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Research Article

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Increased Hmgb1 Associated With Low Zinc and Symptom Severity in Children with Autism

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Abstract

Autistic children often have a high prevalence of immune-related pathologies, such as allergies and autoimmune diseases, and there is compelling evidence that immune dysfunction is related to the etiology of autism. High-mobility group box proteins (HMGB1) constitute a family of non-histone and ubiquitous molecules with a pro-inflammatory function. In this study, we measured HMGB1 levels in autistic individuals and compared these levels to plasma zinc concentration. We found that in individuals with autism increased levels of HMGB1 was associated with low zinc levels, as well as increased selected symptom severity. These results suggest that there is a relationship between Zinc and HMGB1levels in autistic individuals, and that low zinc levels may be exasperating inflammation in these patients.

Introduction

Many gastrointestinal, psychiatric and autoimmune disorders have been found to be associated with ASDs. In fact, autistic children often have a high prevalence of immune and inflammatory related pathologies such as allergies and autoimmune diseases (1-8).

High-mobility group box proteins constitute a family of non-histone and ubiquitous molecules with a pro-inflammatory function and therefore they often are considered biomarkers of inflammation. They belong to the high mobility group family of proteins. There are four categories of HMGB, from 1 to 4. High-mobility group box 1 (which is known also as HMGB-1, HMG1, HMG-1, HMG 1, amphoterin, p30) is the most represented of the whole HMG family proteins and is encoded in human by the HMGB1 gene (13q12) (9,10).

HMGB1 is released in the surrounding tissues as a consequence of a cell's damage or inflammation (11), and although its purpose is to activate the immune response, over expression might lead to damaging inflammation (12).

HMGB1 is a "cytokine-type" RAGE ligand (18). Zinc may down regulate RAGE expression (19) and therefore decrease HMGB1 inflammatory function.

This study measured HMGB1 levels in autistic individuals and compared these levels with plasma zinc concentration. We found that increased levels of HMGB1 was associated with low zinc levels, as well as increased selected symptom severity in individuals with autism.

Methods

ELISA to measure plasma HMGB1 (eBiosciences, San Diego, CA) All reagents were used at room temperature. A 1:50 dilution of the patient samples was prepared using plasma diluent. One hundred microliters of all samples were added to the appropriate microwells of a microculture plate (each well contained affinity purified polyclonal IgG to HMGB1. The plate was incubated for 60 minutes (± 5 min) at room temperature, then washed 4 times with wash buffer. One hundred microliters of pre-diluted HRP conjugated anti-human IgG, was added to all wells, incubated for 30 minutes (± 5 min) at room temperature, then washed 4 times with wash buffer. One hundred microliters of enzyme substrate were added to each microwell. The reaction was stopped fter approximately 30 minutes at room temperature, by adding 50µl of 1M sulfuric acid, The plate was read at 405 nm with an ELISA reader (BioRad Laboratories, Inc., Hercules, CA, USA).

Serum/Plasma and Zinc Measurement

All experimental and control plasmas were refrigerated (4 C) immediately after collection and cell/serum separation. Zinc concentration (LabCorp, Warrenville Il) was measured within 4 hours using inductively-coupled plasma-mass spectrometry.

Subjects

Plasma EGFR was measured in 33 autistic children (23 male +/- 10.2 years of age) and 34 neurotypical controls (26 male +/- 9.1 years of age) using an ELISA. Plasma zinc levels were measured in all of the above by LabCorp, Inc. using inductively-coupled, plasma-mass spectrometry.

It should be noted that the diagnostic measures used in this study are defined by DSM-IV criteria. In 2012, the separate diagnostic labels of Autistic Disorder, Asperger's Disorder, and PDD-NOS were replaced by one umbrella term "Autism Spectrum Disorder".

Plasma from consecutive individuals with diagnosed autism was obtained from patients presenting at the Health Research Institute (HRI) *. The autistic individuals in this study were diagnosed using The Autism Diagnostic Interview-Revised - ADI-R before presenting for treatment at the Health Research Institute of the Pfeiffer Treatment Center. This study was approved by the IRB of the HRI.

Zinc and Anti-oxidant Therapy

Individuals in this study were tested for zinc, copper and anti-oxidant levels. Based on abnormal levels zinc and copper, they were then prescribed the appropriate dose of zinc and anti-oxidants. Pre-therapy patients were not previously taking any zinc or anti-oxidants. Post-Therapy patients received anti-oxidant therapy (Vitamin C, E, B-6, Magnesium, and Manganese if warranted), and zinc supplementation (as zinc picolinate), daily, for a minimum of 8 weeks.

