly higher in the supplemented group (17.72 µmol/l SEM 1.83 µmol/l vs. 12.96 umol/l SEM 0.69 µmol/l).

Discussion

The current study examined plasma zinc levels in patients with ASD in comparison to a control group. Overall, 82% of patients with ASD were classified as deficient (< 11.5 umol/L), 57% had plasma zinc levels below 10.5 umol/L and over 25% had zinc levels below 8.80 umol/L. The low plasma zinc level appeared to be having systemic effects with lymphocytes correlating with zinc levels above 10.5 umol/L (P<0.04), and below 10.5 umol/l total lymphocyte count was lower than the group mean, and lower than the population mean (P<0.04).

In comparison to unhealthy controls, patients with ASD had significantly lower plasma zinc levels (10.01 µmol/L vs. 11.67 µmol/L (P<0.0001). The findings withstood correction for age and sex, and zinc levels in those with or without dietary modification or supplementation were similar. Zinc deficiency is likely common in autism. Whether such deficiency is integral to the pathophysiology, or indeed aetio-pathophysiology, or is a consequence of chronic illness remains unknown.

Is zinc deficiency a risk factor for the development of autism?

Given the diverse role of zinc throughout the body, zinc deficiency could go some way to explain the myriad of biological findings already identified in autism. Immune, neurological and gastrointestinal abnormalities have been reported in both prenatal/perinatal and infant zinc deficiency and reported separately in autism, and there is considerable symptom overlap.

Immune function: Whilst zinc is known for its effects on immune function, our understanding of the extent and nature of prenatal, perinatal and infantile zinc deficiency on immune function in humans remains limited. Wong et al. describe specific immune effects of prenatal zinc deficiency in a transgenic model following a maternal immune insult suggesting prenatal zinc deficiency may lead to epigenetic and immune effects responsible for maintaining a chronic inflammatory response [64]. Further transgenic work suggests that under zinc deficient conditions lysosomes containing inflammatory mediators lose integrity leading to a pro-inflammatory environment [65]. Such chronic inflammatory response has been reported in the elderly in response to zinc deficiency with an improvement of inflammatory cytokines following zinc supplementation [66]. Specifically, microglial activation has been reported *in-vitro* in response to zinc deficiency and reported *in-vivo* in patients with autism [67-70]. In animal models zinc has conferred protection against LPS induced maternal insult preventing aberrant behavior in object recognition tasks preventing abnormal sickness behaviour following immune challenge and has recently been demonstrated to prevent communication deficits in an autism mouse model [71-73]. From an immunological standpoint, developing a zinc-based intervention may provide protection or reduce the harm associated with prenatal, maternal and paternal factors contributing to the development of autism.

Neurological function: Neurological findings in zinc deficiency suffer similar limitations as immune findings. There are limited studies investigating the effects of prenatal, paternal or maternal zinc deficiency on neuropathological processes in human offspring. In a transgenic zinc deficiency model impaired glutathione metabolism was reported, perhaps explaining the protective effect of zinc supplementation on spatial and object memory following a maternal ethanol insult [74]. A significant reduction in transcription factors crucial for cell differentiation and synaptic plasticity (AP-1, NF-KB and NFAT) were reported in an experimentally induced zinc deficiency [75]. Abnormalities of the cerebellum have been reported following postnatal zinc deficiency, specifically abnormal metabolism of Purkinje cells [76]. Cerebellum abnormalities including excess Purkinje cell loss in autism have been reviewed recently [77]. Numerous environmental insults can alter glutathione metabolism, levels of transcription factors and neuronal cell loss. Perhaps then, zinc deficiency lowers the threshold for such environmental insults to lead to long-term neurodevelopmental disorders such as autism. Transgenic models have provided some evidence to suggest this may be the case. Pesticide induced neuropathology of the cerebellum and cerebrum was successfully reduced when zinc supplementation was given immediately following a 4-week exposure, and neuro-behavioural abnormalities also improved [78]. 4-months of lithium-induced cerebrum and cerebellum lipid peroxidation was reduced following 4-months of zinc supplementation with improved levels of Glutathione-S-transferase [79]. Zinc administered together with a sub-acute organophosphate exposure over three days conferred complete protection over abnormalities in the Forced Swimming Test versus no zinc supplemented controlled exposure. There was also a corresponding protection against lipid peroxidation and impaired glutathione metabolism in the cerebral cortex, and protection against impaired glutathione metabolism in the hippocampus [80]. Zinc conferred a similar protective effect against aluminium-induced damage to the blood brain barrier in an acute toxic insult model [81]. Following ten weeks of postnatal protein restriction, 3 weeks of zinc supplementation improved oxidative stress markers and neuro-behavioural deficits – specifically locomotor activity and memory and learning [82]. Zinc provided significant protection against lead-induced neurotoxicity in a mouse model of postnatal and adult sub-acute insults via a reduction in oxidative stress and improvement in monoamine metabolism [83,84]. As yet, there are no studies exploring whether zinc confers a neuroprotective effect over exposure to air pollution or volatile organic chemicals.

