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Comparative evaluation of salivary zinc and sialic acid concentration in autistic and healthy children- An in vivo study

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Abstract

Background: Autism spectrum disorder (ASD) presents complex challenges rooted in genetic and environmental factors. Diagnosing ASD relies on observing behaviour, lacking specific early indicators. Saliva offers a non-invasive, cost-effective biomarker source. Zinc and sialic acid, essential for nervous system health, are implicated in ASD development.

Aim: To evaluate and compare Zinc and Sialic acid concentration levels in unstimulated saliva of autistic and healthy children.

Method: Fifty children, including twenty-five with ASD and twenty-five healthy controls, were selected for saliva collection using the passive drooling/spitting method. Zinc and Sialic acid amounts were determined using an inductively coupled plasma mass spectrometry (ICP-MS) and spectrophotometer.

Result: After statistical analysis, the saliva's Zinc and Sialic acid levels were statistically significantly lower in autistic children than healthy controls.

Conclusion: It was discovered that children with autism have lower zinc and sialic acid levels than children without autism. Henceforth, Zinc and Sialic acid estimation can act as a useful biomarker for the diagnosis of autism spectrum disorder (ASD).

Keywords: Autism Spectrum Disorder (ASD), Zinc, Sialic Acid, Salivary Biomarkers, Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Spectrophotometer.

Introduction

Understanding autism spectrum disorder (ASD) is a multifaceted challenge that transcends mere medical, professional, or emotional interests. Linked to genetic susceptibility and prolonged exposure to environmental toxins [1], ASD is characterised by early difficulties in social interaction, communication, and rigid behavioural patterns [2]. First identified by Kanner [3] in 1943, ASD symptoms typically emerge in early childhood, often subtly progressing after developmental milestones [4].

Despite extensive research efforts, diagnosing ASD still relies solely on observing behavioural and cognitive development, lacking specific early indicators or definitive laboratory tests [5]. Early intervention, critical for improving outcomes, is hindered by the average age of diagnosis—currently over four years old—despite advocacy and educational efforts.

Earlier detection of ASD could significantly benefit affected children and their families by enabling timely access to tailored interventions and support services [6]. The potential use of saliva as a non-invasive biomarker source presents a promising avenue for research and diagnostic development, offering simplicity, safety, and cost-effectiveness compared to traditional blood tests.

Saliva, a complex fluid containing various organic and inorganic components, has gained attention for its utility in diagnosing diverse medical conditions [7]. Trace elements like Zinc and Sialic acid, essential for neurodevelopment and immune function, have been implicated in ASD pathophysiology [6]. Zinc plays a crucial role in immune response and neurogenesis, while Sialic acid is integral to brain development and cognition [8].

This study aims to explore the concentrations of Zinc and Sialic acid in the saliva of children diagnosed with ASD, addressing a significant research gap in pediatric populations. By leveraging saliva as a research tool, this investigation seeks to deepen our understanding of ASD's biochemical underpinnings and potentially pave the way for innovative diagnostic strategies and targeted interventions.

Material & Method

This in vivo study comprises 50 sample sizes which included 25 institutionalized autistic (Experimental group) children who were free of any other physical or systemic illness at that specific time and were not on any medications and 25 non-autistic (Control group) children of age 6-14 years. The ethical committee approval was taken prior to study no. DJC/IEC/2021/01. To evaluate zinc and sialic acid, saliva was collected in all children using the drooling or spitting method.

Procedure for Saliva Collection

Whole, unstimulated saliva was collected, from 50 children inclusive of both groups. During saliva collection, the child was required to be seated in a room that was well-ventilated and well-lit. The participants were instructed not to speak and their head was positioned at a 45° flexion, with one hand securely holding the container, within a tranquil environment to replicate unstimulated conditions. Two millilitres of unstimulated whole saliva were obtained from both groups for the study (Fig: 1a & 1b), collected via passive drooling/spitting method into sterile plastic containers. After the collection of an adequate volume of saliva. The lids of the containers were tightly closed and immediately sent to the clinical laboratory.