Statistics

Unpaired t-test and odds ratios with 95% confidence intervals was used for statistical analysis.

Severity of Disease

An autism symptom severity questionnaire was used to evaluate symptoms. The questionnaire (HRI Questionnaire) asked parents or caregivers to assess the severity of the following symptoms: Awareness, Expressive Language, Receptive Language, (Conversational) Pragmatic Language, Focus, Attention, Hyperactivity, Impulsivity, Perseveration, Fine Motor Skills, Gross Motor Skills, Hypotonia (low muscle tone), Tip Toeing, Rocking/Pacing, Stimming, Obsessions/Fixations, Eye Contact, Sound Sensitivity, Light Sensitivity, and Tactile Sensitivity. The symptoms were rated by parents/guardians on a scale of 0-5 (5 being the highest severity) for each of these behaviors.

*The Health Research Institute is a comprehensive treatment and research center, specializing in the care of with neurological disorders, including autism.

Results

Using an ELISA, we measured HMGB1 in the plasma of 24 individuals with autism (mean age 9.8 years, 18 male) and 10 neurotypical controls (mean age 12 years, 8 males). HMGB1 levels in the individuals with autism were significantly higher (118.3 ng/µl +/- 43.3) than the HMGB1 levels of neurotypical controls (52.3 ng/µl +/- 35.3) (p = 0.01) [Figure 1]

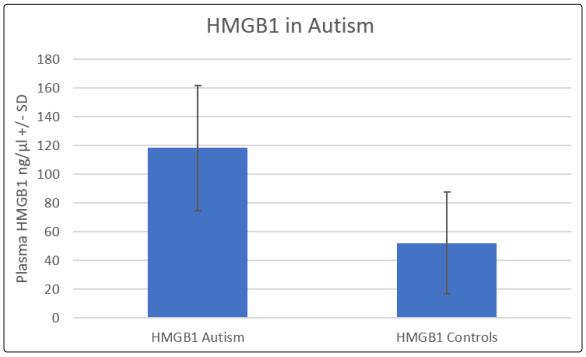

Figure 1: HMGB1 levels in the individuals with autism were significantly higher (118.3 ng/µl +/- 43.3) than the HMGB1 levels of neurotypical controls (52.3 ng/µl +/- 35.3) (p = 0.01)

In autistic patients, HMGB1 levels were significantly higher in patients with low zinc (all below 90 µg/dL zinc; mean +/- SD 71.1 ± 5.3) (125 ng/µl +/- 50.2) compared to patients with normal zinc levels (all above 90 µg/dL zinc; mean +/- SD 113.1 ± 7.3) (63.7 ng/µl +/- 41) (p=0.05) [Figure 2]

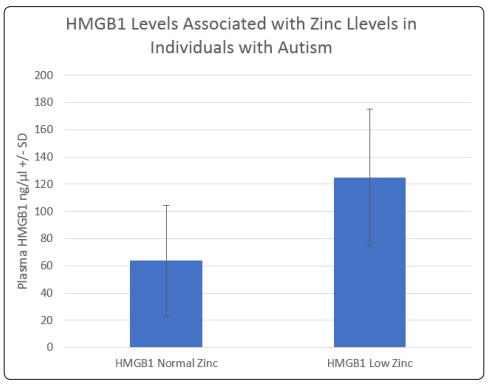

Figure 2: In autistic patients, HMGB1 levels were significantly higher in patients with low zinc (all below 90 µg/dL zinc; mean +/- SD 71.1 ± 5.3) (125 ng/µl +/- 50.2) compared to patients with normal zinc levels (all above 90 µg/dL zinc; mean +/- SD 113.1 ± 7.3) (63.7 ng/µl +/- 41) (p=0.05)

We found that individuals with autism had significantly higher focus/attention (p=0.031), gross motor symptom severity (p=0.002) and Hypotonia (p=0.01) (Figures 3-5).

Autistic individuals with low zinc and high HMGB1 have significantly higher focus/attention symptom severity (4+/- 0.93) than those with normal zinc and low HMGB1 levels (2.8 +/- 0.58) (p=0.031) [Figure 3].

