ORIGINAL ARTICLE

Evaluation of serum level of tumor necrosis factor-alpha and interleukin-6 in patients with congenital heart disease

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ABSTRACT

Background: The objective of the study is to assess the levels of tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) in patients with congenital heart diseases (CHDs) and control. **Patients and Methods:** In this case–control study, sixty patients with CHD with ages of 1 month to 15 years and thirty healthy subjects were assessed. All objects measured in height, weight, age, sex, and body mass index (BMI). Patients diagnosed by echocardiography and patients' blood samples were 3 ml and taken in the catheterization laboratory through catheter and kept for 60 min at a room with normal temperature and separated serum has been held. All samples in compliance with the cold chain carried out to biochemistry laboratory and finally the levels of serum TNF-α and IL-6 were measured by Elisa Kit. Data were analyzed with Statistical Package for Social Sciences version 20. Nonparametric tests by considering 95% confidence interval were applied. **Results:** The mean of age in cyanotic patients was 4.28 ± 3.44 years, a cyanotic was 3.12 ± 3.87 years and for the control group was 3.30 ± 3.61 years. Comparison of TNF-α (Mann–Whitney U-test = 56.62, $P < 0.001$), IL-6 (Mann–Whitney U-test = 313.5, $P < 0.001$), right ventricular (RV) pressure (Mann–Whitney U-test = 27, $P < 0.001$), pulmonary artery (PA) pressure (Mann–Whitney U-test = 618, $P = 0.015$), and BMI (Mann–Whitney U-test = 214.5, $P < 0.001$) in the case and control groups resulted in significant differences. To compare TNF-α (Chi-square = 57.82, $P < 0.001$), IL-6 (Chi-square = 54.70, $P < 0.001$), RV pressure (Chi-square = 71.35, $P < 0.001$), PA pressure (Chi-square = 5.92, $P = 00.052$), oxygen saturation (Chi-square = 74.70, $P < 0.001$), and BMI (Chi-square = 34.90, $P < 0.001$) in cyanotic, acyanotic, and control groups resulted that there were significant differences between these three groups except PA pressure. **Conclusion:** The findings of this study showed that in patients with CHD, serum levels of TNF-α increased but IL-6 not changed when compared to control and this increase in necrosis tumoral factor-α would be related with hypoxia and remarkable left to right shunt and caused growth retardation in these patients.

Key words: Acyanotic, congenital heart diseases, cyanotic, interleukin-6, tumor necrosis factor-alpha

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INTRODUCTION

The prevalence of congenital heart diseases (CHDs) has been reported in 4–50 of 1000 live births. Congenital defects have a broad spectrum from severity points of view in children. About 2–3 infants in every 1000 births have the symptoms of heart diseases in the 1st year of their life.[1]

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The common cause of malnutrition and growth retardation is cardiac malformation in children.[2]

Many studies have been conducted in respect to the relationship between cytokine levels in patients with cyanotic and acyanotic. They found that in patients with CHD seems that cytokines have a regulating role in feeding, growth, and weight and energy intake.[3,4] Cardiac cachexia syndrome occurs due to shunt in patients with chronic congestive heart failure or chronic hypoxemia. Among cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) can be noted that they are secreted due to several chronic diseases and they had an important role in the cardiac cachexia and caused the worse prognosis.[5] Cytokines are multifunctional and have great immunologic actions on some of the body's organs such as IL-6, and they are more important catabolic factors.[6,7] The most important cytokines are TNF-α and IL-6 in which they have known for modulating immune reactions and acute response phase in inflammation.[8] It seems that necrosis tumoral factor-alpha (NTF-α) and IL-6 are reasons of cachectic in patients with CHD, but this cause has not been confirmed.[9] The levels of serum TNF-α and IL-6 are higher in CHD patients compared to controls when the serum of TNF-α levels in patients with acyanotic is higher than in patients with cyanotic and for IL-6 is not observed any differences between acyanotic and cyanotic patients.[10] It seems that cardiac cachexia syndrome in children with chronic congestive heart failure and chronic hypoxemia occurs due to shunt.[5] Growth retardation in children with cyanotic is higher than healthy children.[11] The severity of CHDs is directly associated with the level of TNF-α and IL-6.[12] Cytokines are secreted in response to stress hypoxia and tissue destruction by the body's cells and gradually release its effect on body's organs and caused cachexia, weakness, and malnutrition and demonstrated that after ingestion of apoptotic cells many of anti-inflammatory cytokines are secreted.[13] CHD also is a cause of malnutrition and growth retardation in children. Since CHD as an important disease has a high effect on the normal life of children and because it is curable, we aimed to conduct a study to understand its relationship with TNF-α and IL-6.

