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Burden of Zinc (Zn) deficiency: A high volume clinical laboratory data analysis
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Abstract
A cross-sectional survey was done at the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, AKUH, Karachi, to determine frequency of Zn deficiency, the second major micronutrient deficiency in developing countries, in subjects coming for investigations. Data of plasma Zn, from Jan 2013 to Dec 2014 was analyzed and deficiency was labeled at <65ug/dl. Total 469 subjects were tested for plasma Zn levels over the period of 24 months and complete information was available for 422 subjects, included in final analysis. Median age of subjects was 13 yrs (IQR 7-35) and 57% were male. Mean plasma Zn levels of the total subjects were 105±42.6ug/dl. Out of the total subjects 13.5% (n=57) had Zn deficiency (mean plasma Zn 52±11 ug/dl), and most of them were of age group 6-16yrs. These findings advocate that diet of Pakistani children should be revised in order to provide sufficient amounts of Zn.

Keywords: Plasma Zinc, Deficiency, Pakistan.

Introduction
During the past five decades, zinc (Zn) deficiency has emerged as a major micronutrient deficiency in developing countries and the understanding of its consequences has advanced considerably. Zinc deficiency is an important cause of morbidity in developing countries. Adolescents and children are especially prone to Zn deficiency due to increased growth rate and even mild deficiency of Zn can affect growth and development in children. While in adolescents it manifests with decreased serum testosterone level, decreased lean body mass and oligospermia in males.

Three indicators have been recommended by World Health Organization (WHO), the United Nations Children’s Fund (UNICEF), the International Atomic Energy Agency (IAEA), and the International Zinc Nutrition Consultative Group (IZiNCG) for assessing the Zn status, which includes nutritional assessment of dietary zinc intake, serum or plasma zinc concentration, and outcome related functional factor determined by estimating percentage of stunted growth in <5 year old children. The IZiNCG suggests that estimated global prevalence of Zn deficiency is 31%, it ranges from 4% to 73% and is more common in low-income countries. One reason stated for the increased Zn deficiency in developing countries is diet primarily composed of cereals and legumes, which contains high phytates concentration inhibiting Zn absorption from the gut. It is now recognized that during the flour milling process a lot of minerals are lost.

In Pakistan the National Wheat Flour Fortification Programme was launched in 2005 but the programme operations were suspended during 2010 to September 2013 and again resumed in October 2013. There is paucity of literature to reflect the actual Zn status of Pakistani population. However, limited studies reported 54.2% children and 37.1% preschool children to be Zn-deficient. In this study we present the estimate of Zn deficiency in subjects being tested at our clinical laboratory which serves the country with more than 200 phlebotomy centers and satellite laboratories spread all over Pakistan. Another aim was to assess the Zn status amongst two flour fortification periods in subjects from various parts of the country tested at a clinical laboratory.

Subjects and Methods
This observational study was conducted at Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Karachi Pakistan. Laboratory data of plasma Zn from 1st January 2013 till 31st December 2014 was retrieved from laboratory integrated systems. Only initial test results were included in analysis for subjects while repeated testing samples for Zn analysis were excluded. To maintain confidentiality patient’s identification were deleted and codes were given to them and exemption was sought from institutional ethical review committee.

The Zn status was assessed on plasma samples. Plasma Zn was analyzed by flame atomic absorption spectrometry on 240 FS Agilent Atomic Absorption Analyzer (Agilent Technologies, US). Three level quality control materials were run with each batch of samples. During the study period our laboratory also participated in external...
proficiency testing programme of College of American Pathologists, with 3 surveys per year and performance was acceptable (>80%) in all of them. For evaluating the burden of Zn deficiency among the study population, plasma Zn levels 65-150ug/dl were taken as optimal, with <65ug/dl taken as zinc deficiency and >150ug/dl as elevated levels.

The statistical analyses were performed using the SPSS version 19. Subjects were classified into the following three age groups, 0-6 years, 6-16 years, and >16 years and to assess the effect of flour fortification, subjects were categorized into two groups according to flour fortification periods; Jan-Oct 2013 (Non-Fortified Flour Period) and Nov 2013 - Dec 2014 (Fortified Flour Period). Mean and standard deviation was derived for quantitative variables (age, plasma Zn).

Results

A total of 422 subjects underwent testing for plasma Zn levels over a period of 24 months. The median age of the study subjects was 13 yrs with interquartile range of 7-35 and majority of the subjects were males (56.5%). The overall mean Zn level of the study group irrespective of the age was 105±42.6 ug/dl.

