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Research Article

Increased Epidermal Growth Factor Receptor (EGFR) in Children with Autism Normalized with Zinc Therapy

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Abstract

Background: Autism spectrum disorders (ASDs), resulting in dysfunctional social interactions, affect 1 in less than 100 children in the US. There is much support for the role of growth factors and their receptors in the etiology of autism. Recent research has shown that epidermal growth factor receptor (EGFR) activation may be affected by zinc and is associated with nerve cell development and repair. This study was designed to determine if EGFR levels in autistic children are associated with zinc levels and if these levels are affected by zinc therapy.

Subjects and Methods: Plasma EGFR concentration was measured in autistic children and neurotypical, age and gender similar controls using an ELISA. Plasma zinc levels were measured in all of the above by LabCorp, inc. using inductively-coupled plasma-mass spectrometry.

Results: In this study, we found plasma EGFR levels in autistic children, significantly higher than neurotypical controls. These EGFR levels normalized in these individuals after zinc supplemental therapy.

Conclusion: These results suggest that zinc therapy may have an effect on EGFR levels in individuals with autism.

Keywords: *EGF; Zinc; Autism; ASD; Symptom Severity*

Abbreviations

EGFR: Epidermal Growth Factor Receptor; HGF: Hepatocyte Growth Factor; EGF: Epidermal Growth Factor; ELISA: Enzyme Linked Immunosorbent Assay

Introduction

Autism spectrum disorders (ASDs) are characterized by problematic social interactions, verbal and nonverbal communication, and stereotyped and repetitive behaviors [1].

Epidermal growth factor receptor (EGFR) is a member of a family of receptor tyrosine kinases (RTKs) [2]. EGFR binds to specific ligands, including EGF, and becomes activated and changes to a homodimer [3]. This stimulates its intrinsic intracellular kinase activity [4]. This activates many pathways, including the MAPK, Akt and JNK pathways, which leads to DNA synthesis and cell proliferation [5].

EGFR stimulation is associated with cell development and repair. It also stimulates astrocytes into reactive cells [6]. Increased EGFR has been shown to be related to Alzheimer's disease. Erlotinib, an EGFR Inhibitor, delays Alzheimer's progression in mice [7-9]. Inhibition of EGFR also suppresses microglia activation which slows damage due to inflammation which, in turn, slows spinal cord injury in a rat model [9].

Zinc is associated with the structure and regulation in cells [10]. $Zn2^{+}$ ions have been found to have a regulatory effect on intracellular signaling molecules such as EGFR [11-13].

Variants of kinase receptors have been associated with the etiology of autism [14,15]. Growth factors which are ligands for receptor kinases, such as hepatocyte growth factor (HGF) and epidermal growth factor (EGF), are decreased in autism [16,17].

Attachment of growth factors to their receptors regulates the processes of neuronal growth, differentiation, and proliferation, as well as neuronal survival, neuronal migration, the formation or elimination of synapses [18], as well as modulation of the immune response [19-21]. Nervous system immune deficiencies have been found in children with autism [22-26]. This abnormal function may be associated with dysregulation of growth factor activity.

Specifically, EGF plays an important role in controlling proliferation and differentiation of nervous tissue during neurogenesis [26-28].

Studies have shown a possible association between EGF and autism. Single nucleotide polymorphisms of EGF are increased in children with autism [29]. Studies have found lower plasma EGF levels in adults with autism [30], but also increased as well as decreased EGF in autistic children [31,32].

Our lab has previously shown that EGFR levels are significantly increased in our autistic population [33]. This study was designed to determine if higher EGFR levels are associated with plasma zinc levels and if these zinc concentrations are affected by zinc therapy.

Materials and Methods

ELISA to measure plasma EGFR (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 HGF or GABA). The plate was incubated for 60 minutes (± 5 minutes) 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 minutes) 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 after 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/ Pfeiffer Treatment Center*. 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 Health Research Institute, Pfeiffer Treatment Center. All patients involved in this study gave patient consent.

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.

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

Results

In this study, we found that plasma levels of EGFR in children with autism (post zinc therapy) were significantly lower (m = 12541 +/- 1844 pg/µl) than EGFR levels of autistic individuals pre zinc therapy (p = 0.03) (Figure 1).