Gut microbiomes: Zinc has been shown essential to microbiota composition and maternal zinc deficiency has even been hypothesised to influence the development of the gastrointestinal tract in autism leading to an impaired gut-brain connectivity [85,86]. Beyond the immune and neurological effects of zinc deficiency discussed above, and the impact such effects will have on the normal mechanisms for microbiota regulation, there are some direct microbiological effects of zinc. It appears that certain species of microbiota tend to have high affinity zinc transporters, capable of utilising low levels of zinc [87]. Certain species of clostridia have been shown to increase fermentation in response to additional zinc and the pathogenesis of clostridia difficile has been reported as being zinc dependent [88]. This is consistent with the consensus of opinion that the majority of zinc absorption occurs in the small intestine, leaving lower levels of zinc available for the colon and hence the microbiota situated there, possibly limiting the growth of certain species such as clostridia. Zinc deficiency has also been suggested as a risk factor in the development of Environmental Enteric Dysfunction (EDD) [89]. Impaired absorption of zinc leading to increased transit of zinc to the colon may alter the composition of gut microbiota going forward. Equally the impairment of immune function found with zinc deficiency may be directly impeding the ability of the intestinal epithelial cells, dendritic and T-regulatory cells' immune regulation mechanisms. In this regard zinc may be a risk factor for the development of unique pattern of microbiota in autism.

Given that the majority of dietary zinc is absorbed in the small intestine, it seems unlikely the principle cause of zinc deficiency is excessive consumption of zinc by the abnormal microbiota present in the colon. Perhaps colonisation in the small intestine occurs or perhaps certain microbes gaining a slight selective advantage for another reason have the capacity to alter zinc transporter expression or pancreatic function in a bid to improve carbohydrate, zinc and preferred nutrient transition to the large bowel. A similar mechanism may be behind the lower level of disaccharidases previously identified [90].

Chronic illness: Equally zinc deficiency is expected in chronic disease, and more so where bowel symptoms predominate [85,87]. Indeed, the non-ASD control group had a sub-normal

mean zinc level (11.67 µmol/l vs. the national average of 14.7µmol/l), presumably from the chronic illness they attended clinic for the more marked zinc deficiency identified in the ASD-group (10.01 µmol/l) may simply relate to a more marked chronic disease state in the ASD group [87-91].

Physical co-morbidity: In adulthood the most likely cause of premature demise in ASD patients relates to seizure disorders with over 30 times increased risk of death from seizures regardless of co-existing intellectual disability [92]. Zinc deficiency has been explored as a risk factor for the development of seizures particularly intractable seizure disorders [93-95]. Cancer is another elevated risk for patients with ASD, and again there is evidence of increased risk through poor zinc status. It seems likely that poor zinc status will not only increase mortality rates in ASD, but also increase total morbidity and disease burden [96,97]. This remains to be proven as there have been no studies investigating the relationship between zinc status and the level of morbidity in ASD.

Future Studies

The results of this retrospective controlled analysis suggest further investigation into the relationship between zinc and autism may well be fruitful. A prospective controlled trial would enable improved data collection of both ASD patients and controls and mitigate any potential selection bias in those undergoing nutritional screening. Perhaps the most poignant question posed by this and other similar studies pertains to whether or not zinc supplementation prenatally, pre-paternally, maternally or in early infancy can provide any degree of protection over the eventual level of disability associated with autism or indeed perhaps even protect against it altogether in some cases. A population based longitudinal study of zinc levels during these periods may provide the crucial steer as to when zinc deficiency occurs and hence where in the pathophysiology of autism does zinc become key, and perhaps then when zinc supplementation may provide a useful harm-reduction intervention.

Clinical Implications

This study represents the largest controlled trial of serum zinc levels in ASD patients so far. To date, all studies exploring zinc status in ASD have demonstrated poor zinc status in ASD patients. Pending further investigations, clinicians should actively assess all patients with ASD, at diagnosis and throughout their life, for zinc deficiency. From the best available evidence, it is likely that adequate zinc status will reduce the burden of disease going forward. Supplementing zinc through a multi-vitamin is unlikely to be sufficient. Detailed analysis of the Cochrane reviews in this area reveal a loss of positive effect when zinc is given as part of a multi-vitamin formula and the authors could find no evidence multi-vitamins raise zinc levels [98-104]. Our results, whilst limited in number and detail of supplementation, suggest general supplementation alone is not adequate. Instead, oral zinc sulphate liquid titrated to serum response is recommended, preferably given in two divided doses daily. Clinicians should be aware of the potential impaired absorption of zinc caused by hypochlorhydria, small intestinal bacterial overgrowth, inflammatory bowel disease and excessive grain consumption, and hence failure for zinc deficiency to respond to oral zinc supplementation should prompt further investigation. As per tolerance, zinc should be administered away from meals containing grains, and additional vitamin C may improve absorption [105].

Conclusion

There have been a number of studies reporting zinc deficiency in ASD and the results of our moderately powered, controlled analysis support these findings. Indeed, 82% of patients with ASD were characterized as deficient, and there was a significant difference with controls ($P<0.001$). Zinc deficiency appears common in autism and should be considered in all patients presenting with autism or early autism features. Our results also suggest poor zinc status in patients with autism may affect their immune function. Other studies have identified correlations between low or deficient zinc levels and neurological function in patients with autism. Whilst it remains unknown whether correct supplementation of zinc will reduce the severity of autism symptomology going forward, it is likely correction of zinc deficiency will reduce co-morbid illness and improve general health. Our recommendation is to be mindful of potential zinc deficiency in patients with autism and actively manage it when found. To help determine whether zinc deficiency is involved in the aetio-pathogenesis of autism, and therefore help determine if timely zinc supplementation can reduce the disease burden or even protect against the disability associated with ASD, a population based longitudinal study is required.

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