PATIENTS AND METHODS

This case–control study was performed on sixty patients that distributed in two cyanotic and acyanotic groups, thirty participants in each group with the age of 1 month to 15 years. These patients compared with thirty healthy children with matching age and gender. Healthy children were collected randomly among those who referred to the hospital for the routine checkup. Required sample size considered based on the studies by Afify and Yilmazs,[7,14] and sampling performed based on accessibility accordance with the exclusion criteria after inclusion criteria consideration.

The study was performed in the clinic of Ali Asqar and Pediatric Ward of Ali ebne Abi Taleb Hospital belongs to Zahedan University of Medical Sciences from March 2014 to February 2015 February. The study protocol was approved by the research Ethics Committee of Zahedan University of Medical Sciences, and informed consent was obtained from parents before the study enrollment. Patients with primary pulmonary hypertension, metabolic diseases, renal, endocrine and chronic inflammatory diseases, and malnutrition were excluded. Some common causes of increasing these mediators, particularly in children, are fever or infections in the three recent weeks. Therefore, this criterion was used as an exclusive factor. For thirty subjects in the control group, statistical methods used to generate the right ventricular (RV) and pulmonary artery (PA) pressure randomly. According to the Moss and Adams' Heart Disease in Infants, Children, and Adolescents, 2013 page 264 (Allen HD, Driscoll D, Shaddy RE, Feltes TF. Moss and Adams Heart Disease in Infants, Children, and Adolescents Including the Fetus and Young Adults. 8th ed., Vol. 13. Tehran: Teymurzade; 2013. p. 577-618), we considered mean and standard deviation as 25 and 5 for RV and 22 and 4 for PA, respectively.

For participants, height, weight, age, sex, body mass index (BMI) were determined and recorded in a form. Weight was measured by Japanese MIKA scale factor with an error of 10 g for children <2 years and for elders a construction of RASA scale factor with error 100 g was used. Measuring height in children <2 years was performed in the supine position on a scaled wooden table and in elders measuring was done in standing position with a balance with a scale ruler and BMI was calculated by the following equation: Weight (kg)/height^2 (m). Diseases diagnosed by echocardiography MyLab 60 made in Italy with a transducer of 3–8. Children's parents were asked to provide their verbal agreement to take blood samples from their children. Patients' blood samples were 3 cc and taken in the cath lab via vessel catheter and kept for 60 min at a room with normal temperature and separated serum was held in a −70°C freezer in a laboratory of Zahedan University of Medical Sciences belong to the Clinical Research Center. After collecting the required data, all samples in compliance with the cold chain were carried out to biochemistry laboratory and finally the levels of serum TNF-α and IL-6 were measured by Eliza method with a KIT of Bender Med Company made of America. Ethical codes of 3, 8, and 17 were considered and approved by the Ethics Committee of Zahedan University. For the analysis, Statistical Package for Social Sciences version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) was applied. First of all, normality test was used and then parametric and nonparametric tests, ANOVA, and Kruskal–Wallis with Kendal coefficient correlation by considering 0.04 error for the level of significant in 95% confidence interval were applied.

