

*Original Article***Relationship between echocardiographic findings and laboratory serum biomarkers in patients with and without low cardiac output**

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**Abstract**

**BACKGROUND:** Cardiac dysfunction is seen in many patients and could be evaluated with echocardiography and serum biomarkers. The aim of this study was evaluation of the relationship between echocardiographic findings and laboratory serum biomarkers in children with and without low cardiac output.

**METHODS:** Thirty patients older than 1 month with and without low cardiac output were enrolled in this study. It composed of 13 patients with dilated cardiomyopathy (DCM), 7 with end stage renal disease (ESRD) and 10 who had a Fontan operation. Echocardiography was performed with emphasis on shortening fraction (SF) and ejection fraction (EF). Blood samples were collected for measurement of atrial natriuretic peptide (ANP), high sensitivity C-reactive protein (hs-CRP) and alkaline phosphatase (Alk-P). Both echocardiographic findings and laboratory data were compared with control levels in twenty-seven normal children. Pearson correlation and regression analysis were conducted to evaluate the aforementioned associations.

**RESULTS:** The mean of ANP and hs-CRP in the case group was statistically higher than control group ( $p < 0.001$ ). The mean of ANP and hs-CRP were different in all groups ( $p < 0.001$ ). There was a reverse linear regression between the SF and ANP in the case group ( $r = -0.594$ ,  $p < 0.001$ ).

**CONCLUSIONS:** Determination of the plasma ANP and hs-CRP level may be helpful for decisions related to early diagnosis of patient with low cardiac output.

**KEYWORDS:** Ejection Fraction, Shortening Fraction, Atrial Natriuretic Peptide, High Sensitivity C-reactive Protein.

J Res Med Sci 2011; 16(11): 1397-1404

The heart can be viewed as a pump with an output proportional to its filling volume and inversely proportional to the resistance against which it pumps. As ventricular end-diastolic volume increases, a healthy heart increases cardiac output until a maximum is reached and cardiac output can no longer be augmented. Cardiac output can be calculated as the product of heart rate and stroke volume. The primary determinants of stroke volume are the afterload (pressure work), preload (volume work) and contractility (intrinsic myocardial function).<sup>1</sup>

Echocardiographic techniques are the most useful ones in assessing ventricular function. The most commonly used parameter in children is fractional shortening, a single dimensional variable, determined as the difference between end-systolic and end-diastolic diameter divided by end-diastolic diameter. Normal fractional shortening is between 28% and 40%. In adults, the most commonly used parameter is ejection fraction which uses two-dimensional data to calculate a three-dimensional volume and the normal range is 54-75%.<sup>1</sup>

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Three peptides of the natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide and C-type natriuretic peptide participate in the control of circulation. The atrial natriuretic peptide is primarily produced by the atrial myocardium, while the brain natriuretic peptide is synthesized by the ventricular myocardium. The main stimulus for their release is stretching of the myocardium. Other stimuli include endogenous vasoactive factors, neurotransmitters, pro-inflammatory cytokines, and hormones. The vascular effects of atrial and brain natriuretic peptides are similar. Both of them reduce sympathetic tone through suppression of sympathetic outflow from the central nervous system, catecholamine release reduction of autonomic nerve endings and probably damping of baroreceptors.<sup>2</sup>

Acute phase reactants (APR) are secreted into blood as the results of a major pathophysiological phenomenon that accompanies systemic inflammation.<sup>3</sup> The most extensively studied biomarker of inflammation in cardiovascular diseases is C-reactive protein (CRP), for which standardized high-sensitivity assays (hs-CRP) are widely available.<sup>4</sup> CRP is an acute phase protein that is produced predominantly by hepatocytes under the influence of cytokines such as interleukin 6 (IL-6) and tumor necrosis factor- $\alpha$ .<sup>3</sup> Increased concentrations of inflammatory markers have been reported in patients with heart failure and have been associated with an adverse prognosis.<sup>5</sup> Several experimental and clinical studies suggested that inflammation contributes to the pathogenesis of both acute and chronic heart failure (CHF).<sup>6</sup> Activation of the immune system, leading to production of pro-inflammatory cytokines, participates in the development of left ventricular dysfunction, vascular abnormalities, cardiac cachexia and other pathologic features in patients with CHF.<sup>7</sup> The inflammatory marker that presently seems most suitable to assess inflammation is hs-CRP. Elevated hs-CRP has been shown to be associated with an adverse

prognosis in patients with CHF and, based in these findings, the use of hs-CRP as a prognostic marker is increasingly advocated, in addition to other prognostic parameters in particular B-type natriuretic peptide and peak oxygen consumption.<sup>8</sup>

Alkaline phosphatase (Alk-P) refers to a group of enzymes that catalyze the hydrolysis of a large number of organic phosphate esters at an alkaline pH optimum. Although alkaline phosphatase is found in many locations throughout the body, its precise function is not yet known.<sup>9</sup>

Serum total alkaline phosphatase (TALP) activity, an indicator of osteoblastic function particularly in preadolescence, may be a marker of low cardiac output after a Fontan operation.<sup>10</sup>

Early diagnosis of patients with low cardiac output by the use of serum biomarkers (atrial natriuretic peptide, hs-CRP, Alk-P) and echocardiography could be helpful to perform appropriate treatment for them. In patients with chronic heart failure, plasma atrial natriuretic peptide provides important prognostic information.<sup>11</sup> In this study we tried to find if there is any relationship or prediction equation between laboratory biomarkers and echocardiographic data in patients with low cardiac output.

