Serum Levels of Ghrelin, Leptin, IGF-I, IGFBP-3, Insulin, Thyroid Hormones and Cortisol in Prepubertal Children with Iron Deficiency

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Abstract. The aim of the present study is to investigate possible alterations in ghrelin and other hormone levels related to appetite and somatic growth in children with iron deficiency anemia. Twenty-five patients and 25 healthy controls that were prepubertal and within normal limits regarding height and BMI standard deviation scores were recruited. Ghrelin, leptin, IGF-I, IGFBP-3, insulin, thyroid hormones and cortisol levels were studied. Ghrelin, insulin and IGF-I levels were significantly low in the study group (ghrelin 13.58 ± 16.32 vs. 35.39 ± 23.69 ng/ml, p<.001; insulin 3.41 ± 2.42 vs. 5.67 ± 1.09 mU/ml, p = .008 and IGF-I 126.94 ± 92.82 vs. 203 ± 105.1 ng/ml, p = .015). We concluded that low ghrelin and insulin levels might be causes of the appetite loss in iron deficiency and as a result of appetite loss and undernutrition as well as by direct effects they might be related with growth retardation, which could be also influenced by low IGF-I levels.

Key words: Ghrelin, Hormones, Child, Iron deficiency anemia

IRON deficiency anemia (IDA) is one of the most widespread diseases of infancy and childhood affecting the performance and quality of life significantly [1]. Insufficient intake of iron containing foods is its first-line cause in developing countries and in Turkey as well [2]. Loss of appetite and unfavorable course of growth and development are typical components of the clinical picture. Several studies showed hormonal changes in subjects with IDA [3–5]. Hormonal basis for appetite loss is not yet explained [6].

Ghrelin is a growth hormone secretagogue peptide recently showed to be secreted from human and mouse stomach and is the natural ligand of growth hormone secretagogue receptor [7]. Besides other physiological functions, ghrelin’s most important effects are to stimulate food intake as well as growth hormone (GH) secretion [8, 9]. Ghrelin levels were studied extensively in normal subjects of different age groups, in various body adiposity pictures like anorexia nervosa, obesity, post-gastric bypass surgery subjects and chronic disease conditions like diabetes, pulmonary, renal and hepatic diseases [10–17]. Serum ghrelin is decreasing with age, low in obesity and post-gastric bypass surgery, high in anorexia nervosa and after diet-induced correction of obesity and varying in chronic diseases.

In the present study we hypothesized that IDA might affect ghrelin secretion and the relationship of hematological parameters with growth factors, cortisol, thyroid hormones, insulin, leptin and serum ghrelin levels could give some clues to explain the appetite loss and subsequent growth retardation in IDA. To eliminate the effects of loss of adiposity or puberty on hormonal parameters and the probability of concomitant disorders of GH-axis we selected a group of IDA children who are otherwise healthy, prepubertal and in normal limits for age and sex regarding anthropometric parameters.
Materials and Methods

The study group consisted of 16 boys and 9 girls aged 2.65–9.16 years and the control group 12 boys and 13 girls of a similar age range (3.14–11.05 years). Informed consent was obtained from at least one parent and the project was approved by the local ethics committee. Subjects in the study group were selected out of the attendees from outpatient clinics of pediatric hematology or general pediatrics. Control subjects were selected from cases of urology, otolaryngology, pediatric surgery and orthopedics outpatient clinics that have mild and isolated surgical complaints and cases referred to our pediatric endocrinology outpatient clinic that are suspected for minor endocrine problems (e.g. goiter) but found to be normal.

Inclusion criteria

- Being prepubertal
- Height, weight and BMI in normal range for age and sex
- Established IDA according to Dallman criteria for the study group [18]

Exclusion criteria

- Any acute or chronic condition including infectious diseases related or not related to gastrointestinal system (Except for IDA in the study group)
- Any acute or chronic medication
- Occult gastrointestinal bleeding was excluded by fecal examination and other bleeding disorders by anamnesis in the study group.

Before laboratory studies, subjects of both groups underwent a detailed physical examination of all systems with light clothing including height measurement using Harpenden stadiometer, weight measurement using a digital scale and evaluation of body temperature and pubertal signs.

Laboratory studies

Fasting morning blood and urine samples were obtained from patients and controls and following parameters were studied: Urinanalysis, whole blood count, blood sedimentation rate, CRP, blood smear, iron, total iron binding capacity (TIBC), transferrin, ferritin, hemoglobin electrophoresis, fT3, fT4, T3, T4, TSH, cortisol, insulin, IGF1, IGFBP-3, leptin and ghrelin. Samples were put in EDTA K3 tubes for hematological parameters and plain tubes for biochemical parameters and studied on the same day. Samples for hormonal parameters were collected in EDTA tubes and plasma was stored at –70°C until performance. Hematological tests were done using Sysmak SE-9000 autoanalyseur, iron and TIBC were measured spectrophotometrically using Technicon RA-XT autoanalyseur, ferritin using automated chemiluminescence system (ACS 180 plus). IGF-I, IGFBP-3 and leptin were studied using IRMA and ELISA commercial kits (DSL-5600, DSL-6600 and DRG international, Inc., USA, respectively).