Out of the total subjects, 85 (20.1%) belonged to age group 0-6years, while 175 (41.4%) and 163 (38.5%) were in age groups 6-16years and >16 years respectively. Overall Zn deficiency was observed in 57 (13.5%) patients, 31 of them belonged to age group 6-16 years. Optimal Zn levels were seen in 316 subjects (75%), while only 49 subjects (11.6%) had higher then optimal levels, majority of age group >16 years. Distribution of Zn deficiency across the three age groups is shown in Table-1. Significant difference in Zn status was observed during the flour fortification and non-fortification periods (p value <0.001); shown in Table-2.

Discussion

Our study documents the extent of Zn deficiency in this part of the world and provides evidence for the existence of Zn nutritional deficiency among all age groups, highest in the adolescents. These findings show that the growing age group (6-16 years) is most susceptible for developing Zn deficiency and this is the age group which will benefit most from food fortification with Zn.

In Pakistan the National Wheat Flour Fortification Programme was launched in 2005, with funding support from Global Alliance for Improved Nutrition and Pakistan Flour Mills Association. The programme operations were suspended due to lack of funding during 2010 to September 2013 and again resumed in October 2013. Additionally to assess the effect of flour fortification on Zn status, we distributed all subjects into two groups according to fortification status and analyzed their Zn levels. Significant difference in Zn status was observed during the two time periods (Table-2). These findings advocate that flour fortification may be an effective public health intervention means to improve the Zn status of the population. Use of Zn fortified fertilizer and development of bio fortified seeds is another agricultural strategy for increasing the micronutrient content of wheat. Additionally health care providers should continue supporting and promoting exclusive breastfeeding.

Table 1: Zinc status across different age groups (n=389).

<table>
<thead>
<tr>
<th>Zinc Status</th>
<th>Overall Frequency (%)</th>
<th>Overall Zn (ug/dl) Mean± SD</th>
<th>Age Groups Frequency (%)</th>
<th>Mean Zn (ug/dl)</th>
<th>Frequency (%)</th>
<th>Mean Zn (ug/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency (&lt;65ug/dl)</td>
<td>57(13.5%)</td>
<td>52±11</td>
<td>10(17.5%)</td>
<td>54±7.9</td>
<td>31(54%)</td>
<td>52±12</td>
</tr>
<tr>
<td>Optimal (65-150 ug/dl)</td>
<td>316(75%)</td>
<td>101±23</td>
<td>64(20%)</td>
<td>109±22.5</td>
<td>138(44%)</td>
<td>95±21</td>
</tr>
<tr>
<td>Toxicity (&gt;150ug/dl)</td>
<td>49(11.6%)</td>
<td>189±42</td>
<td>11(22.4%)</td>
<td>181±32.2</td>
<td>6(12.2%)</td>
<td>180±38.8</td>
</tr>
</tbody>
</table>

Data is presented as frequency (%).

Table 2: Distribution of all subjects according to flour fortification status.

<table>
<thead>
<tr>
<th>Zinc Status</th>
<th>Jan-Oct 2013 (Non-Fortified Flour Period) (n=195)</th>
<th>Nov 2013 - Dec 2014 (Fortified Flour Period) (n=194)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Mean± SD</td>
<td>Frequency</td>
<td>Mean± SD</td>
</tr>
<tr>
<td>Deficiency (&lt;65ug/dl)</td>
<td>35(17.9%)</td>
<td>54±8.1</td>
<td>15(7.7%)</td>
</tr>
<tr>
<td>Optimal (65-150ug/dl)</td>
<td>153(78.4%)</td>
<td>94±19.6</td>
<td>137(70.6%)</td>
</tr>
<tr>
<td>Toxicity (&gt;150ug/dl)</td>
<td>7(3.5%)</td>
<td>164±6.7</td>
<td>42(21.6%)</td>
</tr>
</tbody>
</table>
during the first six months of life and complementary feeding thereafter. The breast milk is a natural source of bio-available zinc during this period and breastfeeding protects against diarrhoea, which causes excessive Zn losses.

There are certain limitations of this data; as a small sample-size and dataset are not representative of the whole population. Additionally reason for testing plasma Zn was not known.

**Conclusion**

In this study a high burden of Zn deficiency in all age groups was noted, highest in the adolescents. Zinc supplementation may be an effective public health intervention means to improve the Zn status of the population. The commonly consumed staple foods in Pakistan are rich in phytates, which inhibit the absorption and utilization of Zn. In order to tackle the problem several efficacious interventional programmes, such as supplementation and fortification, for this micronutrient deficiency must be considered to address the problem at mass level.

**Disclosure of Conflict of Interest:** The authors have no multiplicity of interest to disclose.

**Disclaimer:** Abstract has been previously presented in following conferences:

**References**