

Changes in serum level of ghrelin, tumor necrosis factor- α and interleukin-6 in children with congenital heart disease

Shiv Kumar Yadav^{1,2}, Qi-jian YI²

¹Key Laboratory of Child Development and Disorder Co-founded by Provincial Government & Ministry of Education, Key Laboratory of Pediatrics in Chongqing, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Chongqing 400014, China, ²Heart Center, Children's Hospital of Chongqing Medical University, Chongqing 400014, China

Submitted: 06-02-2015

Revised: 16-03-2015

Published: 01-05-2015

ABSTRACT

Objective: To investigate the changes in serum levels of ghrelin, tumor necrosis factor- α and interleukin-6 in Children with Congenital Heart Disease (CHD) at pre and post percutaneous management of the lesions. **Material & Methods:** We measured the serum levels of ghrelin, tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) by enzyme linked immunosorbent assays (ELISA) in 67 patients with CHD and in 20 control subjects; and compared these markers of inflammatory reaction between groups of pre-closure (37 patients) and at the end of 5 minutes (37 patients), 1 month (22 patients) and 3 month (8 patients) post percutaneous management of the lesions. **Results:** Our results showed that the patients with CHD had significantly higher serum level of Ghrelin, TNF- α and IL-6 in comparison of the controls but gradually return towards the baseline levels at the end of 3 months post percutaneous management of the lesions. **Conclusion:** The inflammatory reaction is activated in pre-closure and self-limited post-closure in children with CHD. Percutaneous management could improve the inflammatory process, which is safe and effective in children with CHD.

Key words: Ghrelin, Tumor Necrosis Factor- α , Interleukin-6, Percutaneous

Access this article online

Website:

<http://nepjol.info/index.php/AJMS>

DOI: 10.3126/ajms.v6i5.12129

INTRODUCTION

Ghrelin is a hormone that is produced mainly by the stomach and is not secreted into the gastrointestinal tract like digestive enzymes but into blood vessels to circulate throughout the body. It 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. It has effects on nutrient intake and growth hormone release, subsequently on physical development and growth.^{3,4} Nagaya et al⁵ have shown that plasma ghrelin level is increased in cachectic patients with congestive heart failure as a compensatory mechanism in response to anabolic-catabolic metabolic.

Tumor necrosis factor α (TNF- α) and Interleukin-6 (IL-6) are pleiotropic cytokines with numerous

immunologic and metabolic actions.^{6,7} IL-6 is generally considered to be an important cytokine in the network of cytokines that regulate immune reactions and acute phase responses.⁸

The relationship between congenital heart disease (CHD), malnutrition and growth retardation is well documented.^{9,10} The children in early age with CHD are very easy to have malnutrition due to many reasons including decreased energy intake, increased energy requirement or both. Different types of cardiac malformations can affect nutrition and growth to varying degrees.^{11,12} The main aim of the study to evaluate the serum level of ghrelin, TNF- α and IL-6 in children with CHD at pre and post-percutaneous procedure of the lesions, and effectiveness of percutaneous management for these markers to be discussed.

Address for Correspondence:

Qi-Jian Yi, MD. PhD. Heart Center, Children's Hospital of Chongqing Medical University, No. 136, Zhongshan 2nd Road, Yuzhong District, Chongqing, PR China, 400014. **Tel no:** +86-23-63624344.

E-mail: QJYI2003@126.COM

© Copyright AJMS

MATERIAL & METHODS

Study population

The study was conducted on 67 Children with congenital heart disease (CHD) and 20 healthy children as control subjects. All patients were completed physical examination, checking their weight and height. They were supposed to be abnormal if they were below the 5th percentile compared with standard reference data for age-matched children.¹³ All patients' cardiac diagnoses were made on the basis of clinical examinations, laboratory investigations, electrocardiography, echocardiography and catheterization procedure. Informed consent was obtained from the parents of the patients. The patients have treated with percutaneous procedure at the Heart Center, Children's Hospital of Chongqing Medical University, Chongqing, P. R. China between January 2013 to September 2013. During the percutaneous procedure, no death occurred. We retrospectively selected into 4 groups: Group A (Pre-closure), Group B (post-closure 5 minutes), Group C (post-closure 1 month), Group D (post-closure 3 months) and control subjects group; and compared the parameters (Ghrelin, TNF- α and IL-6) between the groups.