## Methods

### *Patients*

The study population consisted of 57 patients older than 1 month in two groups. Case group consisted of patients with and without low cardiac output divided in three subgroups of dilated cardiomyopathy (DCM), end-stage renal disease (ESRD) and patients who had a Fontan procedure that referred to the pediatric cardiology center in Al-Zahra hospital in Isfahan, Iran. Low cardiac output is determined by ejection fraction less than 50% and shortening fraction less than 28% in echocardiographic assessment.<sup>12</sup> In our study, patients with DCM were stable at the time of study and duration of their disease had been at least 6 months and possible diagnosis was idiopathic DCM. We

performed echocardiographic assessment for 44 patients with ESRD, 7 of them had systolic dysfunction which included in this study while most of the other ESRD patients (who were not included in this study) had ventricular hypertrophy and/or diastolic dysfunction. Ten patients in Fontan palliation subgroup comprised of 1 patient with pulmonary atresia and unbalanced ventricles, 3 patients with pulmonary atresia and single ventricle with LV morphology, 1 patient with pulmonary atresia and undetermined ventricle, 1 patient with tricuspid atresia and normally related great arteries, 1 patient with tricuspid atresia and malposition of great arteries, 2 patients with complete atrioventricular septal defect and malposition of great arteries and unbalanced ventricles, 1 patient with d-transposition of great arteries and unbalanced ventricle. These patients had their Fontan procedure at least 1 year before participation in this study. Control group consisted of normal children that were matched with the same age range in the case group. Patients with other conditions that affect on the serum biomarkers such as fever and infections or patients with acute low cardiac output were excluded from the study.

#### **Clinical assessment**

The case and control groups underwent a complete clinical examination, and a comprehensive medical history was recorded. Echocardiography was performed by pediatric cardiology fellow with a MEDISON SONOACE X8 instrument with a 3-5 MHz transducer. The findings of ejection fraction (EF) and shortening fraction (SF) were obtained by echocardiography although a complete echocardiographic assessment was done.

#### **Biochemical measurements**

Blood samples were transferred to chilled plastic tubes and immediately after were placed on ice and promptly centrifuged. An aliquot of plasma was frozen immediately at  $-70^{\circ}\text{C}$  and thawed only once at the time of assay, which was performed within 2 months after sampling. In physiology research center of Isfahan

University of Medical Sciences, Alk-P, hs-CRP and atrial natriuretic peptide (ANP) plasma concentration were determined using ELISA test (Alk-P: Pars azmoon, Iran; hs-CRP: IBL, Germany; ANP: Bachem, Germany). For each determination, a minimum of 0.2 ml plasma was needed.

#### **Statistics**

In this cross-sectional study participants were selected using nonrandom sampling. All the statistical analyses were performed using SPSS for Windows 15.0 (SPSS Inc., Chicago, Illinois). Differences between the groups were assessed using the independent student's t-test, the one-way ANOVA and in subgroups it was assessed by Duncan test. Pearson correlation and regression analysis were performed to assess echocardiographic findings relationship with serum biomarkers.

#### **Results**

The median age of the study population was  $13.3 \pm 7.6$  years in case group (ranging from 0.58 to 27.17). In control group it was  $11.4 \pm 8.3$  years (ranging from 0.67 to 26.3). The echocardiographic findings and serum biomarker levels in both groups are presented in table 1. Accordingly, the mean SF and EF in case group was significantly lower than control group ( $p < 0.001$ ). On the contrary, plasma ANP and hs-CRP concentrations were higher in case group ( $p < 0.001$ ).

In the case of subgroups, the DCM subgroup consisted of 13 patients (43.3%) with mean age of  $10.6 \pm 8.4$  years and the ESRD subgroup was consisted of 7 patients (23.3%) with mean age of  $15.9 \pm 3.1$  years. The Fontan procedure subgroup was consisted of 10 patients (33.3%) with mean age of  $15.1 \pm 8.1$  years. The echocardiographic findings and serum biomarker levels in cases subgroups are presented in table 2.

