

QUASI-EXPERIMENTAL EVALUATION OF ZINC SUPPLEMENTATION ON MEAN SERUM INSULIN LIKE GROWTH FACTOR-1 (IGF-1) AND INSULIN LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3) LEVELS IN PAKISTANI SHORT STATURED CHILDREN

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ABSTRACT

Objective: To investigate the influence of supplemental zinc on the levels of insulin like growth factor-1 (IGF-1) and insulin like growth factor binding protein-3 (IGFBP-3) in short statured Pakistani children of 3-6 years of age.

Study Design: Quasi-experimental study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology Rawalpindi, from Jun 2016 to Jun 2017.

Methodology: Children were divided into two groups; group-1 short stature with zinc deficiency and group-2 without zinc deficiency. Group-1 was given zinc supplementation (oral Zn sulfate-50mg/day elemental Zn) for 12 weeks. Anthropometric measurements and biochemistry analysis of zinc (umol/l) on Atomic Absorption Spectrometry, insulin like growth factor-1 (ng/ml) and insulin like growth factor binding protein-3 (ng/ml), serum growth hormone stimulation analysis by chemiluminescent analyzer were done in all groups.

Results: Of totally 360 screened children, 96 met the eligibility criteria who were equally divided into two groups according to their zinc status. Gender distribution showed that group 1A included 29 (60%) male and 19 (40%) female while group 2 consisted of 28 (60%) male and 20 (40%) females. Most of the children in group 1A were 5years old while in group 2 greater numbers of children were of 6 years. Inferential statistics revealed that when group 1A and 1B was compared with paired t-statistics for height, weight, BMI, Zinc, insulin like growth factor-1 and insulin like growth factor binding protein-3, the mean increase from the baseline to weeks 12 was highly significant ($p < 0.001$) for all these parameters.

Conclusions: Zinc supplementation improved short stature clinically and biochemically in zinc deficient patients.

Keywords: Growth hormone-insulin growth factor-1 system, Growth, Oral zinc supplementation.

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INTRODUCTION

Growth is principally regulated and controlled by growth hormone - insulin like growth factor (GH-IGF) system in the body. Zn supplementation seems to have supportive influence on growth and insulin like growth factor-1 (IGF-1) levels in different groups of children with Zn deficiency¹. Zinc is an essential trace element vital for growth and development. It is present in the cells of all metabolically active tissues and organs and its concentration in the bone matrix is very high as compared to other tissues². Zn is second to iron as the most abundant trace element in the

body and has a role not only in cell division, protein synthesis and growth but is also involved in gene expression and a variety of reproductive and immunologic functions³ and are mandatory for growth hormone production and secretion⁴ and by activating protein kinase C, it regulates the intracellular transduction pathways of growth hormone. The dysfunction of zinc-finger proteins are likely to cause changes in growth hormone action and secretion. Zn also has an important role in the biosynthetic pathway of IGF-1 and insulin-like growth factor-binding protein 3 (IGFBP3)⁵. It is essential to take Zn regularly in diet because Zn cannot be produced or stored in the body⁶. Nonetheless, the precise mechanism of Zn deficiency and how Zn therapy influence GH secretion and IGF-1 levels is not famed. However

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it has been reported by some studies that Zn therapy has supportive effect on growth in various groups of children with Zn deficiency. But, in other studies, this outcome was not catch-out^{1,7}. The levels of serum IGF-1 and IGFBP-3 are lower in short statured children having zinc deficiency, and improve following zinc therapy. As there were limited studies available in Pakistan which showed the association of zinc supplementation on growth, we had planned this quasi-experimental study to investigate the influence of supplemental zinc on the levels of IGF-1 and IGFBP-3 in short stature Pakistani children between ages of 3-6 years.