Laboratory investigation and immunoassay

All blood samples were drawn during the percutaneous procedure by venipuncture and the control group blood have collected in the OPD (Out Patient Department) laboratory; each blood sample was three milliliter. Serum was separated by centrifugation at 3500 r.m.p. for 5 minutes and then stored at -20°C. Quantitative determination of ghrelin, TNF- α and IL-6 in serum was done using ELISA kits (Shanghai Hushang Biological Technology Co., Ltd. China).

Statistical analyses

All data were analyzed by SPSS software, version 17.0 for Windows. Data were presented as mean \pm standard deviation. The given data were compared between groups using student t-test. Correlations between the parameters were explored with Pearson correlation. *P* values less than 0.05 were considered statistically significant.

RESULTS

Sample description

Patients' and controls' baseline characteristics are presented in Table 1. Sex, age, weight were not statically different between the groups: Group A, Group B, Group C, Group D and control subjects. None of the patients presented with pulmonary hypertension during the cardiac catheterization (pulmonary artery pressure >30 mm Hg and/or pulmonary mean pressure >20 mm Hg). The

children with type of congenital heart diseases are listed in the Table 2.

Baseline Ghrelin, TNF- α , IL-6 and controls

The serum level of ghrelin were significantly higher in children with CHD at the pre-percutaneous procedure compared to the healthy subjects (controls) (*P* = 0.0004). On comparison the serum level of ghrelin in group A with group B, group C and group D, there were no any significant difference where the *P*-value is *P* = 0.8854, *P* = 0.1931 & *P* = 0.1449 respectively. The serum TNF- α levels in the children with CHD were significantly higher than in the control subjects (*P* < 0.0001) and group D (*P* = 0.0139). The serum level of TNF- α in group A vs group B (*P* = 0.7795), vs group C (*P* = 0.2502) have no any significant differences. At the same time, the serum level of IL-6 was significantly higher in the children with CHD to compare with the control group (*P* < 0.0001). To compare for the serum level of IL-6 between the group A vs group B, vs group C and vs group D (*P* = 0.8201, *P* = 0.1113 & *P* = 0.0835 respectively) have no any significant differences (Table 3)

At the end of the first month, a mild decrease in serum level of ghrelin, TNF- α and IL-6 were noted. By end of the third month post-percutaneous procedure, we found that the serum level of ghrelin, IL-6 progressively decrease as well.

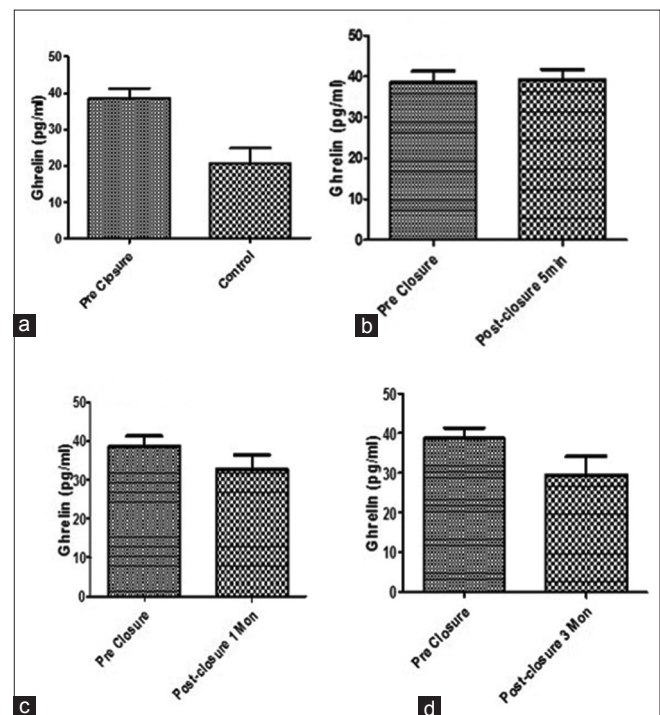


Figure 1: Correlation of serum ghrelin level between the groups: a. Pre-closure(37) vs controls (20) *P*-value = 0.0004; b. Pre-closure(37) vs Post-closure 5 minutes (37) *P*-value=0.8854; c. Pre-closure (37) vs Post-closure 1 month (22) *P*-value = 0.1931; and d. Pre-closure (37) vs Post-closure 3 month (8) *P*-value=0.1449

