Serum Levels of Ghrelin, Tumor Necrosis Factor-α and Interleukin-6 in Infants and Children with Congenital Heart Disease

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Summary

Objective: To estimate serum levels of ghrelin, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in infants and children with congenital heart disease (CHD), compared with levels in age-matched controls, and to correlate the levels of ghrelin with TNF-α and IL-6.

Design: Case–control study.

Setting: Suzan Moubarak Hospital of Al-Minya University, Egypt.

Patients: We measured serum ghrelin, TNF-α and IL-6 levels using ELISA in 60 patients with CHD (40 acyanotic and 20 cyanotic) and in 20 control subjects.

Results: Our results showed that patients with CHD, regardless of the presence or absence of cyanosis, had significantly higher serum ghrelin, TNF-α and IL-6 than controls (p = 0.000). Serum levels of ghrelin and TNF-α in the acyanotic patients were significantly higher than in the cyanotic patients (p = 0.000). On the other hand, there was no significant difference in serum levels of IL-6 between the acyanotic and the cyanotic patients (p = 0.126). In acyanotic and cyanotic patients with CHD, there was a positive correlation between ghrelin and TNF-α (r = 0.424; p = 0.006 and r = 0.577; p = 0.008, respectively). Ghrelin levels were not correlated to IL-6 in the acyanotic and cyanotic patients with CHD (r = –0.211; p = 0.216 and r = –0.341; p = 0.08, respectively).

Conclusion: Serum ghrelin, TNF-α and IL-6 levels are elevated in patients with CHD whether acyanotic or cyanotic. Increased ghrelin levels represent malnutrition and growth retardation in these patients. The relation of ghrelin with TNF-α may be explained by the possible effect of chronic congestive heart failure and chronic shunt hypoxemia.

Key words: ghrelin, cytokines, children, congenital heart disease.

Introduction

Ghrelin is a hormone that is produced mainly by the stomach. Ghrelin is not secreted into the gastrointestinal tract like digestive enzymes but into blood vessels to circulate throughout the body. Ghrelin might also be synthesized in other organs, where it might have autocrine or paracrine effects [1, 2]. Ghrelin causes weight gain by increasing food intake and reducing fat use. Ghrelin has effects on nutrient intake and growth hormone release, subsequently on physical development and growth [3, 4].

Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are pleiotropic cytokines with numerous immunologic actions and they are potentially important catabolic factors [5, 6]. IL-6 is generally considered to be an important cytokine in the network of cytokines that regulate immune reactions and acute phase responses [7].

The relationship between congenital heart disease (CHD), malnutrition and growth retardation is well documented [8, 9]. Different types of cardiac malformations can affect nutrition and growth to varying degrees [10, 11]. The aim of this study was to estimate serum levels of ghrelin, TNF-α and IL-6 in infants and children with CHD, compared with levels in age-matched controls, and to correlate the levels of ghrelin with TNF-α, and IL-6, which has not, as yet, been documented in literature.

Subjects and Methods

The study population comprised 60 infants and children with CHD (mean age = 32.4 ± 18.8 months) classified into 2 groups: 40 acyanotic and
Patients were subjected to a complete physical examination, checking their weight and height. They were considered abnormal if they were below the 5th centile compared with standard reference data for age-matched children [12]. All patients’ cardiac diagnoses were made on the basis of clinical examination, laboratory investigations, electrocardiography and echocardiography; and none of the patients had pulmonary hypertension. Body mass index (BMI) was calculated as the ratio of body weight (kg) and squared height (m). The study also included 20 age- and sex-matched healthy infants and children as a control group (mean age = 31.3 ± 15.2 months). Informed consent was obtained from the parents of the subjects.

Specimen collection was done at 8 to 9 a.m. by venipuncture. Three milliliter of blood was allowed to clot at room temperature for 60 min. Serum was separated by centrifugation at 5000 r.p.m. for 10 min, and then stored at -20°C. Quantitative determination of ghrelin, TNF-α and IL-6 in serum was done using ELISA kits (TNF-α and IL-6 kits were purchased from Bio-Source International Inc, 542, Flynn Road, Camarillo, CA, USA; Ghrelin kit from Phoenix International, Inc, USA).

Statistical methods
Analysis of the data was done using SPSS, version 12. The following statistical tests were used:

(i) Mean and standard deviation (SD) to describe quantitative data.
(ii) Student t-test was used to compare between two groups as regards parametric data.
(iii) Chi-square test was used to compare between two groups as regards nonparametric data.
(iv) Pearson correlation was used to correlate two quantitative variables.

