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Evaluation of Brain Natriuretic Peptide, Tumor Necrosis Factor and Interleukin-6 Plasma Levels in Infants and Children with Congenital Heart Disease

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Abstract

Background
Congenital heart disease (CHD) is the most common cause of major congenital birth defect. The aim of this study was to investigate the association of CHD with serum levels of Tumor necrosis factor-α (TNFα), Interleukin 6 (IL-6), and B-type Natriuretic Peptide (BNP) in infant and children.

Materials and Methods
The present study was conducted in Heshmat heart hospital and 17 Shahrivar hospital Rasht city, Iran. In 50 children, 25 with CHD and 25 age and sex-matched healthy children (were selected from companions or patients without heart disease), 3 ml vein blood samples of right hand were taken by a trained nurse and samples were sent to a private laboratory. After separation, the plasma placed at temperature of -20 °c. ELISA kits used for measuring BNP and eBioscience Bender MedSystems GmbH kit for measuring IL-6 and TNF-α. The results were entered into SPSS software (version 20.0).

Results
Serum levels of TNF-α and IL-6 and BNP in the healthy and patient groups were evaluated and by comparing results of the two groups there was no significant difference in serum levels of TNF-α and IL-6, but the serum levels of BNP has significant difference between two groups (80.7 ± 52.5 pg/ml vs. 455.4 ± 550.4 pg/ml, P=0.003). There was no significant relationship between BNP changes with changes in serum TNF-α, IL-6 levels.

Conclusion
Our study provided conclusive evidence BNP level is elevated in children with CHD; and significant relationship was observed between incidents of CHD respect to changes in serum BNP level.

Key Words: BNP, Children, Congenital heart disease, Interleukin 6, TNF-α.

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1- INTRODUCTION

Congenital cardiovascular abnormalities are caused by abnormal organogenesis during the embryonic period (1, 2); and the largest group of abnormalities in humans at birth and one of the causes of death in the first year of birth (3). Prevalence of Congenital heart disease (CHD) in the first year of life was 0.821% from 1998 to 2005. In 2010, overall prevalence of CHD was 1.311% in children and 0.612% in adults. CHD prevalence increased by 11% in children and 57% in adults from 2000 to 2010 (4).

It is estimated that 8% of cardiovascular abnormalities are due to genetic and chromosomal abnormalities, 2% due to peripheral teratogenic agents, such as medications, and the rest are due to the interaction between genetic and environmental factors (multi-factorial causes). To prevent this disorder, researchers are now trying to identify the risk factors; for example, the genes regulating the development of the heart are determined and their mapping is identified, and mutations that lead to heart defects are detected (5). Among the environmental risk factors, infections, thyroid disorders, maternal diabetes, nutritional factors, viruses, especially rubella and drugs such as thalidomide, and socioeconomic and cultural status can be mentioned (5-11).

Given that inflammatory factors increase in adult patients with heart disease, there is evidence that there are pre-inflammatory cytokines in children with congenital heart disease (12, 13); in general it seems that the main cause of the disease must be examined at the cellular-molecular level (14). Given the available evidence, there seems to be a trace of inflammatory factors in the pathogenesis of CHD. However, high levels of brain natriuretic peptide (BNP) have been reported in this disease, but so far these associations have not been studied and its proof can support anti-inflammatory treatment in patients. However, the effects of these factors on each other can also be used to determine the prognosis of disease for treatment of the patient. Therefore, the aim of this study was to investigate serum levels of tumor necrosis factor (TNF-α), interleukin6 (IL-6), and BNP, their relationship and their association with CHD in infants and children with CHD compared with healthy children.

2- MATERIALS AND METHODS

The present study is a case-control study on 50 children (25 patients with CHD and 25 healthy children). The group of patients was children under the age of four-year-old who were referred to the pediatric cardiology clinic of Heshmat hospital in Rasht, Iran and performed with a physical examination of the pediatric cardiologist and electrocardiogram (ECG), chest X-ray, echocardiography and, if necessary, angiography and cardiac catheterization, and diagnosis of CHD was given for them and were placed in the group of patients. Twenty-five other children who were matched according to age and sex were selected from companions or patients in 17 Shahrivar hospital in Rasht and after examination and echocardiography by a specialist, heart disease was rejected for them and entered the study as a control group.

The children under the study had not a history of infectious, inflammatory, cardiomyopathy, heart failure, renal and pulmonary disease during the first month before the study. Demographic data including age and sex were recorded, then from each child, 3 ml vein blood samples of right hand were taken by a trained nurse. Samples were sent to a private laboratory for separation. After separation, the plasma placed at temperature of -20 °C and serum levels of TNF-α and IL-6 were measured by ELISA kits (eBioscience Bender Medsystems GmbH kits with

normal ranges of 0-15 pg/ml for TNF-α and 0-12.7 pg/ml for IL-6 respectively). Serum level of BNP was determined using enzyme-linked fluorescent assay (ELFA) technique (Biomerieux Vidas ProBNP kits with normal ranges 0-150 pg/ml. The results were analyzed using SPSS software version 20.0 and descriptive and analytical analyzes was performed.