METHODOLOGY

It was a quasi-experimental study which was conducted in department of Chemical Pathology and Endocrinology Armed Forces Institute of Pathology Rawalpindi from June 2016 to June 2017. Formal approval was taken from hospital ethics committee before commencement of study. Children were taken from AFIP endocrine clinic. All children with short statures between ages 3 to 6 years were included. Short stature was defined by (height <2.3rd percentile) as per WHO guideline for short stature^{8,9}. Children with diabetes mellitus, hypothyroidism and other endocrine problems, diarrhea, liver diseases, renal disorders, malignancy, sickle cell anemia, history of zinc therapy in last six months and use of medication that may affect GH-IGF axis, bone dysplasias, dysmorphic syndromes, chronic malabsorption were excluded from study after detailed history, medical examination and relevant investigations for evaluation of short stature (plasma random glucose (mmol/l), serum ALT (u/l), serum albumin, serum urea (mmol/l), serum creatinine (umol/l), blood complete picture and C-reactive protein (CRP) in mg/dl. First informed written consent was obtained on consent proforma and hospital ID numbers were allotted to all children included in the study. Of totally 360 screened children, 96 met the eligibility criteria, consented and were enrolled and divided into two groups according to their zinc status. Group-1A consisted of 48 children who were

short stature and zinc deficient. Group-2 consisted of 48 children who were short stature and without Zn deficiency. Then children of group-1A were supplemented with oral Zn sulfate (50-mg/day elemental Zn) for 12 weeks. Out of these 48, six lost follow up and one with-draw consent, so after 12 weeks, 41 participants were characterized in group-1B post zinc supplementation group (fig-1).

Anthropometric measurements height (cm), weight (kg), BMI (kg/m²) were done in all groups. 5ml blood was taken in gel tube from both groups at the start of experiment and from participant of group 1 after 12 weeks of zinc supplementation for biochemistry analysis of zinc (umol/l) on Atomic Absorption Spectrometry,

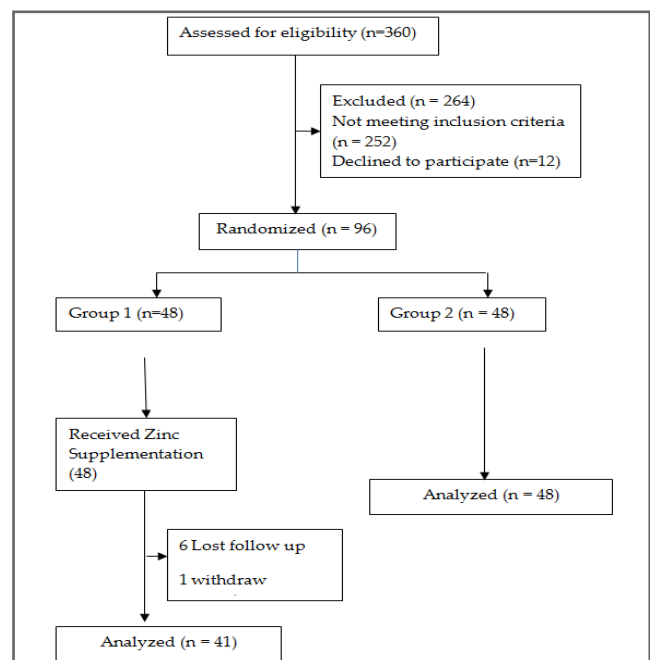


Figure-1: Schematic distribution of study population.

IGF-1 (ng/ml) and IGFBP-3 (ng/ml), serum growth hormone stimulation analysis by chemiluminescent analyzer (Adviacenture). Data was entered and analyzed by using SPSS-21. For qualitative variables; frequency and percentage were calculated and for quantitative variable after normality check by KS test, mean and standard deviation were calculated. Inferential statistic in the form paired t statistics (between pre and post

zinc supplementation in zinc deficient group) and multiple group's comparison by ANOVA was done.

RESULTS

Gender distribution showed that group 1A included 29 (60%) male and 19 (40%) female while group 2 consisted of 28 (60%) male and 20

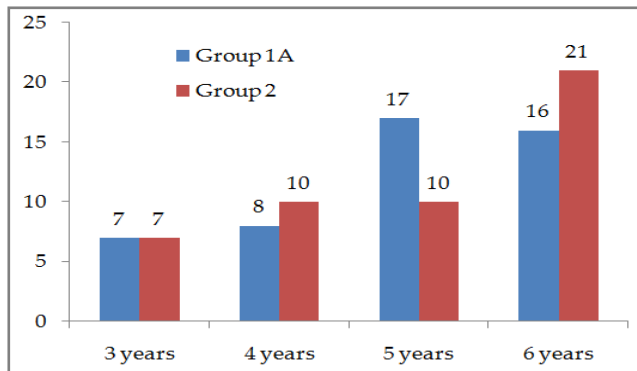


Figure-2: Age distribution of study population.