For all tests, a probability (p) of <0.05 was considered significant.

Results
Of the 60 patients with CHD, 36 (60%) were below the 5th centile for weight, 23 (38.3%) were below the 5th centile for height. Age and anthropometric data of the patients and controls are shown in Table 1. There was no significant difference between groups (the acyanotic and the cyanotic) in terms of mean age, sex, weight, height and BMI (all p > 0.05). The specific cardiac lesions of patients are listed in Table 2.

Patients with CHD, regardless of which group, cyanotic or acyanotic, had significantly higher serum levels of ghrelin, TNF-α and IL-6 than controls. Serum levels of ghrelin and TNF-α in the acyanotic group were significantly higher than in the cyanotic group. On the other hand, there was no significant difference in serum levels of IL-6 between the acyanotic group and the cyanotic group (Table 3).

In both groups, serum ghrelin levels were negatively correlated with BMI (Figs 1 and 2, respectively). TNF-α was not correlated with BMI in the acyanotic and cyanotic patients with CHD (r = -0.199; p = 0.231 and r = -0.137; p = 0.572, respectively). Similarly, IL-6 was not correlated BMI in the acyanotic and cyanotic patients with CHD (r = -0.034; p = 0.813 and r = -0.206; p = 0.213, respectively). Ghrelin levels were positively correlated with TNF-α in the acyanotic and cyanotic groups (Figs 3 and 4, respectively). Ghrelin levels were not correlated with IL-6 in the acyanotic and cyanotic patients with CHD (r = -0.211; p = 0.216 and r = -0.341; p = 0.08, respectively).

### Table 1

**Age and anthropometric data of the patients and the controls**

<table>
<thead>
<tr>
<th></th>
<th>Acyanotic patients (n = 40)</th>
<th>Cyanotic patients (n = 20)</th>
<th>Controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>31.2 ± 17.2</td>
<td>33.5 ± 20.2</td>
<td>31.3 ± 15.2</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>16/24</td>
<td>11/9</td>
<td>9/11</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>11.8 ± 6.8</td>
<td>10.5 ± 8.1</td>
<td>15.1 ± 3.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>89 ± 24</td>
<td>84.5 ± 29.5</td>
<td>95.4 ± 17.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>14.6 ± 1.5</td>
<td>14.3 ± 2</td>
<td>16.6 ± 1.1</td>
</tr>
</tbody>
</table>

### Table 2

**Diagnosis of the patients**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acyanotic patients</strong></td>
<td></td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>31</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>8</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>1</td>
</tr>
<tr>
<td><strong>Cyanotic patients</strong></td>
<td></td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>14</td>
</tr>
<tr>
<td>Transposition of great arteries</td>
<td>3</td>
</tr>
<tr>
<td>Tricuspid atresia</td>
<td>2</td>
</tr>
<tr>
<td>Truncus arteriosus</td>
<td>1</td>
</tr>
</tbody>
</table>

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FIG. 1. Correlation of ghrelin with BMI in acyanotic patients with CHD.

FIG. 2. Correlation of ghrelin with BMI in cyanotic patients with CHD.

FIG. 3. Correlation of ghrelin with TNF-α in acyanotic patients with CHD.

FIG. 4. Correlation of ghrelin with TNF-α in cyanotic patients with CHD.

TABLE 3
Serum levels of Ghrelin, TNF-α and IL-6 in patients with CHD and controls