Table 1: Age and anthropometric data of the patients and the controls

	Group-A & B (n=37)	Group-C (n=22)	Group-D (n=8)	Controls (n=20)	P-value
Age (years)	4.214±2.400	6.272±4.261	5.043±3.391	5.800±3.334	0.0995
Male:female	19/18	8/14	1/7	12/8	0.099*
Weight (kg)	15.61±4.499	15.89±5.429	18.24±4.794	17.75±4.697	0.2712

Note: There are no any significant difference between groups; P>0.05

Table 2: Diagnosis of the patients

	Group-A & B	Group-C	Group-D
VSD	16	3	1
PDA	9	14	4
ASD	8	5	3
PS	1	0	0
ASD+PDA	1	0	0
PDA+VSD	1	0	0
ASD+VSD	1	0	0

Note: VSD: Ventricular Septal Defect; PDA: Patent Ductus Arteriosus; ASD: Atrial Septal Defect; PS: Pulmonic stenosis

Table 3: Serum level of Ghrelin, TNF-α & IL-6 in each groups (mean±SD)

	Ghrelin (pg/ml)	TNF-α (ng/L)	IL-6 (ng/L)
Controls (n=20)	20.73±4.155	53.00±11.41	32.55±6.585
Group A (n=37)	38.64±2.668#	103.3±5.374#	73.79±5.751#
Group B (n=37)	39.17±2.555*	101.1±5.785*	72.05±4.950*
Group C (n=22)	32.65±3.835*	92.67±7.663*	59.23±6.522*
Group D (n=8)	29.53±4.561*	88.21±13.06**	58.37±9.921*

Note: #Compared with Controls, p<0.05; *Compared with Group A, p>0.05, **Compared with Group A, p<0.05

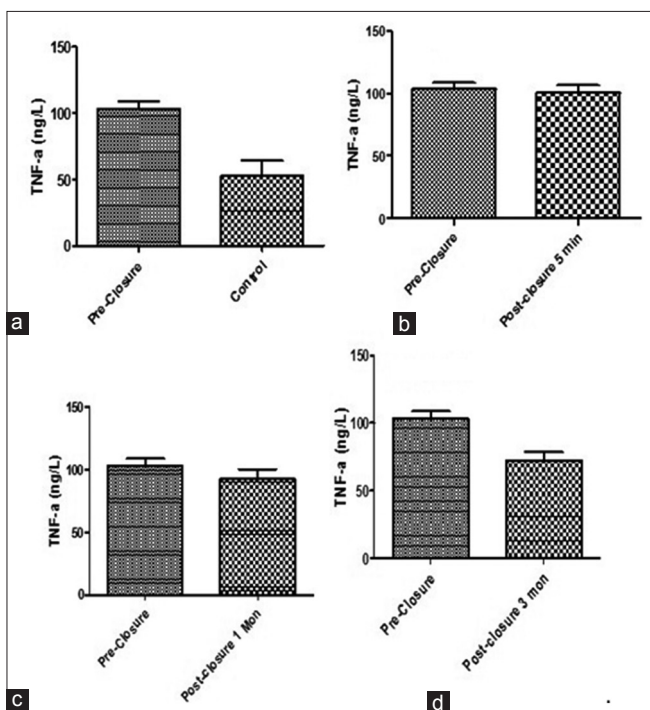


Figure 2: Correlation of serum TNF-α level between the groups: a. Pre-closure (37) vs controls (20) P-value < 0.0001; b. Pre-closure (37) vs Post-closure 5 minutes (37) P-value = 0.7795; c. Pre-closure (37) vs Post-closure 1 month (22) P-value = 0.2502; and d. Pre-closure (37) vs Post-closure 3 month (8) P-value = 0.0139

Although, the serum level of these three markers were not decrease very significantly (except TNF-α) but it showed that it is heading toward the baseline of as control subjects level.