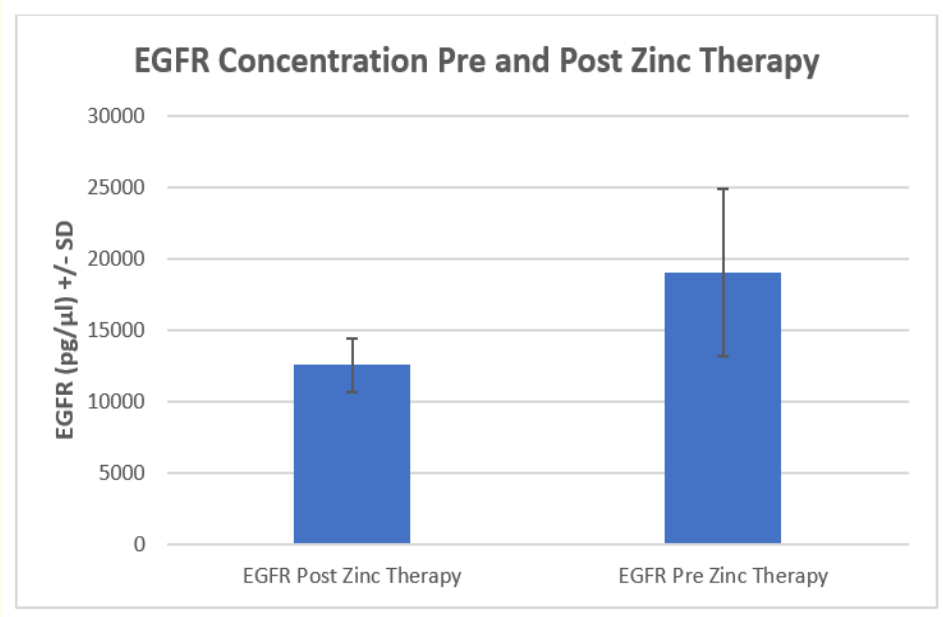

Figure 1: *Plasma levels of EGFR in children with autism (post zinc therapy) were significantly lower (m = 12541 +/- 1844 pg/µl) than EGFR levels of autistic individuals pre zinc therapy (p = 0.03).*

Plasma zinc concentration in the above autistic individuals rose significantly after zinc therapy (Pre zinc therapy, zinc concentration m = 75.8 +/- 9.5 mg/dL) (Post zinc therapy, zinc concentration m = 94.1 +/- 10.7 mg/dL) (p = 0.01) (Figure 2).

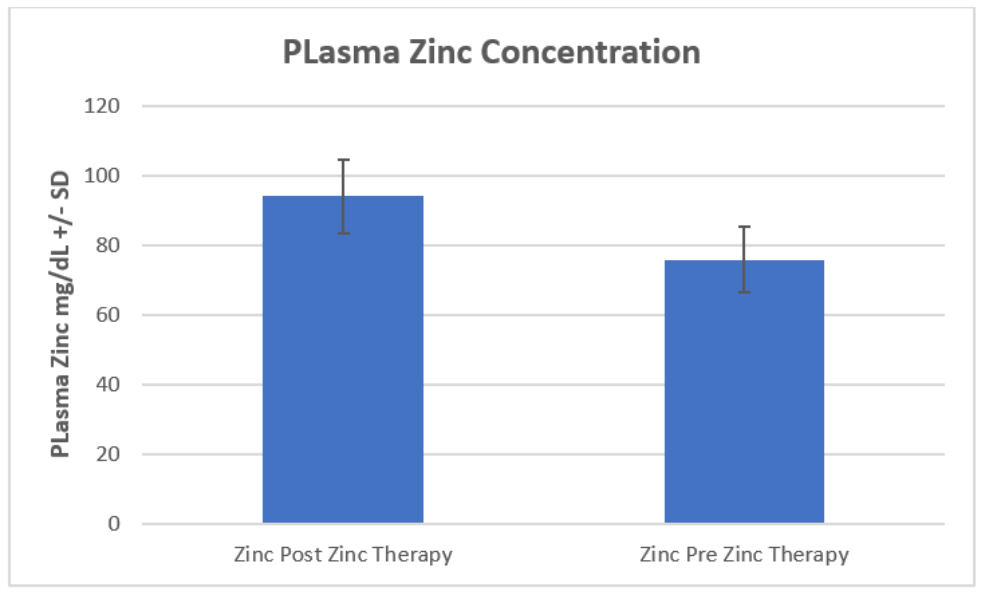

Figure 2: *Plasma zinc concentration in autistic individuals rose significantly after zinc therapy (Pre zinc therapy zinc concentration m = 75.8 +/- 9.5 mg/dL) (Post zinc therapy zinc concentration m = 94.1 +/- 10.7 mg/dL) (p = 0.01).*

Discussion

The EGFR receptor tyrosine kinase (RTKs), when signaled by their respective ligands, HGF and EGF, respectively, though a cascade of signaling reactions, modulate the ERK and PI3K intracellular regulatory pathways.

ERK, by interacting with other kinases, helps control cell functions such as cell cycle, cell differentiation, cell survival and motility.

Methylation regulates transcription of key genes involved in met proto-oncogene-RTK signaling (MET RTK signaling), which has been implicated in ASD risk [10,14].

Different genetic routes to altered receptor tyrosine kinases (RTKs), such as EGFR, function by way of modulation of ERK/PI3K signaling pathways. Biochemical stressors, they may alter the degree of dysfunction associated with the etiology of autism [10,14].

EGF is involved in growth and differentiation of cells of the CNS and the gastrointestinal tract [13,17] as well as the migration and differentiation of neural progenitor cells into astrocytes and neurons [34]. EGF appears to be necessary for normal development of intestinal mucosa [35], this growth factor also promotes wound healing [36], possibly through anti-inflammatory action [37,38].

A recent study compared the pathway enrichment analysis results of various brain regions. Several cross-brain regions pathways were detected for ASD signaling by constitutively active EGFR in the anterior caudate [33].

We previously found that individuals with autism have significantly increased levels of EGFR [39].

Conclusion

In this study, we found that zinc levels significantly rose after zinc therapy and EGFR levels normalized after this zinc therapy (decreased to the levels of controls) in individuals with autism. This suggests that zinc has a significant role to play with respect to influencing EGFR levels in an autistic population and zinc supplementation may also be a benefit in other pathologies that have etiologies influenced by high zinc levels.

Authors' Contributions

AR carried out the immunoassays, participated in the design of the study and performed the statistical analysis. AR, AM, JB conceived of the study and participated in its design and coordination. AM, JB provided clinical records of patients and controls. AR, AM, JB drafted and approved the final manuscript.

Competing Interests

The authors have no competing interests.

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