Saliva samples were transported in an ice box with an ice pack within one hour to the Lab for estimation of Zinc and Sialic acid.

Fig. 1a: Collection of saliva sample (autistic patient**)**

Fig 1b: Collection of saliva sample (healthy patient)

Laboratory Evaluation (Fig: 1c & 1d)

Using a micropipette to quantify the quantities of solution and reagents, each subject's saliva sample was diluted with distilled water at a ratio of 1:4 to determine the content of Zinc and Sialic acid in saliva. After that, an examination using inductively coupled plasma emission spectroscopy & spectrophotometer respectively was performed on this diluted saliva sample.

Zinc estimation was done by adding Nitro-PAPS reagent using inductively coupled plasma mass spectrometry (ICP-MS).

Sialic acid estimation was done by adding an acidic Ninhydrin reagent using a spectrophotometer.

Fig1c: Addition of reagents

Fig 1d: Zinc and sialic acid estimation value on a spectrophotometer

evaluated by using inductively coupled plasma mass spectrometry (ICP-MS)

Result and Observation

On statistical evaluation, it was found that the average Zinc levels in individuals with autism (Experimental Group) were 0.617 ± 0.773, while in those without autism (Control Group), they were 4.172 ± 0.867. The comparison between the two groups yielded a statistically significant difference when analysed using an independent t-test, with a p-value of less than 0.001. (Table:1) (Graph:1).

On statistical evaluation, it was found that the average Sialic acid levels in individuals with autism (Experimental Group) were 10.888 ± 3.041, whereas in those without autism (Control Group), they were 19.848 ± 3.117. The comparison between the two groups revealed a statistically significant difference when analysed using an independent t-test, with a p-value of less than 0.001. (Table: 2) (Graph:2)

Table: 1 Intergroup comparisons of the level of Zinc using t-test.

Group	N mean	Mean value of Zn (mg/l)	Max. value of Zn (mg/l)	Min. value of Zn (mg/l)	T calculated	P value
Experimental group (Autism)	25	0.617	1.93	0.01	15.293	0.001 (Significant)
Control group (Healthy)	25	4.172	5.5	2.02		