Ghrelin was measured using human Ghrelin RIA kits (Phoenix Pharmaceuticals, Inc, Belmont, CA). During this method 125 I labeled bioactive ghrelin is used as tracer and the rise of rabbit polyclonal antibodies against octanoylated C-terminal of human ghrelin is used for the measurement of total ghrelin concentration. The intra-assay coefficient of variation (CV) was <5% and the interassay CV was <14 (%) for the kit.

Statistics

The pocket program of SPSS for Windows version 10.0 was used for statistical analyses. Student’s T test and Mann Whitney U test were used for comparisons between two groups. The correlations between parameters were analyzed using Spearman method since there were nonparametric variables. Results were considered significant when p<0.05.

Results

Mean age was younger in the study group; sex distribution as well as standard deviation scores (SDS) of height, weight and BMI on the other hand were comparable between groups as expected according to our patient selection criteria (Table 1).

Hb, Htc, MCV, MCH, MCHC, iron, TIBC, transferrin and ferritin were significantly lower in the study group as expected according to the definition of the groups (Table 2).

Insulin, IGF-I and ghrelin levels were significantly lower and T3 significantly higher in the study group (Table 3, Figure 1). Neither of the hormone levels showed sex differences.

We looked for correlations of age, height and BMI
Table 1. General characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>m/f</td>
<td>16/9</td>
<td>12/13</td>
<td>NS*</td>
</tr>
<tr>
<td>mean age (dec. yrs.)</td>
<td>5.64 ± 2.01</td>
<td>7.41 ± 2.24</td>
<td>.005†</td>
</tr>
<tr>
<td>[min–max]</td>
<td>[2.65–9.16]</td>
<td>[3.14–11.05]</td>
<td></td>
</tr>
<tr>
<td>height SDS (mean ± SD)</td>
<td>–.86 ± .77</td>
<td>–.42 ± 1.09</td>
<td>NS†</td>
</tr>
<tr>
<td>BMI SDS (mean ± SD)</td>
<td>.32 ± .86</td>
<td>–.13 ± .75</td>
<td>NS†</td>
</tr>
</tbody>
</table>

* Nonsignificant
† Student’s T test

Table 2. Comparison of hematological parameters between the groups (mean ± SD, student’s T test)

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>9.68 ± 1.49</td>
<td>12.6 ± .78</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>30.03 ± 3.4</td>
<td>37.2 ± 2.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>67.64 ± 6.5</td>
<td>81.88 ± 3.73</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fe (mcg/dL)</td>
<td>12.4 ± 4.73</td>
<td>64.6 ± 25.55</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>TIBC (mcg/dL)</td>
<td>378.72 ± 51.66</td>
<td>275.95 ± 48.26</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>9.7 ± 6.34</td>
<td>41.24 ± 12.81</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>3.96 ± 2.74</td>
<td>24.84 ± 12.62</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 3. Comparison of hormonal parameters between the groups

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 (mg/ml)</td>
<td>9.18 ± 1.48</td>
<td>8.99 ± 1.06</td>
<td>NS†</td>
</tr>
<tr>
<td>ft4 (ng/dl)</td>
<td>1.35 ± .17</td>
<td>1.42 ± .12</td>
<td>NS†</td>
</tr>
<tr>
<td>T3 (ng/ml)</td>
<td>1.91 ± .23</td>
<td>1.7 ± .25</td>
<td>.007†</td>
</tr>
<tr>
<td>ft3 (pg/ml)</td>
<td>4.66 ± .63</td>
<td>4.53 ± .69</td>
<td>NS†</td>
</tr>
<tr>
<td>TSH (mU/ml)</td>
<td>2.17 ± 1.53</td>
<td>2.01 ± .67</td>
<td>NS†</td>
</tr>
<tr>
<td>Cortisol (mcg/dl)</td>
<td>11.58 ± 4.53</td>
<td>11.5 ± 5.31</td>
<td>NS†</td>
</tr>
<tr>
<td>Insulin (mU/ml)</td>
<td>3.41 ± 2.42</td>
<td>5.67 ± 4.72</td>
<td>.008‡</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>126.4 ± 92.82</td>
<td>203 ± 105.1</td>
<td>.015†</td>
</tr>
<tr>
<td>IGFBP-3 (ng/l)</td>
<td>4.81 ± 1.05</td>
<td>5.43 ± 1.09</td>
<td>NS†</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>10.38 ± 15.14</td>
<td>8.34 ± 6.73</td>
<td>NS‡</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>13.58 ± 16.32</td>
<td>35.39 ± 23.69</td>
<td>&lt;.001‡</td>
</tr>
</tbody>
</table>

† Student’s T test
‡ Mann Whitney U-test

SDS with laboratory parameters and for correlations between each laboratory parameter separately. Parameters related to iron status were not correlated with hormonal parameters. Ghrelin was inversely related with age in the study group (r = –.458, p = .024).