DISCUSSION

Ghrelin has received attention as an appetite stimulant, but receptors are found in heart and vessels and numerous

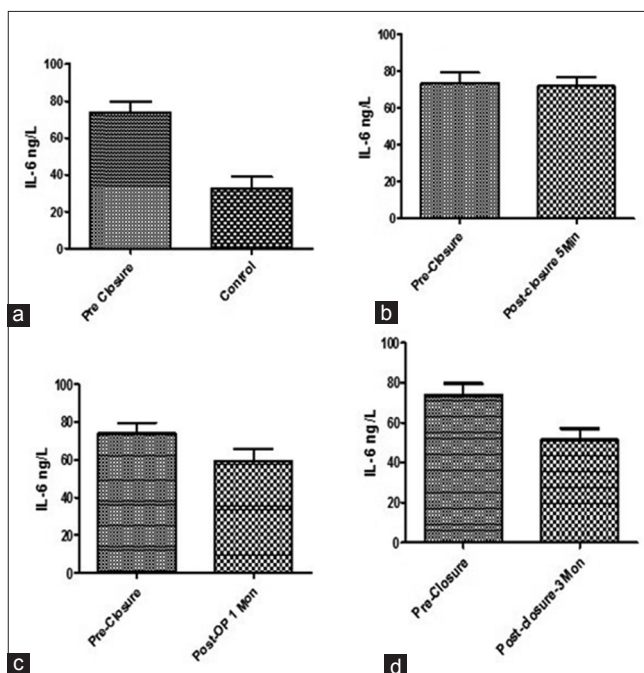


Figure 3: Correlation of serum IL-6 level between the groups: a. Pre-closure (37) vs controls (20) P-value < 0.0001; b. Pre-closure (37) vs Post-closure 5 minutes (37) P-value = 0.8201; c. Pre-closure (37) vs Post-closure 1 month (22) P-value = 0.1113; and d. Pre-closure (37) vs Post-closure 3 month (8) P-value = 0.0835

cardiovascular effects have been described.¹⁴ It is well known that malnutrition accompanies and contributes to morbidity in CHD. The cause of growth retardation in CHD is multifactorial.¹⁵⁻¹⁷ 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 CHD¹⁸ and growth failure is a strong independent risk factor for mortality in patients

with CHD.¹⁹ In our study, we have not found increasing body weight significantly at the end third month of post-percutaneous management.

In this study, the serum levels of ghrelin were investigated in the children with congenital heart diseases pre-percutaneous procedure, post-percutaneous procedure (at 5 minutes, 1 month and 3 month) and control subjects. We got to know that the serum level of all these three markers at pre-percutaneous procedure were very high in children with CHD in the comparison of control subjects. Contrarily, at end of first month after percutaneous procedure, there was no significant change in ghrelin concentrations but has decrease mildly. While at the end of third month after percutaneous procedure moderately decrease was found in the serum level of ghrelin in comparison of the pre-percutaneous procedure levels. The increased concentrations of ghrelin found in children with CHD could be the main cause for poor development in the present study subjects, have poor body weight. A larger number of patients and long series over 6 month to one year of the study could have given the significant deference in the weight of patients. Unfortunately, we are lacking patients of third month (8 cases) follow up.