Graph: 1 Mean difference of the level of Zinc in both groups

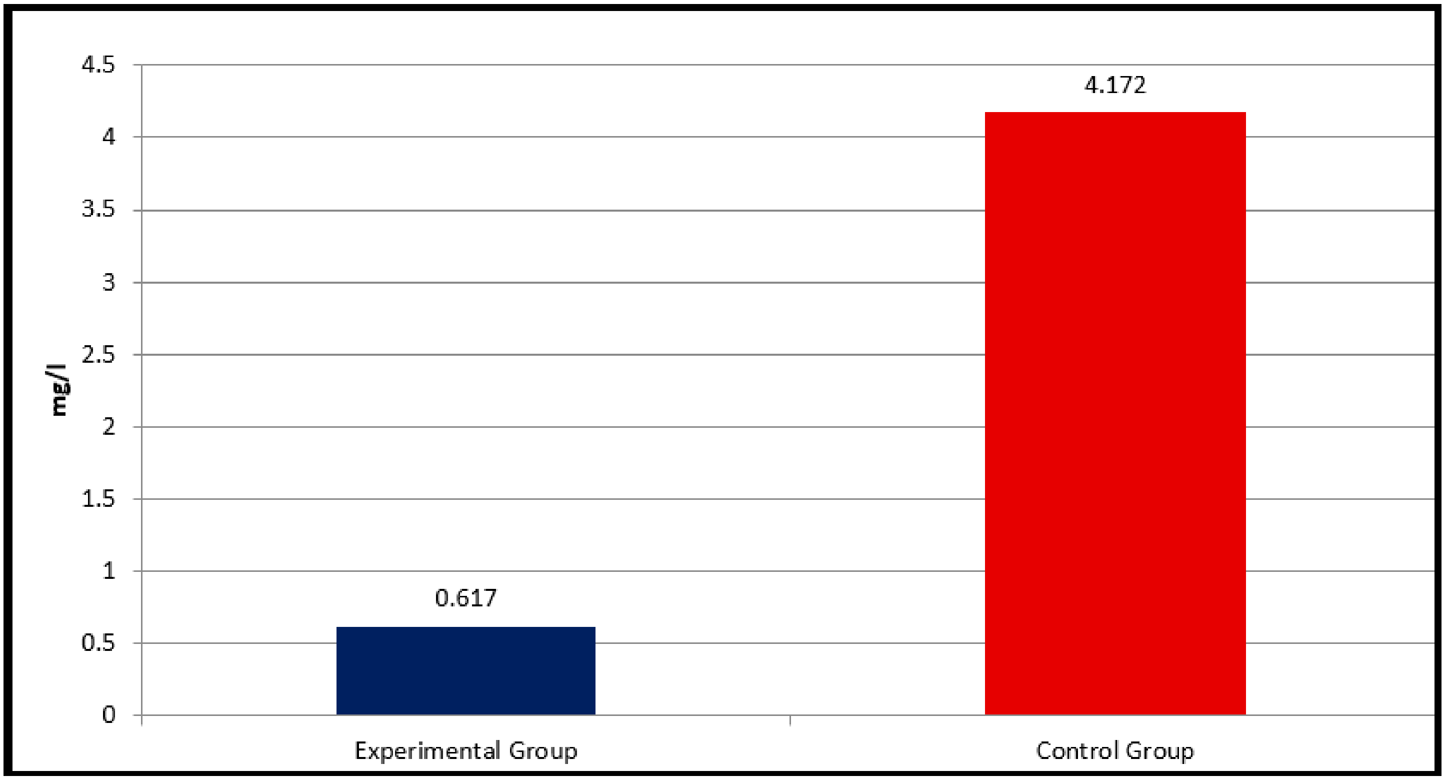
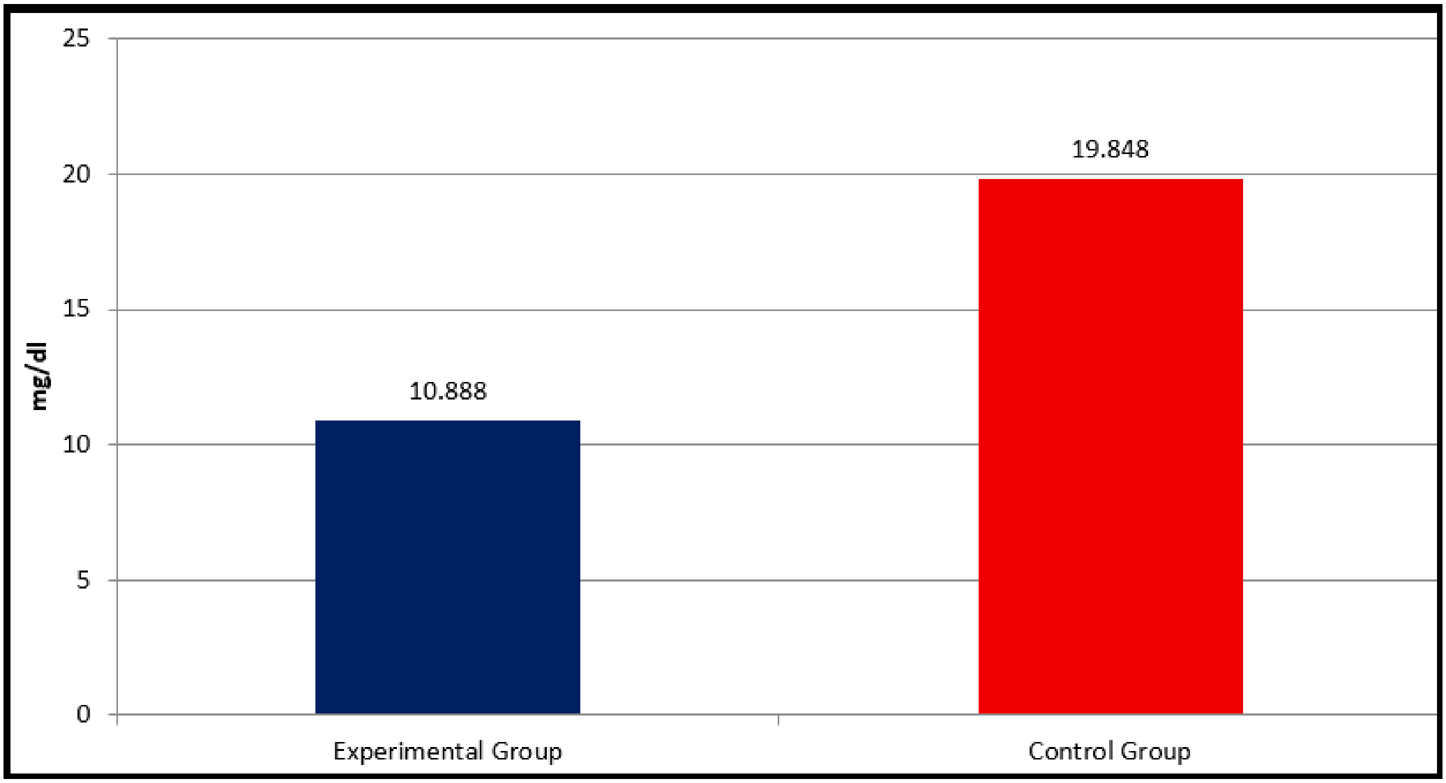


