A study of serum levels of leptin, ghrelin and tumour necrosis factor-alpha in child patients with cyanotic and acyanotic, congenital heart disease

Iraj Shahramian,1 Noor Mohammad Noori,2 Mohammad Hashemi,3 Elham Sharafi,4 Abdolvahab Baghbanian5

Abstract
Objective: To investigate the serum levels of leptin, ghrelin and tumour necrosis factor-alpha in children with cyanotic and acyanotic congenital heart disease.

Methods: The prospective cohort study, was conducted at imam Ali Hospital, Zahedan University of Medical Sciences, Iran, in 2009-10 and comprised 64 subjects, including patients and controls. Using enzyme-linked immunosorbent assay kits, serum levels of ghrelin, leptin and tumour necrosis factor-alpha were measured and compared among patients (both cyanotic and acyanotic) and the controls, SPSS version 20 was used for statistical analysis.

Results: Of the 64 subjects, 24 (37.5%) were cyanotic, 21(32.8%) were acyanotic and 19(29.68%) were healthy controls. The three groups were homogenous in terms of age and gender characteristics. There was no significant difference among the groups leptin, ghrelin and tumour necrosis factor-alpha serum levels (p>0.05). There were also no significant differences in terms of weight, height and body mass index (P>0.05).

Conclusion: Serum levels of ghrelin, leptin and tumour necrosis factor-alpha did not change in acyanotic and cyanotic patients with congenital heart disease, suggesting that other crucial factors may regulate individuals’ nutrient intake, growth, weight and energy intake and output.

Keywords: Congenital heart disease, Ghrelin, Leptin, Tumour necrosis factor-Alpha. (JPMA 63: 1332; 2013)

Introduction
Congenital heart disease (CHD) is a type of defect or malformation in one or more structures of the heart or blood vessels that occurs before birth - in the early weeks of pregnancy when the heart is in the process of being formed.1 Such an abnormality is often present in any part of the heart at birth, and may produce symptoms at birth, during childhood, and sometimes not until adulthood. This defect is among the most common birth defects and is a leading cause of death where it affects 5-8 of every 1,000 live births.2,3 The overall incidence of CHD does not include mitral valve prolapse, premature infants with patent ductus arteriosus and bicuspid aortic valves.4 According to the National Heart, Lung, and Blood Institute, CHD affects about 35,000 infants (1 of every 125 live births) in the United States each year. This abnormality may be so slight that the baby appears healthy at birth even for many years, or might be so severe that encounters infants with a life-threatening condition. Almost 40-50% of infants with CHD are diagnosed within the first week, while 50-60% will be detected during the first month.3

Several potential genetic and environmental risk factors can contribute to congenital CHD but in most cases the reason is not known, and may include gene mutations, drinking alcohol, using cocaine or taking certain medications during pregnancy. Thalidomide may increase the risk of having a baby with a heart defect.2,5

Research on patterns of congenital anomalies and their correlation with serum levels of leptin, ghrelin and tumour necrosis factor-alpha (TNF-α) in patients with cyanotic and acyanotic, CHD has been uneven across different countries.6-9 Ghrelin, a bioactive peptide of 28 amino acids and an orexigenic hormone of gastric origin, and the adipocyte hormone leptin are two peripherally produced hormones regulating individuals’ nutrient intake, growth, weight, and energy intake and output. TNF-α is a pleiotropic, pro-inflammatory cytokine with numerous immunologic and metabolic actions.6-9

In patients with CHD, leptin, ghrelin and tumour TNF-α serum levels appear to regulate nutrient intake, growth, weight and energy intake and output. It is also claimed that there is a relationship between CHD, malnutrition and growth retardation among children.7,9,10 Such people are prone to malnutrition for several reasons, such as decreased energy intake, increased energy requirements,
or both. Different types of cardiac malformations can affect nutrition, energy homeostasis and growth in human beings to varying degrees. Yet, the direct or positive influence of serum levels of leptin, ghrelin and TNF-α on CHD or the relationship among them is documented unevenly in different sources of data. This study aimed at investigating and comparing the serum levels of leptin, ghrelin and TNF-α in patients with cyanotic and acyanotic CHD. This study has the potential to shed light on the matter by developing a different statistical analysis, and to expand human understanding of the issue.

**Patients and Methods**

The prospective cohort study was conducted at Imam Ali hospital, affiliated with Zahedan University of Medical Sciences (ZaUMS), Iran in 2009-10. Sixty four randomly-selected children, aged six months to 12 years and admitted to the hospital, were selected. The sample size was calculated based on previous studies. Patients with pulmonary hypertension, gastrointestinal diseases, autoimmune disorders, renal failure and other associated abnormalities were excluded. Others with mitral valve prolapse without valvular regurgitation or those with bicuspid aortic valve without regurgitation or stenosis were also excluded.