Present study found that the serum levels of TNF- α in child with CHD before percutaneous procedure were significantly higher than the control subjects. Similarly the serum level of IL-6 in the children with CHD before percutaneous procedure was significantly higher than the control subjects. Given that TNF- α constitutes one of the important stimuli for IL-6 peripheral production,²⁰ the solitary increase of IL-6 levels appears as a 'paradox'. IL-6 emerges as a risk factor and sensitive marker of various cardiovascular disorders including congenital heart diseases²⁰⁻²³ and an independent predictor of future cardiovascular events in high-risk patients.²⁴ TNF- α is increasingly expressed in the failing myocardium, it affects cardiomyocyte contractility and participates in the left ventricular dysfunction and hypertrophy.²¹ There is evidence that in asymptomatic or with mild symptoms congenital heart disease patients, TNF- α is not elevated, in contrast to IL-6.²⁵

Taken all together, these data may suggest that IL-6 represents a more sensitive inflammatory marker of myocardial dysfunction which increases during the initial steps of the inflammatory process, whereas TNF- α could be released to the peripheral circulation after heart failure reaches an advanced stage. This may be attributed to a different, than the TNF- α , initial stimulus of the IL-6 release in congenital heart diseases.

During the follow up period, we observed that there were slightly decrease in the serum level of TNF- α and IL-6

at the end of the first month post-closure and a marked decrease in the serum level of these markers, which is reaching toward control levels at the end of third month. These findings suggest that percutaneous closure does not deteriorate the inflammatory status in patients with the congenital heart diseases. However, we assume that the serum level of ghrelin, TNF- α and IL-6 could go as far as to normalize the serum level of TNF- α and IL-6 with time going. Unfortunately, relative data in the post-closure 3 month of patients are lacking.

Our results need to be tested in a larger sample of patients where subgroup comparisons could be allowed (i.e. children vs adults, different functional status). Furthermore, a longer follow up with end point data is necessary in order to determine if ghrelin, TNF- α and IL-6 levels can be used as predictive factors for late complications (i.e. arrhythmias) or deterioration of functional status. A study with a longer follow-up period would have allowed us to explain the results of a number of studies demonstrating the increased ghrelin, TNF- α and IL-6 levels in adult patients long-term after the percutaneous closure. We should observe, however, that in a number of studies supporting the latter assumption, the study sample included a variety of cardiac abnormalities with a rather small cohort of patients with CHD and the treatment of choice should be percutaneous closure.

Finally, the possible correlations between IL-6 and TNF- α may be attributed to another transcriptional factor not evaluated by this study. Therefore, the pathophysiology involving ghrelin and cytokine expression of IL-6 & TNF- α in CHD patient remains to be clarified.

CONCLUSION

In our study, we found that the serum level of ghrelin, TNF- α and IL-6 elevated in children with CHD. Probably increased ghrelin levels represent growth retardation in these patients. The inflammatory reaction is activated in pre-closure and self-limited post-closure in children with CHD. Percutaneous closure could improve the inflammatory process that changed the serum level of ghrelin, TNF- α and IL-6 gradually toward the control subjects, which is safe and efficient in children. We speculate that the longer duration of the study like at the end of 6 month, 1 year and 2 year post-closure, the investigations of these markers will probably give the great result.

ACKNOWLEDGEMENT

To my supervisor, Prof. Qi-jian YI, for his professional guidance, his patience in supervisions.