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin (ng/ml)</th>
<th></th>
<th>TNF-α (pg/ml)</th>
<th></th>
<th>IL-6 (pg/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>p-value</td>
<td>Mean ± SD</td>
<td>p-value</td>
<td>Mean ± SD</td>
<td>p-value</td>
</tr>
<tr>
<td>All patients with CHD</td>
<td>36.1 ± 16.7</td>
<td>0.000</td>
<td>16.6 ± 6.3</td>
<td>0.000</td>
<td>16.4 ± 7.1</td>
<td>0.000</td>
</tr>
<tr>
<td>vs. Controls (n = 20)</td>
<td></td>
<td></td>
<td>vs.</td>
<td></td>
<td>vs.</td>
<td></td>
</tr>
<tr>
<td>Acyanotic patients with CHD (n = 40)</td>
<td>44.2 ± 13.9</td>
<td>0.000</td>
<td>18.9 ± 6</td>
<td>0.000</td>
<td>14.9 ± 6.4</td>
<td>0.000</td>
</tr>
<tr>
<td>vs. Controls (n = 20)</td>
<td></td>
<td></td>
<td>vs.</td>
<td></td>
<td>vs.</td>
<td></td>
</tr>
<tr>
<td>Cyanotic patients with CHD (n = 20)</td>
<td>19.7 ± 6.5</td>
<td>0.000</td>
<td>12.9 ± 3.5</td>
<td>0.000</td>
<td>17.8 ± 7.6</td>
<td>0.000</td>
</tr>
<tr>
<td>vs. Controls (n = 20)</td>
<td></td>
<td></td>
<td>vs.</td>
<td></td>
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</tr>
<tr>
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<td>44.2 ± 13.9</td>
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<td>0.000</td>
<td>14.9 ± 6.4</td>
<td>0.000</td>
</tr>
<tr>
<td>vs. Cyanotic patients with CHD (n = 20)</td>
<td>19.7 ± 6.5</td>
<td></td>
<td>12.9 ± 3.5</td>
<td></td>
<td>17.8 ± 7.6</td>
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<tr>
<td>vs.</td>
<td></td>
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</table>
Discussion

It is well known that malnutrition accompanies and contributes to mor bidity in CHD. The cause of growth retardation in CHD is multifactorial [13–15]. Inadequate caloric intake, malabsorption and increased energy requirements caused by increased metabolism may all contribute. However, inadequate caloric intake appears to be the most important cause of growth failure in CHD [16].

Previous studies have investigated the effect of cardiac lesion type on growth and nutrition, and several have reported that degree of cyanosis is not correlated with severity of growth impairment [17]. However, degree of growth impairment was found to be closely associated with severity of the hemodynamic impairment [14, 18, 19]. Linde and colleagues [20] found a more pronounced retardation in both height and weight in children with cyanosis than in those with acyanotic heart disease. In contradiction to the report by Linde et al., Salzer and colleagues [21] showed that infants with left to right shunt tended to gain less weight and to be leaner than those with cyanotic heart disease. In our study, there was no significant difference between the acyanotic and cyanotic patients with CHD regarding the weight, the height and the BMI.

Ghrelin is accepted as a good marker of the nutritional state, mainly in situations of malnutrition, like anorexia nervosa [22]. The inverse correlation between ghrelin levels and BMI is well defined [23, 24]. We observed the mentioned correlation, both in children with acyanotic CHD and in children with cyanotic CHD.

We found that patients with CHD, whether in one group or classified into acyanotic and cyanotic, had significantly higher serum ghrelin than controls. Growth failure in cyanotic children has not been shown to be proportional to the severity of cyanosis, suggesting that multiple factors are involved in the pathogenesis of their growth disturbance [25]. Alteration of endocrinal mediators of growth has been implicated as a possible mechanism of growth failure in cyanotic patients [26].

We found that serum TNF-α significantly increased in both the acyanotic and the cyanotic patients. Similarly, serum IL-6 was increased in both groups. TNF-α and IL-6 appear to be important cachectic process mediators, although this association is not completely established [27, 28]. Cardiac cachexia describes wasting primarily due to loss of lean body mass. Cachexia results in decreased muscle strength and function and compromised immune function [29, 30]. This syndrome is likely to occur in children who have chronic congestive heart failure and chronic shunt hypoxemia [31]. In addition to inadequate calorie and protein intake, there is evidence that this syndrome may be caused by circulating tumor necrosis factor, which stimulates catabolism [32].

In this study, ghrelin correlated positively with TNF-α in acyanotic and cyanotic patients. The relation of ghrelin with TNF-α raises the possibility of the direct effect of TNF-α upon ghrelin or the impact of heart failure severity upon both ghrelin and TNF-α. Nagaya et al. [33] have shown that plasma ghrelin level is increased in cachectic patients with congestive heart failure as a compensatory mechanism in response to anabolic–catabolic imbalance. Because growth failure is a strong independent risk factor for mortality in patients with CHD [17], it would be interesting to investigate whether additional supplementation of ghrelin attenuates the development of growth failure in patients with CHD.

Conclusion

Serum ghrelin, TNF-α and IL-6 levels are elevated in patients with CHD whether acyanotic or cyanotic. Increased ghrelin levels represent malnutrition and growth retardation in these patients. Additionally, the relation of ghrelin with TNF-α may be explained by the possible effect of chronic congestive heart failure and chronic shunt hypoxemia.

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