(40%) females. Age distribution was 3-6 years, most of the children in group 1A were 5 years old while in group 2 greater numbers of children were of 6 years. Group-1A age distribution that

children of 3rd year were 7 (14.6%), 4th year were 10 (20.8%), 5th year were 10 (20.8%) and sixth years were 21 (43.8%) as shown in fig-2. Growth hormone deficiency when checked by growth hormone stimulation tests was detected in 26 (54%) patients of group-1A and 21 (51%) patients of group-1B, whereas 22 (46%) patients of group-2. Inferential statistics revealed that when group-1A and 1B was compared with paired t-statistics for height (cm), weight (kg), BMI (kg/m²), Zinc (umol/l), IGF-1 (ng/ml) and IGFBP-3 (ng/ml) the mean increase from the baseline to weeks 12 was highly significant ($p < 0.001$) for all these parameters. ANOVA (table-II) revealed significant difference in serum Zinc (umol/l), IGF-1 (ng/ml) and IGFBP-3 (ng/ml) i.e. $p = 0.001$ and for height $p = 0.001$ values in all three groups meaning that all three groups (zinc deficient-baseline, zinc deficient after supplementation and group without zinc deficiency) were significantly different from each other and even after zinc supplementation, these biochemical parameters could not increase IGF-1 and IGFBP-3 up to normal levels which were present in non-zinc

Table-I: Baseline characteristics of children in each group.

Parameters	Group 1- with zinc deficiency		
	Pre supplementation (n=48)	Post supplementation (n=41)	Paired t-statistic (p-value)
Height (cm)	91.39 ± 8.137	92.98 ± 8.218	<0.001
Weight (kg)	12.75 ± 2.046	12.90 ± 2.008	<0.001
BMI (kg/m ²)	15.28 ± 1.937	14.91 ± 1.501	<0.001
Bone age (year)	3.01 ± 0.959	3.06 ± 0.988	<0.001
IGF-1 (ng/ml)	5.13 ± 2.690	7.25 ± 4.462	<0.001
IGFBP-3 (ng/ml)	39.35 ± 18.769	46.192 ± 27.113	<0.001
Zn (umol/l)	8.27 ± 1.880	11.16 ± 1.465	0.001

Table-II: Results of ANOVA.

Parameters	Pre supplementation (n=48)	Post supplementation (n=41)	Zinc non deficient (n=48)	ANOVA (p-value)
Height (cm)	91.39 ± 8.137	92.98 ± 8.218	93.73 ± 7.750	<0.001
Weight (kg)	12.75 ± 2.046	12.90 ± 2.008	12.53 ± 2.207	0.709
BMI (kg/m ²)	15.28 ± 1.937	14.91 ± 1.501	14.14 ± 0.649	0.001
Bone age (year)	3.01 ± 0.959	3.06 ± 0.988	2.84 ± 1.067	0.560
IGF-1 (ng/ml)	5.13 ± 2.690	7.25 ± 4.462	12.28 ± 4.066	<0.001
IGFBP-3 (ng/ml)	39.35 ± 18.769	46.192 ± 27.113	88.33 ± 18.092	<0.001
Zn (umol/l)	8.27 ± 1.880	11.16 ± 1.465	13.65 ± 1.396	<0.001

children of 3rd year were 7 (14.6%), 4th year were 8 (6.7%), 5th year were 17 (35.4%) and sixth years were 16 (33.3%) and group 2 showed that chil-

deficient group while bone age result was non-significant ($p = 0.560$) showing that it was same in all short stature and was not affected by zinc

deficiency and short term zinc supplementation. Base line characteristics of study population was shown in table-I.

DISCUSSION

Regarding zinc deficiency and effects of its supplementation on GH-IGF axis our study revealed following results;

Firstly zinc deficiency is common in preschool children which was supported by others^{8,9}. It has been reported that zinc inadequacy is wide spread globally, especially in those children living in under developed countries. Reduced intake of foods of animal origin and limited zinc content in diets rich in phytates, that are widely consumed in developing countries, is the cause of developing zinc deficiency¹⁰. During childhood the demand of zinc is increased, because of accelerated growth and development, that may not be met leading to suboptimal Zn status¹¹. At the time it is noteworthy, that childhood is also very important period in terms of eating habits which once developed during this time remain continue in the later stages of child's life. Un-healthy eating habits adopted during childhood can have long term consequences¹².