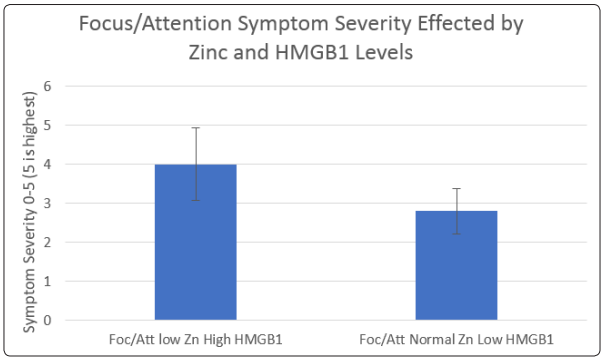

Figure 3: Autistic individuals with low zinc and high HMGB1 have significantly higher focus/attention symptom severity (4+/- 0.93) than those with normal zinc and low HMGB1 levels (2.8 +/- 0.58) (p=0.031)

Autistic individuals with low zinc and high HMGB1 have significantly higher Gross Motor symptom severity (2.91+/- 0.84) than those with normal zinc and low HMGB1 levels (1.07 +/- 0.86) (p=0.002) [Figure 4].

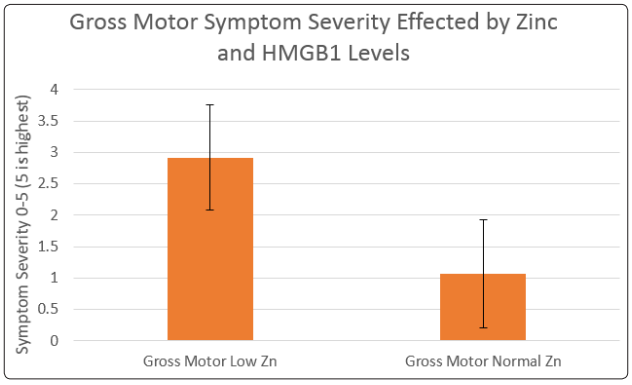

Figure 4: Autistic individuals with low zinc and high HMGB1 have significantly higher Gross Motor symptom severity (2.91+/- 0.84) than those with normal zinc and low HMGB1 levels (1.07 +/- 0.86) (p=0.002)

Autistic individuals with low zinc and high HMGB1 have significantly higher Hypotonia symptom severity (2.5+/- 1.2) compared to those with normal zinc and low HMGB1 levels (0.81+/- 0.73) (p=0.01) [Figure 5].

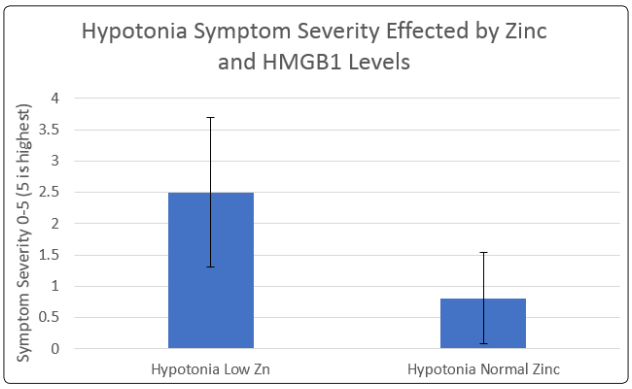

Figure 5: Autistic individuals with low zinc and high HMGB1 have significantly higher Hypotonia symptom severity (2.5+/- 1.2) than those with normal zinc and low HMGB1 levels (0.81+/- 0.73) (p=0.01)

Discussion

Our data supports other recent studies showing that HMGB1 is significantly increased in ASD and can promote neurite outgrowth and cell migration (13). Treatment with HMGB1inhibitors was found being able to reduce the inflammatory response in a wide range of preclinical autism models (14).

Our lab has previously found that HMGB1 is associated with reduced levels of plasma epidermal growth factor (15) and increased epidermal growth factor receptor (16) in individuals with autism.

Since zinc plays an important role in cell mediated immunity and is also an antioxidant and anti-inflammatory agent (17), it is logical that zinc levels might be inversely associated with HMGB1, an inflammatory marker.

In summary, we found that individuals with autism have significantly higher levels of HMGB1 and higher selected symptom severity and that autistic individuals in this population with normal zinc levels have significantly lower HMGB1 as well as lower symptom severity. This suggests that zinc supplementation may be a therapeutic method for lowering inflammation as well as improving symptom severity in individuals with autism [1-19].

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