Table: 2 Intergroup comparisons of the level of Sialic acid using t test.

Group	N mean	Mean value of Sialic acid (mg/dl)	Max. value of Sialic acid (mg/dl)	Min. value of Sialic acid (mg/dl)	T calculated	P value
Experimental group (Autism)	25	10.888	15.7	5.3	10.285	0.001 (Significance)
Control group (Healthy)	25	19.848	26.2	15.9		

Graph: 2 Mean difference of the level of Sialic acid in both groups



Discussion

Autism spectrum disorder (ASD) is a neurological condition characterized by distinctive behavioural patterns, including challenges in communication, social interaction, and engaging in limited and repetitive behaviours, interests, and activities. **(American Psychiatric Association, 2013)** [9].

To date, no particular markers have been pinpointed that could facilitate early intervention as well as no specific laboratory test available for diagnosing autism at very early stage.

Early diagnosis of Autism offers benefits for both the child and the family, providing timely information on education and support. Thus, the need for relevant non-invasive biomarkers for early detection of autism can be a breakthrough tool for these special children.

Saliva is gaining recognition as a valuable diagnostic tool for the early detection of a range of diseases including pre-cancer, oral cancer, and conditions like benign migratory glossitis, oral submucous fibrosis, recurrent herpes labialis, type 1 diabetes mellitus, dental caries, oral lichen planus, periodontal health, and Autism etc. Unstimulated saliva, naturally produced without external stimuli such as chewing or tasting, provides a baseline reflection of the physiological state, making it suitable for monitoring chronic conditions and long-term changes in health.

Moreover, Saliva is chosen as a biomarker because it can be collected non-invasively, providing a convenient and easily accessible source for various analytes [10].

Many blood and plasma tests may serve as a valuable source of information, involving piercing the skin with a needle and turning it into an invasive procedure. Which causes discomfort and also raises the risk of infection.

Present study, in comparison with healthy children, we found the level of Zinc in saliva was significantly lower in autistic children than in healthy children, which is in support of the studies by **Deshpande RR et al. (2019)** [6]

investigating Zinc content in saliva in children with mixed dentition ages who were autistic and healthy, using a sample size of twenty.