RESULTS

Ninety subjects were involved in three groups of thirty in three groups of participants, and the sex distributions were as 50%, 56.7%, and 53% of females in cyanotic, acyanotic, and controls, respectively. Mean age in cyanotic patients was 4.28 ± 3.44 years and for acyanotic was 3.12 ± 3.87 years. Mean age for the control group was 3.30 ± 3.61 years. The mean age of patients (cyanotic patients and acyanotic) as the case group was 3.68 ± 3.71 years. Mean of BMI in cyanotic patients and acyanotic was 15.17 ± 2.77 and 14.54 ± 2.66, respectively, and for the case was 14.86 ± 2.72 and 18.86 ± 2.43 kg/m^2 for controls. Cyanotic patients had higher height (0.896 ± 0.24 m) compared with acyanotic patients (0.84 ± 0.25 m) and controls (0.83 ± 0.24 m). Mean of weight shows that it was highest for controls (13.73 ± 8.15) and lowest for acyanotic patients (10.70 ± 6.10). Mean of weight observed of 12.5 ± 5.86 for cyanotic patients. For both weight and height, significant differences were not observed between groups. Table 1 shows the results of normality. All major variables were normally not distributed. Using nonparametric test of Mann–Whitney U-test applied to compare TNF-α (Mann–Whitney U-test = 56.62, $P < 0.001$), IL-6 (Mann–Whitney U-test = 313.5, $P < 0.001$), RV pressure (Mann–Whitney U-test = 27, $P < 0.001$), PA pressure (Mann–Whitney U-test = 618, $P = 0.015$), and BMI (Mann–Whitney U-test = 214.5, $P < 0.001$) in the case and control groups and resulted that there were significant differences between case and controls [Table 2]. Using nonparametric test of Kruskal–Wallis applied to compare TNF-α (Chi-square = 57.82, $P < 0.001$), IL-6 (Chi-square = 54.70, $P < 0.001$), RV pressure (Chi-square = 71.35, $P < 0.001$), PA pressure (Chi-square = 5.92, $P = 00.052$), oxygen saturation (Chi-square = 74.70, $P < 0.001$), and BMI (Chi-square = 34.90, $P < 0.001$) in cyanotic, acyanotic, and control groups and resulted that there were significant differences between these three groups except PA pressure [Table 3]. Table 4 shows the matrix correlation of the variables. Table 4 shows that IL-6 had a significant correlation with all other variables. According to Table 4 TNF-α (Pearson's correlation = −0.157, $P = 0.139$) and PA pressure (Pearson's correlation = −0.129, $P = 0.224$) had not significantly correlated with BMI.

Table 1: Test of normality for major variables in the study

Variables	Mean	SD	Kolmogorov-Smirnov Z-test	*P*
TNF-α	75.16	123.33	2.68	0.000
IL-6	6.51	4.77	1.91	0.001
weight	12.31	6.83	1.47	0.027
height	0.86	0.24	1.42	0.035
Oxygen saturation	82.07	18.43	2.70	0.000
RV pressure	56.58	34.93	1.68	0.007
PA pressure	21.83	16.80	2.93	0.000

IL-6 – Interleukin 6; PA – Pulmonary artery; RV – Right ventricular; TNF-α – Tumor necrosis factor alpha; SD – Standard deviation

Table 2: Comparison means ranks of parameters in case and control groups

Parameters	Group	Mean	SD	Mean rank	Mann-Whitney U-test	Asymptotic significant (two-tailed)
TNF-α	Case	100.69	144.41	56.62	233	0.000
	Control	24.09	14.45	23.27		
IL-6	Case	8.24	4.96	55.28	313.5	0.000
	Control	3.03	1.06	25.95		
RV pressure	Case	73.07	31.76	60.05	27	0.000
	Control	23.60	2.28	16.40		
PA pressure	Case	25.2	19.72	50.20	618	0.015
	Control	15.10	2.34	36.10		
BMI	Case	14.86	2.72	34.08	214.5	0.000
	Control	18.86	2.43	68.35		

IL-6 – Interleukin-6; PA – Pulmonary artery; RV – Right ventricular; TNF-α – Tumor necrosis factor-alpha; SD – Standard deviation

Table 3: Comparison means ranks of parameters in three cyanotic, acyanotic, and control groups