The participant information sheet was distributed to provide the children's parents with a comprehensive explanation of the research and its procedures before informed consent was sought for investigation. Ethics approval was obtained from the Human Research Ethics Committee at ZaUMS.

Thorough clinical and laboratory examinations and procedures were performed to diagnose patients with CHD. These included the use of Body Mass Index (BMI) to assess children's growth; use of specific immunoassay enzyme-linked immunosorbent assay (ELISA) kits to measure the serum levels of ghrelin, leptin and TNF-α; and echocardiography examination to confirm clinical diagnosis and suspected cases of CHD.

Initially, the evaluation of suspected cases of congenital heart disease involved a systematic process with three main steps: First, CHD was classified into two main categories based on the presence or absence of cyanosis, which were revealed/determined by physical examination using a pulse oximeter. Second, the two categories were further sub-divided into smaller groups based on the pattern of their chest radiography, showing evidence of increased, normal or decreased pulmonary vascular markings. Third, the evaluation was followed by electrocardiography as a technique for determining the hypertrophy of the left and/or right ventricles. Echocardiography examination was used to identify the final diagnosis, which might further be confirmed or ruled out by cardiac catheterisation.

To accurately measure the weight of children — aged less than two years — the Iranian-made scale called ‘Rasa’ was used with 10g error, while the Japanese scale, ‘Seca’ was used for the measurement of weight in children over two years old with 100g error. Height of children less than five years old was measured lying down, while standing height was used for children over five.

Blood samples were taken from patients at 07-08am in Imam Ali Hospital Pathology Lab and stored at -20°C until the procedure was completed. Only 5ml of blood was drawn from each patient for pathological analysis. This was done by a particular technician at the laboratory. Once the separation of the blood components was completed, the plasma or serum of each sample was transferred to the Clinical Research Development Centre at the ZaUMS and was stored at -70°C until the procedure was performed. Considering the cold chain process, all samples were then taken to the Bio-chemistry Lab at ZaUMS to measure the serum levels of leptin, ghrelin and TNF-α. The serum levels were analysed by ELISA kits: leptin serum was measured by specific kits made by Diagnostic Biochem Canada Inc.; ghrelin serum was measured by Ghrelin Biovendor ELISA kits; and Human TNF ELISA Med System kits were used to measure the serum level of TNF-α.

Data was analysed by SPSS version 20 mainly using Kruskal-Wallis and Spearman’s correlation, since the data was not normally distributed. Kruskal-Wallis was used to compare the groups whereas the Spearman’s correlation was applied to explore the correlation between the parameters. Data sets were presented as mean ± standard deviation. Findings that yielded P-values less than 0.05 were considered statistically significant.

**Results**

In total, 64 children met the inclusion criteria. Of them, 24 (37.5%) children were with cyanotic, CHD (male=10; 41.66% and female=14; 58.33% ); 21(32.8%) children were with acyanotic CHD (male=12; 57.14% and female=9; 42.85%); and 19 (29.68%) healthy children formed the control group (male=14 and female=5). The three groups were homogenous in terms of age and gender characteristics. Mean age of the study groups was respectively 4.49±3.38, 4.12±3.21 and 3.49±3.22 years. Similarly, mean weight of the study groups was respectively 10.68±6.18 kg (Table). No significant difference was
found among the study groups in terms of mean age, weight, height and BMI (p>0.05). There were also no significant differences between ghrelin, TNF-α or leptin levels of patients with CHD and control group (p>0.05) (Figure-1-3).

Although our findings revealed a significant correlation between serum level of leptin and BMI in patients with CHD (r=0.183; 95%; p=0.0042), no significant correlation was found between the serum levels of ghrelin or TNF-α with BMI (r=-0.095; 95%; p=0.499) and control group (r=0.169; 95%; p<0.193), respectively. Serum levels of ghrelin was not also associated with leptin in acyanotic and cyanotic patients (r=-0.308; 95%; p<0.026) (Figures-4-6).
The prospective cohort study was designed and done to investigate the serum levels of leptin, ghrelin and TNF-α in patients with cyanotic and acyanotic CHD. Overall, the review of findings showed no significant correlation between serum levels in the three study groups. The evidence of a significant difference between the acyanotic patients, the cyanotic patients and healthy people in terms of leptin, ghrelin and TNF-α serum levels has been more consistent, but this was not the case in this study. There was also no significant difference between the groups in terms of mean age, weight, height and BMI.

While it has been recognised that malnutrition and growth failure in patients with cyanotic and acyanotic CHD are multi-factorial, previous research has attempted to isolate single factors. Recent studies in the area of cardiovascular diseases have identified many relevant risk factors such as inadequate caloric intake, mal-absorption and increased energy requirements caused by increased metabolism, but depending upon the source and study, inadequate caloric intake and increased metabolism appear to be the most dominant causes of growth retardation in CHD. In this study, no significant association was observed.