The study's findings demonstrated that children with autism have less Zinc in their saliva than children lacking autism.

Research by **Li So et al. (2014)** **[1]** used a sample size of 60 children with ASD to examine the serum concentrations of Zinc and copper. The results showed that compared to children without ASD, children with ASD had significantly lower mean blood Zinc levels and a Zn/Cu ratio.

During childhood and adolescence, Zinc plays a vital role in numerous growth and developmental processes, as emphasized by Dogus Vuralli (2017) and Vijay Kumar (2021), particularly in the maturation of the nervous system [11]. Moynahan was among the first to observe that untreated children with a genetic Zinc deficiency disorder exhibited abnormal visual behaviour and mood swings, symptoms consistent with certain characteristics of autism. Zinc is crucial for the metabolism of lipids, proteins, and carbohydrates, which are essential for cellular function [12]. It is believed to act as a neurotransmitter or neuromodulator within the central nervous system, where it integrates into the active sites of various metalloenzymes [13]. Zinc is stored in presynaptic boutons and released with glutamate upon membrane depolarization in a calcium-dependent manner [14]. Additionally, Zinc modulates the activity of both glutamate and gamma-aminobutyric acid receptors [13]. Vesicular Zinc ions are suggested to play a direct role in neuronal death during excitotoxic damage following conditions like ischemia, traumatic brain injury, and seizures [15]. Moreover, Zinc deficiency may alter brain function through changes in neurotransmitter levels and receptor function [16].

Zinc is a critical trace element for the metabolism of biogenic amines, particularly dopamine and serotonin, during early brain development. These amines are hypothesized to influence neuronal development, with serotonin reported to affect processes such as neuroblast division, cell migration, and synapse formation, which are potentially relevant to the origins of autism [17]. No studies have been conducted on saliva to assess sialic acid levels in autism so far; however, several studies have explored its presence in plasma, hair follicles, cancer detection, polysialylated glycan synthesis, and other contexts.

A study by **Yang X et al. (2018)** **[18]** used 142 children with ASD as a sample size to examine the levels of anti-ganglioside and Sialic acid antibodies. The results showed that the control group's plasma Sialic acid level was much greater than the ASD group's.

Children with autism spectrum disorder had their salivary sialic acid levels measured by **Demirci E. et al. (2019)** **[5]**. According to the findings, the ASD group's Sialic acid level was considerably lower than that of the healthy controls.

No studies have yet investigated the levels of sialic acid in saliva concerning both typically developing children and those with autism spectrum disorder.

Sialic acid (Sia), a vital nutrient for brain development and a constituent of glycoproteins and gangliosides, has been investigated concerning autism spectrum disorder (ASD). Some studies suggest alterations in Sialic acid levels in plasma or the metabolism of sialoglycoconjugates in individuals with ASD. Sialic acid has a role in the formation of neurons and the immune system, among other things.

Therefore, it is evident that comprehensive studies are warranted to ascertain whether Sialic acid levels, linked with polysialic acid levels, impact autism-like behaviours.

Past studies linked Sialic acid supports poly sialylated glycan synthesis, affecting neural cell interactions crucial for neuronal outgrowth, synaptic connectivity, and memory. Even more, it has been suggested that Sialic-rich diets elevate Sialic levels in postnatal piglet brains, enhance learning-related gene expression, and improve learning and memory [19].

Hence, being zinc and sialic acid important for neural growth, if measured early in life can help diagnose autism spectrum disorder at an early stage.

Conclusion

Given the constraints of this in vivo investigation and the outcomes, it was determined that: -

The average salivary levels of zinc and sialic acid indicate that children diagnosed with autism have significantly lower amounts of these substances compared to healthy controls.

Henceforth, the estimation of Zinc and Sialic acid in saliva can be a pivotal step forward in non-invasive biomarker-based diagnostics for autism.

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