Parameters	Disease	Mean	SD	Ranks	χ^2	*P*
TNF-α	Cyanotic	157.62	186.30	73.55	57.82	0.000
	Acyanotic	43.76	31.87	39.68		
	Control	24.09	14.45	23.27		
IL-6	Cyanotic	11.88	3.73	73.57	54.70	0.000
	Acyanotic	4.61	2.94	36.98		
	Control	3.03	1.06	25.95		
RV Pressure	Cyanotic	96.83	23.21	73.22	71.35	0.000
	Acyanotic	49.30	18.56	46.88		
	Control	23.60	2.28	16.40		
PA pressure	Cyanotic	28.97	24.56	49.45	5.92	0.052
	Acyanotic	21.43	12.58	50.95		
	Control	15.10	2.34	36.10		
Oxygen saturation	Cyanotic	59.47	15.14	15.80	74.659	0.000
	Acyanotic	91.03	2.77	46.85		
	Control	95.70	1.47	73.85		
BMI	Cyanotic	15.17	2.78	36.37	34.90	0.000
	Acyanotic	14.55	2.67	31.78		
	Control	18.86	2.43	68.35		

IL-6 – Interleukin 6; PA – Pulmonary artery; RV – Right ventricular; TNF-α – Tumor necrosis factor-alpha; SD – Standard deviation

DISCUSSION

In the present study, ninety subjects were involved in three groups. The mean age of cyanotic patients was higher compared with a cyanotic and controls. Levels of TNF-α, IL-6, RV pressure, PA pressure, and BMI in case and control groups resulted in significant differences. Levels of TNF-α, IL-6, RV pressure and oxygen saturation, and BMI in cyanotic, acyanotic, and control groups resulted in significant differences between. The results also revealed the correlation of RV pressure, PA pressure, and BMI with NTF-α and IL-6. There was a significant increase for RV pressure due to an increase for TNF-α. Cytokines are secreted in response to stress, hypoxia,

Table 4: Correlation matrix for basic parameters of patients

	TNF	IL-6	RV	PA
IL-6				
Pearson's correlation	0.536			
Significant (two-tailed)	0.000			
RV pressure				
Pearson's correlation	0.435	0.703		
Significant (two-tailed)	0.000	0.000		
PA pressure				
Pearson's correlation	−0.008	0.235	0.358	
Significant (two-tailed)	0.940	0.026	0.001	
BMI				
Pearson's correlation	−0.157	−0.221	−0.314	−0.129
Significant (two-tailed)	0.139	0.036	0.003	0.224

IL-6 – Interleukin-6; PA – Pulmonary artery; RV – Right ventricular; TNF-α – Tumor necrosis factor-alpha; BMI – Body mass index