REFERENCES

1. Van der Lely A, Tschop M and Heiman E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev* 2004; 25:426-457.
2. Zou CC, Hung K and Liang K. Polymorphisms of the ghrelin/obestatin gene and ghrelin levels in Chinese children with short stature. *Clin Endocrinol* 2008; 8:67-88.
3. Cummings DE and Shannon M. Roles for ghrelin in the regulation of appetite and body weight. *Arch Surg* 2003; 138:389-396.
4. Ueno H, Yamaguchi H and Kangawa K. Ghrelin: a gastric peptide that regulates food intake and energy homeostasis. *Regul Pept* 2005; 126:11-19.
5. Nagaya N, Uematsu M and Kojima M. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation* 2001; 104:2034-2038.
6. B. Beutler and A. Cerami, "Cachectin (tumor necrosis factor): a macrophage hormone governing cellular metabolism and inflammatory response," *Endocrine Reviews* 1988; 9,(1): 57-66.
7. Tracey KJ and Cerami A. "Tumor necrosis factor: a pleiotropic cytokine and therapeutic target," *Annual Review of Medicine* 1994; 45:491-503.
8. Le J and Vilcek J. "Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response," *Laboratory Investigation* 1989; 61(6): 588-602.
9. Forchielli L and McColl R. Children with congenital heart disease: a nutrition challenge. *Nutr Rev* 1994; 52: 348-353.
10. Nydegger A and Bines JE. Energy metabolism in infants with congenital heart disease. *Nutrition* 2006; 22: 697-704.
11. Gingell RL and Hornung MG. Growth problems associated with congenital heart disease in infancy. In: Lebenthal E (ed). *Textbook of Gastroenterology and Nutrition in Infancy*. New York, NY, USA: Raven Press, 1989 pp. 639-49.
12. da Silva VM, de Oliveira Lopes MV and de Araujo TL. Feeding difficulties and growth delay in children with hypoplastic left heart syndrome versus d-transposition of the great arteries. *Pediatr Cardiol* 2008; 29:328-333.
13. El-Naggar M. Clinical assessment of growth and development. In: *Pediatric Clinical Diagnosis*, 5th edn. Cairo, Egypt: University Book Center, 2007, 56-58.
14. Papotti M, Ghe C, Cassoni P, Catapano F, Deghenghi R, Ghigo E and Muccioli G. Growth hormone secretagogue binding sites in peripheral human tissues. *J Clin Endocrinol Metab* 2000; 85:3803-3807.
15. Krieger I. Growth failure and congenital heart disease. *Am J Dis Child* 1970; 120:497-502.
16. Gilger M, Jensen C and Kessler B. Nutrition, growth, and the gastrointestinal system: basic knowledge for the pediatric cardiologist. In: Ganson A, Bricker JT, McNamara PG (eds). *The Science and Practice of Pediatric Cardiology*. Philadelphia: Lea & Febiger, 1990, 2354-2370.
17. Unger R, DeKleermaeker M and Gidding SS. Improved weight gain with dietary intervention in congenital heart disease. *Am J Dis Child* 1992; 146:1078-1084.
18. Abad-Sinden A and Sutphen JL. Growth and nutrition. In: Allan DH, Gutgesell HP, Clark EB, et al. (eds). *Moss and Adams' Heart Disease in Infants, Children, and Adolescents*. Philadelphia, PA, USA: Lippincott Williams & Wilkins, 2001, 325-32.
19. Varan B, Tokel K and Yilmaz G. Malnutrition and growth failure in cyanotic and acyanotic congenital heart disease with and without pulmonary hypertension. *Arch Dis Child* 1999; 81:49-52.
20. El-Menyar A. Cytokines and myocardial dysfunction: state of the art. *J Cardiac Fail* 2008; 14:89-98.
21. Adamopoulos S, Parissis J and Kremastinos D. A glossary of circulating cytokines in chronic heart failure. *Eur J Heart Fail* 2001; 3:517-526.
22. Sharma R, Bolger A, Li W, Davlouros P, Volk H-D, Poole-Wilson P, et al. Elevated circulating levels of inflammatory cytokines and bacterial endotoxin in adults with congenital heart disease. *Am J Cardiol* 2003; 92:188-193.
23. Hudzik B, Szkodzinski J, Romanowski W, Danikiewicz A, Wilczek K, Lekston A, Polonski L, Zubelewicz-Szkodzinska B. Serum interleukin-6 concentration reflects the extent of asymptomatic left ventricular dysfunction and predicts progression to heart failure in patients with stable coronary artery disease. *Cytokine* 2011;54:266-271.
24. Cesari M, Penninx B, Newman A, Kritchevsky S, Nicklas J, Sutton-Tyrrell K, et al. Inflammatory markers and cardiovascular disease (the health, aging and body composition [health ABC] study). *Am J Cardiol* 2003; 92:522-528.
25. Takaya J, Ikemoto Y, Teragushi M, Nogi S and Kobayashi Y. Plasma nitric oxide products correlate with cardiac index of congenital heart disease. *Pediatr Cardiol* 2000; 21:378-381.

Authors Contribution:

SK Yadav: designed the study, collected the laboratory data, analysed the data, drafted the manuscript & reviewed the manuscript; **Qi-jian Yi:** contributed to study design, edited and revised the manuscript.

Source of Support: Nil, **Conflict of Interest:** None declared.