Secondly, Short stature associated with growth hormone deficiency has strong association with zinc deficiency. There is a strong correlation between Zn deficiency and arrested development as evident from our study. According to our findings, growth hormone deficiency was detected in 26 (54%) patients of group 1A (zinc deficient group), whereas 22 (46%) patients of group 2 (zinc sufficient group) were found to be growth hormone deficient, which were also supported¹. By activating protein kinase C, Zn regulates the intracellular transduction pathways of growth hormone. The dysfunction of zinc-finger proteins are likely to cause changes in growth hormone action because zinc-finger function as a DNA binding domain of transcription factor and ultimately gene expression of many proteins. GH dimerization, that is important for its synthesis and storage in Golgi apparatus and GH secretory granules of somatotroph cells of

rats and humans, is not possible without Zn. In the tissues, GH is stored in amyloid-like structure and this amyloid-like aggregation of GH also require zinc ions¹³.

Thirdly, short stature associated with growth hormone deficiency is improved by zinc supplementation. Our study showed significant increases in height, weight, serum Zn, IGF-1 and IGFBP-3 after 12 weeks of Zn therapy. Our findings were supported by other studies^{1,2,14} who reported that Zn supplementation was effective for inducing growth in short children with Zn deficiency. Zn is essential for maintaining the structural integrity and function of multitude of enzymes, especially the ones playing integral role in production & secretion growth hormone along with transcription and translation of DNA and hence the division of cell⁴. Zn also has an important role in the biosynthetic pathway of insulin like growth factor 1 (IGF-1) and insulin-like growth factor-binding protein 3 (IGFBP3)⁵. Several systematic reviews have also highlighted the importance and significant positive effect of zinc on child growth^{15,16}. In meta-analyses in 2009, Brown *et al* showed that zinc supplementation had a positive effect on linear growth in prepubertal children (including infants) in both developed and resource-constrained countries¹⁷. A Cochrane review by Mayo-Wilson *et al*. showed a significant positive effect of zinc supplementation on both height and weight in children⁶ as was found in our study.

Fourthly, Our study revealed that serum IGF-1 and IGFBP-3 were below normal limits in zinc deficient children (93% and 100% respectively) leading to short stature. Zinc is a trace element with various roles in physiological processes¹⁸ and the present knowledge about zinc signaling in the various processes and involved pathways seems to be disconnected by specific types of zinc signal used, with different kinetics and sources of zinc. However, most likely interplay between the different systems may exist by common underlying principles of zinc signaling. Moreover, the exact mechanism of the effects of Zn deficiency and Zn supplementation on GH

secretion, serum IGF-1 levels, and growth is not well delineated¹⁹.

Our findings are also supported by Imamoglu *et al* who reported that supplemental zinc may result in an increased sensitivity to autogenous GH and increased secretion of the physiological GH (i.e. during sleep), without modifying the response of GH to pharmacological stimulant¹³.

Childhood stunting, being short for one's age, has adverse consequences for health, human capital and economic growth. Being stunted in early childhood is associated with slower cognitive development, reduced schooling attainment and adult incomes decreased by 5-53%. The World Health Assembly has endorsed global nutrition targets including one to reduce the number of stunted children under five by 40% by 2025. The target has been included in the Sustainable Development Goals (SDG target 2.2)²⁰. Prophylactic zinc supplementation is also being considered as one of those important interventions which should be implemented at scale to achieve this SDG.

CONCLUSION

In conclusion, serum IGF-1 and IGF1BP-3 levels were found decreased in short statured children having Zn deficiency, and improved following supplemental zinc for 12 weeks but their levels were still not within normal limits in majority of children; so supplemental zinc may be required for extended duration. Furthermore, at the current dosage of 50 mg/day, oral zinc was generally safe and well tolerated and no adverse events were reported. Dietary intervention and awareness programs are required to inculcate good dietary habits and to overcome micronutrient deficiencies in Pakistani children.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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