Chi-square test was used to report qualitative data, frequency and percentage, and to compare them in two groups, Chi-square test was used and to report quantitative data continuously after examining the normal distribution of them by test Kolmogorov-Smirnov, mean and standard deviation and to compare these variables in the two groups, independent t-test was used. Using the Pearson correlation test, the relationship between serum levels of TNF-α, IL-6 and BNP was investigated. To investigate the relationship between the findings of serum levels of TNF-α, IL-6 and BNP and CHD, Phi and Kappa tests were used. The significance level of P-value <0.05 was considered as a significant difference.

This study was approved by the institutional review board and Vice Chancellor for Research of Guilan University of Medical Sciences, Rasht, Iran (project number: 3/132/3172). The research process was explained to the parents before performing examinations and blood sampling. Individuals were free to participate in the study and all parents signed informed written consent prior to participation in the study (Declaration of Helsinki).

3- RESULTS

In this study, 25 boys and 25 girls with an average age of 15.4 ± 14.6 months were present. The healthy children group consisted of 13 boys and 12 girls with an average age of 14.1 ± 9.2 months and a pediatric CHD with an average age of 16.8 ± 18.6 months. Comparison of age between the two groups showed that there was no significant difference between the two groups (P = 0.505) **(Table.1)**. The serum levels of TNF-α, IL-6 and BNP in the healthy group were 14.73 ± 5.72 pg/ml, 6.80 ± 10.52 pg/ml, and 95.60 ± 40.7 pg/ml, respectively, and in the patient group it was 16.89 ± 9.47 pg/ml, 5.44 ± 9.49 pg/ml and 455.4 ± 550.4 pg/ml, respectively. Comparing the results between the two groups **(Table.2)**, there was no significant difference in TNF-α and IL-6 serum levels (P (TNF-α) = 0.333, and P (IL-6) = 0.692), but serum levels of BNP was significantly different in the two groups (P(BNP)= 0.003).

Serum levels of TNF-α, IL-6 and BNP in the study groups showed that 10, 3 and 2 subjects respectively in healthy group (40%, 12% and 8%, respectively), and 12 , 3 and 16 subjects, respectively in the CHD group (48%, 12% and 64%, respectively) had serum levels above the normal range. Significant differences were observed between the two groups in terms of serum BNP levels. The results of the study showed that there is no significant relationship between changes in serum levels of TNF-α, IL-6 and BNP (P>0.05). **Figure.1** shows the values of serum levels measured in all 50 pediatric patients. The numbers 1 to 25 refer to the results of the control group and numbers 26 to 50 of the results of pediatric patients with CHD.

As it is known, however, serum levels of BNP have been significantly increased in children with CHD, but there was no significant relationship with changes in serum TNF-α, IL-6 levels. Also, the results showed that there was a significant relationship and agreement with the incidence of CHD in children with respect to changes in serum BNP level, so that the values obtained from these studies were 0.583 and 0.560, respectively (Phi and Kappa tests numerical p-values of less than 0.001 were obtained for both surveys).

Table-1: Demographic information of all subjects

All Subjects	Study Groups	Gender	Age (Mean ± SD); months	P-value
50 Subjects	Healthy Subjects: n= 25	13 Boys (52%)	14.1 ± 9.2	0.505
		12 Girls (48%)		
	CHD subjects: n= 25	12 Boys (48%)	16.8 ± 18.6	
		13 Girls (52%)		

SD: Standard Deviation; CHD: Coronary Heart Disease.

Table-2: Results of comparing serum levels of TNF-α, IL-6 and BNP between healthy and CHD groups

Serum Levels (pg/ml)	Healthy Subjects (mean ± SD)	CHD Subjects (mean ± SD)	P-value
TNF-α	14.73 ± 5.72	16.89 ± 9.47	0.333
IL-6	6.80 ± 10.52	5.44 ± 9.49	0.692
BNP	95.60 ± 40.7	455.4 ± 550.4	0.003

TNF-α: Tumor Necrosis Factor Alpha; IL-6: Interleukin-6; BNP: Brain Natriuretic Peptide.