While it has been documented that patients with cyanotic CHD, have a different growth deficit or retardation in weight or height compared with acyanotic patients; and that growth impairment is not strongly related to chronic tissue hypoxia. In this study no significant growth deficit or retardation was found among the study groups as demonstrated by decreased height, weight or BMI.

There is robust data that serum levels of leptin, ghrelin and TNF-α correlates significantly with various cardiovascular conditions, including congenital heart diseases, but we found different results.

Previous studies have shown that the serum level of leptin is significantly decreased in patients with cyanotic CHD compared to healthy people, but, in this study the levels were not different between the patients and the controls. This finding is in conformity with the interpretation of a similar study which reported serum levels of Leptin to be normal in both patients with CHD and controls. Our finding also showed that serum leptin concentration does correlate with BMI either in controls or in the patients, which has been reported in other studies. Previous studies have also shown that the serum levels of Leptin vary among patients with chronic heart failure (CHF) and

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Figure-5: Correlation between tumour necrosis factor -alpha (TNF-α) and body mass index (BMI) in patients with congenital heart disease and control group; no significant correlation was found between the serum levels of TNF-α and BMI in cyanotic or acyanotic patients with congenital heart disease and control group (r=-0.095; 95%; p=0.499).

Figure-6: Correlation between serum level of ghrelin and body mass index (BMI) in patients with congenital heart disease and control group; no significant correlation was found between the serum levels of ghrelin and BMI in cyanotic or acyanotic patients with congenital heart disease (r=0.169; 95%; p=0.193).
cardiovascular diseases,21,22 reflecting the controversy over the role of leptin as an essential factor of energy homeostasis and regulation of body weight. Various patho-physiological and/or biochemical parameters may contribute to these changes which can signal a deep rift in our understanding of the issue that warrants broader examination and discussion.

Recent research on malnutrition and growth retardation demonstrates that ghrelin concentration is a strong independent marker of nutritional state;9 but the level of Ghrelin may change throughout the human’s life, and is rather dependent on the food consumption by individuals. Researchers have identified that the serum level of ghrelin correlates with the release of growth hormone, and that Ghrelin plays an important role in the regulation of appetite, energy and bodyweight along with the modulation of cardiovascular functions.8,23 While growth failure and malnutrition are common among patients with CHDs, other factors can also cause the same effects.24 Yet, this study revealed no significant correlation between ghrelin level and BMI among the patients and the controls. This study differs from those which have documented an increase in the serum level of ghrelin and/or the inverse correlation of ghrelin levels with BMI in patients.9,25

In addition, our findings showed that no significant differences exist in serum levels of TNF-α among the study groups. This is in contrast with recent studies that showed an increase in TNF-α level of serum in both cyanotic and acyanotic patients.9 TNF-α, even though proposed as an important mediator of the cachectic process, is unlikely to solely explain the complex mechanism of cardiac cachexia.26,27 Clinically, cachexia is the process of losing weight which is different from malnutrition and anorexia. Cardiac cachexia, most likely as a terminal stage of CHF, is documented as non-oedematous weight loss of a certain percentage (<6%) of the previous normal weight observed over a period of time (>6 months). This involves patients who lose their muscle mass and strength as well as those with reductions in their total fat mass and bone mineral density.6 Yilmaz, et al argue that cardiac cachexia, if diagnosed late or misdiagnosed, can ultimately lead to problems in children with chronic CHF and chronic shunt hypoxaemia.9 It results in decreased blood supply to patients' skeletal muscles as it worsens endothelial dysfunction. This, in turn, yields reduced exercise capacity and lack of essential nutrients.6 Although it has been argued that inadequate calorie and protein intake along with circulating TNF-α can cause cardiac cachexia,9 but in this study no direct antagonism of TNF-α was found among the patients.

Contrary to previous research, in this study the absence of any significant relationship between serum levels of ghrelin, leptin and TNF-α in cyanotic and acyanotic patients with CHD and healthy controls suggests that any influence of serum levels on patients is still a matter of debate among scholars. While there is evidence to suggest that serum level of ghrelin correlates positively with TNF-α serum level and negatively with leptin in cyanotic and acyanotic patients with CHD,28 this association may be explained by the likelihood of the direct influence of TNF-α on ghrelin or the effect of heart disease severity (i.e. chronic CHF and chronic shunt hypoxaemia) upon both Ghrelin and TNF-α. Large-scale clinical trials are warranted to specifically explore various dimensions of this debate.

**Conclusion**

The exact mechanisms of serum levels of leptin, ghrelin and TNF-α in growth failure amongst children with CHD have not been fully elucidated. Further investigations are required to assess the role of different factors in this field.

**References**