Discussion

Ghrelin has been implicated in both meal-time hunger and the long-term regulation of body weight. In humans, plasma ghrelin levels rise shortly before and fall shortly after every meal, a pattern that is consistent with the role in the urge to begin eating [13, 19]. Therefore we hypothesized that the anorexia of IDA might be caused by decreased ghrelin levels and in relationship with other hormonal changes this might be the first step of the cascade towards growth arrest.

Since ghrelin, as well as other parameters display significant changes with age and puberty, we selected a group of subjects strictly before puberty and beyond infancy [20]. Furthermore we tried to establish a group of IDA children in whom significant height and weight arrest did not occur. Thus the anemia in the patient group was not severe. Unfortunately, mean age was significantly younger in the study group, but regarding the overlap of prepubertal and midchildhood characteristics of the two groups, we believe that subject’s age characteristics were homogenous enough. In addition, younger age is a reason to expect higher ghrelin levels in any population. In this context, regarding the significantly lower plasma ghrelin levels in the patient group, we believe that this finding is powerful enough to suggest that ghrelin is low in IDA. Whether this finding is sufficient to conclude that the single most important cause of appetite loss in IDA is low ghrelin levels remains to be elucidated.

When we looked for the changes of plasma ghrelin levels with increasing age, we found significant inverse relationship in the study group which is in accordance
with the literature [12, 20–22]. We couldn’t find any relationship between ghrelin levels and body size (i.e. height, weight and BMI). In a study among children with normal variant short stature ghrelin was increased and negatively correlated with height SDS in short children [23].

Although IDA children had significantly lower ghrelin levels, we couldn’t show any linear relationship between ghrelin and hematologic parameters. This could be explained by the narrow range of hematologic parameters, wide variation in ghrelin levels and the limited number of subjects in the study group.

We found no differences regarding leptin and cortisol levels between two groups. Another group checking for leptin in iron deficiency found similar levels with controls as well [24]. The common relationships between leptin-cortisol and ghrelin is not necessarily to expect in our group since our observations suggest an independent relationship between ghrelin and IDA.

Thyroid functions were similar between groups except for the elevated T3 in the study group which is of little physiological importance if any. In the literature there is no clear evidence of altered thyroid function in IDA [25].

Insulin was measured significantly lower in IDA group. Whether this is the cause or effect of decreased appetite or has no relationship with appetite either cannot be determined in our setting, but to our opinion this is an expected finding, since increased peripheral insulin sensitivity was shown formerly in IDA [5]. Since BMI was not decreased in our patients, we can speculate that their food intake was not affected by means of calories and carbohydrates, and low levels of insulin is not affected by or effecting food intake but rather reflecting the increased peripheral insulin sensitivity.

IGF-I and IGFBP-3 levels are also low in the patient group (the first one significantly and the second one almost significantly). In patient populations with malnutrition, IGF-I and IGFBP-3 levels were also found decreased but leptin is decreased as well in malnutrition [26] and ghrelin is high in anorexia nervosa, starvation and celiac disease [27, 28]. Therefore we cannot compare our group of IDA children with the above mentioned groups. In our study group the relative young age could be a reason for low IGF-I and IGFBP-3 levels but the age difference is not enough to explain this finding. Whether this finding is a consequence of decreased ghrelin which might compromise growth hormone secretion and subsequently IGF-I and IGFBP-3 synthesis remain to be elucidated.

When we summarize our findings, in a group of well defined, prepubertal, midchildhood patients within limits of normal growth and nutritional body characteristics, thus probably with new-onset, mild-moderate IDA we showed that ghrelin is decreased. In our setting it is not possible to explore any clear relationship between ghrelin and appetite and/or iron therapy, since we did not perform objective food intake analyses and prospective ghrelin measurements after institution of iron therapy. As we mentioned in introduction, iron deficiency in developing countries is related to erroneous nutrition, which is resulting from both difficulties in the supply of protein-rich foods in low-income families and deviations in food composition towards carbohydrates affected by advertising of such foods directing towards children, which are not only competing psychologically but even by means of gastrointestinal absorption with protein&iron rich foods. According to our unpublished observations, IDA is also common in overweight and obese children and there is selective appetite loss against meats, vegetables, egg, olive oil and dairy products sparing appetite against starchy and sugary foods.

Regarding the underlying mechanism in our findings, it is difficult to explain the cause-effect relationship. Our finding is interesting, because in conditions affecting appetite and growth adversely, ghrelin has been shown to increase [12, 23]. Although our patient group was in acceptable limits regarding overall appetite and growth, and this was our intention in order to exclude the effects of body size on leptin levels, IDA is crudely a catabolic state. A very recent study is supporting our findings and shows that ghrelin is low in every level of severity in iron deficiency [29]. Ghrelin is known to play a role in a number of different physiological processes including gastric motility and acid secretion as well as pancreatic exocrine function [30]. Supposing low ghrelin levels are the cause of IDA, impaired gastrointestinal processing of iron-containing foods would be an explanation. The setting of our study is not able to explore this cause-effect relationship.

In conclusion, the most important finding of this study is markedly decreased serum levels of ghrelin in IDA and this finding might be related with both of the important signs in IDA, e.g., appetite loss and growth retardation.
References