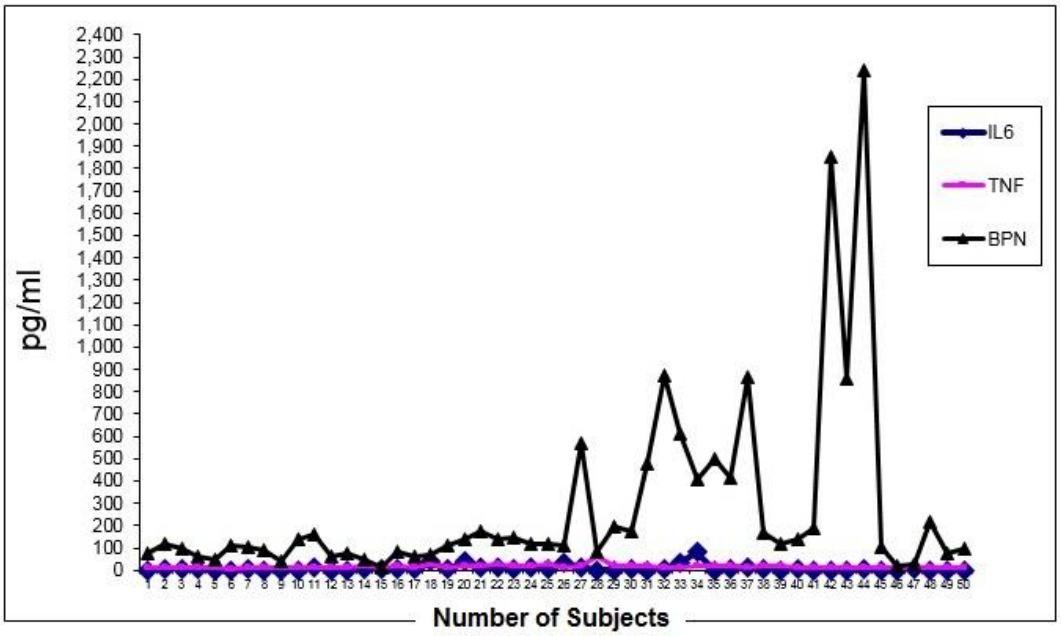

Fig.1: Results of TNF-α, IL-6 serum levels in all children examined. The numbers from 1 to 25 in the horizontal axis are related to the results of the control group and 26 to 50 results for pediatric patients with congenital heart disease.

4- DISCUSSION

Based on the findings of the present study, TNF-α and IL-6 have not changed in CHD, which is different from the Yilmaz study in 2007(15) and Wang study in 2016 (16). In the present study, patients with congenital cyanotic heart disease and also with non-cyanotic CHD have been implicated and can be the possible cause of this difference, although levels of TNF-α and BNP in patients with CHD in the Wang study (16) there was no significant difference between cyanotic and non-cyanotic. Also, the difference in the results may be related to the number of more examples in the above studies. In the present study, serum BNP level in CHD patients was significantly higher than that in control group, which is consistent with the results of Kunii study in 2003 (17), and Koch study in 2006 (18).

TNF-α is a pro-inflammatory cytokine that plays a key role in regulating inflammatory and pro-inflammatory mediators and promotes quick defense of the host against inflammatory (19). In adults with acute coronary heart disease (ACHD), plasma levels of TNF-α are significantly higher in patients with moderate to severe symptoms than those with mild or asymptomatic symptoms (20). A study conducted in Egypt showed that patients with CHD had higher levels of TNF-α than healthy infants and children (21).

TNF-α in the course of inflammatory reactions in the heart can cause the apoptosis to be misplaced and damage the muscle cells of the heart and reduce the function of the heart(22). Despite the increasing evidence that cytokines such as TNF-α may be critically affected by heart damage, the initial cytokine stimulus remains unknown (23). On the other hand, IL-6, is a pre-inflammatory cytokine (22), and is another cytokine involved in congenital heart defects, which is a pleiotropic cytokine with multiple immune activity, which is considered a potential catabolic factor (21). The IL6 system improves inflammatory events by activating and proliferating lymphocytes, differentiating B cells, and serving white blood cells (19). It can be said that TNF-α and IL6 are polypeptide cytokines that are released in response to endotoxin and tissue damage (20). In the case of IL6, there are also studies showing that the serum levels of this inflammatory factor in patients with CHD is higher than that of healthy individuals (15), although no significant changes were made in the present study. Further studies with larger sample size are need to know better biomarkers effectives of mentioned above.

In the course of inflammation, following the release of some inflammatory and non-inflammatory factors, blood flow to the inflammatory zone increases. BNP is a natriuretic peptide, diuretic, and vasodilator cardiac hormone which is mainly secreted by the ventricles in response to cardiac pressure overload and volume overload (18).

Noori et al. found that the BNP levels increased in children with CHD and positive was observed between the BNP level and pulmonary hypertension and recommended to consider the BNP as a biomarker to evaluate the volume and pressure overload in CHD (24). Limited data on the role of BNP in children suggest that measuring BNP levels in children's diseases is also useful. Measuring plasma BNP levels or Pro-BNP accumulation is increasingly predictive of prognosis and evaluation of disease.

5- CONCLUSION

It can be concluded that in pediatric patients with CHD, serum levels of TNF-α and IL-6 have not been associated with CHD, but serum BNP levels increase significantly in these patients. Also, the results showed that there was significant relationship and agreement between serum BNP levels and incidence of CHD in

children. The present study may be important in the prevention and prognosis of CHD.

6- CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

7- ACKNOWLEGEMENT

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