and tissue destruction by the body's cells and gradually release its effect on all the body's organs and caused cachexia, weakness, and malnutrition.[15] Many studies have been done by scholars in association of cytokines with CHD in the various circumstances. CHDs are the key factors of malnutrition and growth disorders. Therefore, malnutrition and growth retardation in CHD patients are common.[16] The reason of malnutrition in these patients is several factors that decrease or increase the magnitude of required energy.[17] Hollyoghli reported that CHD patients who had higher metabolism had more growth disorders, particularly when they suffered from cyanotic.[18] Denlisi approved cardiac cachexia associated with lower BMI in children with CHD.[19] Yilmaz presented that insufficient caloric intake was the main cause of cachexia in cyanotic and acyanotic patients. The degree of growth retardation did not have a relationship to the severity of the hypoxia and cardiac cachexia because of weaken immune function and decreasing muscle strength.[14] Noori *et al*. found that weight in acyanotic patients with pulmonary hypertension was strongly lower than the control group. In cyanotic patients, with and without pulmonary hypertension, the average of height and weight was lower than control which is shown the growth impairment in patients with pulmonary hypertension.[20] The present study in compared with the Noori study showed that the mean of BMI in patients with cyanotic and acyanotic and control groups was quite significant. An average of height and BMI in patients with cyanotic and acyanotic involved with pulmonary hypertension was different with control group significantly. These two studies were comparatively similar. In our study, a decrease in BMI followed by an increase in NTF-α, which is similar with Cummings and Kojima. They showed that TNF-α as one of the cytokines has a strong and effective impact in cachectic process in all kinds of illness but not confirmed in cardiac patients.[21,22] Stocker received to a conclusion that TNF-α and IL-6 serum levels in CHD patients with 34°C and 24°C in body temperature had no different after cardiopulmonary bypass.[16] With a difference in methodology we revealed that TNF-α and IL-6 serum levels were higher in patients, especially cyanotic in compared to controls. It would be consider that this high PA pressure is due to the considerable shunt in acyanotic patients and severe hypoxia in cyanotic patients. It seems that increase in TNF-α and IL-6 would be due to the severity of hypoxia in cyanotic patients. Yilmaz observed a higher increase in serum levels of NTF-α and IL-6 in patients compared to healthy. Increasing of serum levels of NTF-α and IL-6 in cyanotic patients was not correlated with patients' BMI. In that study, acyanotic patients had more weight disorders and cyanotic patients had more growth retardation.[23] According to the results of the present study, significant differences in the serum levels of NTF-α and IL-6 in patients and controls are similar with Yilmaz but in the association of NTF-α and IL-6 with BMI our results are dissimilar with Yilmaz in the case of IL-6. Cummings understood that an increase in TNF-α in patient with congestive heart failure is due to heart disease.[22] This result would be similar to our study because many survived patients had a significant left to right shunt with severe degrees of heart failure. Afify compared the serum levels of TNF-α and IL-6 in CHD patients and observed higher measures compared to controls, but BMI did not show any differences.[10] In our study, the serum levels of TNF-α and IL-6 were significantly higher than control and BMI was significantly lower. Our findings of IL-6 are similar to Afify's results. Shiva concluded that cyanotic heart patients had lower age than a cyanotic, and it was because of early symptoms before surgery and CHD patients had lower BMI than controls but no significant difference between cyanotic and acyanotic. Cyanotic patients had lower SaO_2 than acyanotic and controls which are similar to our findings for this specific terms.[24] In our study, the age of cyanotic patients also was higher than acyanotic and controls. The present study also observed that the BMI of both cyanotic and acyanotic patients was significantly lower than the control group. Cheung aimed of measuring and comparing the levels of cytokines such as IL-6, IL-8, IL-10, and TNF-α at 3 and 6 h after cardiac surgery with control CHD patients. Cheung observed not any differences of IL-6, IL-8, IL-10, and TNF-α in these three groups of patients.[25] We compared IL-6 and TNF-α in CHD patients (cyanotic and acyanotic) and controls which resulted in a significant difference. Our results revealed that increasing of the serum level of IL-6 and TNF-α were significantly higher for cyanotic in compared to acyanotic patients. Our results comparatively are against of Cheung results. Lopes resulted that plasma levels of cytokines were significantly elevated in patients with CHD and pulmonary hypertension compared to controls. TNF-α and IL-6 did not correlate significantly with any of the variables such as age, gender, and clinical presentations,[17] but in dissimilar in our study which resulted that IL-6 has a significant correlation with all variables and TNF-α had a significant correlation with RV pressure. When the plasma levels of

TNF-α and IL-6 were higher in CHD patients similar to lopes results. Shahramian found that ghrelin, leptin, and TNF-α in cyanotic and acyanotic patients had not different with control groups as well as for BMI and not the observed relationship between serum levels of NTF-α and BMI.[3] In comparison to the present study, we concluded that they are dissimilar entirely.

CONCLUSION

The findings of this study showed that in patients with CHD, serum levels of TNF-α and IL-6 increased compared to control and this increase would be related with hypoxia and remarkable left to right shunt and caused growth retardation in these patients. The mean of BMI in both groups of patients was lower than control in our study which demonstrates the growth retardation fact.

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Conflicts of interest
There are no conflicts of interest.

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