One way ANOVA analysis showed that there was statistically significant difference between mean SF and EF in all subgroups ( $p < 0.001$ ). Post Hoc Duncan test showed that mean SF and EF in DCM and ESRD subgroups

**Table 1.** Shortening fraction (%), ejection fraction (%) and serum biomarker levels in case and control groups

variable	Case group	Control group	P-value
	Mean ± SD	Mean ± SD	
Shortening Fraction (%)	23.1 ± 7.1	35.6 ± 3.2	< 0.001
Ejection Fraction (%)	45.9 ± 12.0	66.0 ± 4.4	< 0.001
Atrial Natriuretic Peptide (ng/ml)	655.5 ± 582.4	18.0 ± 16.6	< 0.001
high-sensitivity C-Reactive Protein (µg/ml)	13.5 ± 13.7	2.5 ± 3.0	< 0.001
Alkaline Phosphatase (IU/L)	502.1 ± 329.4	447.2 ± 207.2	0.23

was less than Fontan subgroup and mean SF in Fontan subgroup was less than normal subgroup ( $p < 0.05$ ).

One way ANOVA analysis showed that there was statistically significant difference between mean ANP and hs-CRP plasma concentration in all subgroups ( $p < 0.001$ ). Post hoc Duncan test showed that mean ANP and hs-CRP level in DCM subgroup were greater than what were in ESRD and Fontan subgroups and mean ANP level in ESRD and Fontan subgroups was greater than normal subgroup ( $p < 0.05$ ). There was no statistically significant difference between mean Alk-P plasma concentration in all subgroups ( $p = 0.4$ ).

In the case group, Pearson correlation showed that there was a reverse linear correlation between SF and ANP plasma concentration ( $r = -0.594$ ,  $p < 0.001$ ) (Figure 1). Our findings suggested a probable linear regression equation which is:

ANP plasma concentration (ng/ml) =  $1775.9 - 48.5 \times \text{SF} (\%)$

There was also a reverse linear correlation between EF and ANP plasma concentration ( $r = -0.592$ ,  $p < 0.001$ ) (Figure 2). Our findings suggested a probable linear regression equation which is:

ANP plasma concentration (ng/ml) =  $1974.4 - 28.7 \times \text{EF} (\%)$

In the control group, there was no statistically significant correlation between SF and EF and ANP plasma concentration.

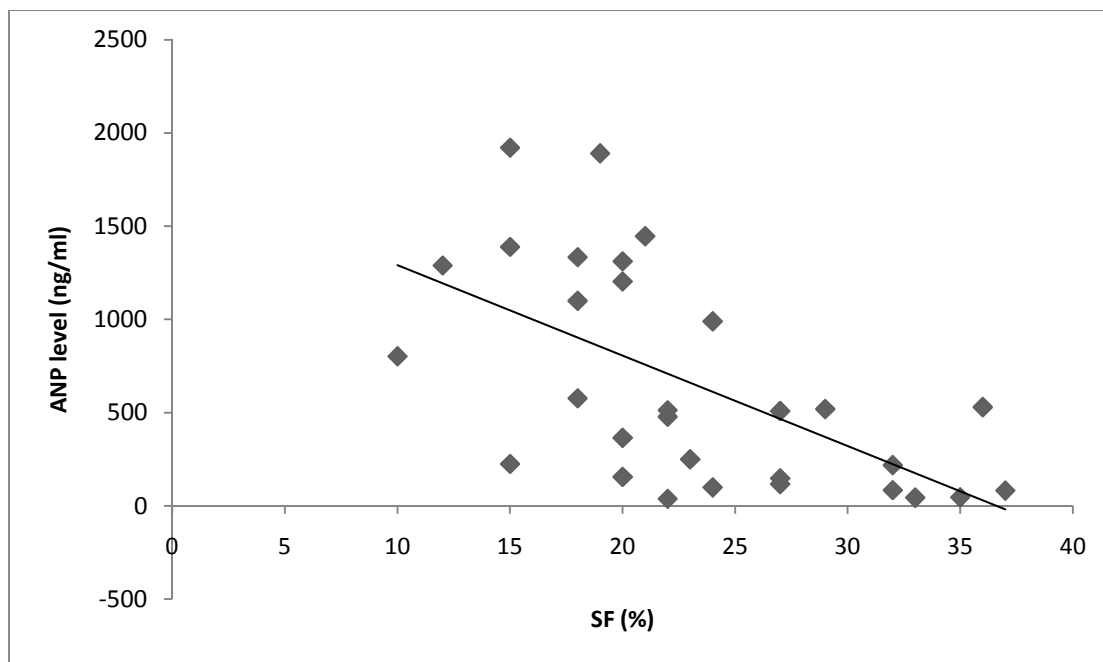
## Discussion

Improvements in technology have positioned echocardiography as the principal diagnostic modality in the field of pediatric cardiology. Serum biomarkers are also important in evaluating cardiac function. In this study, cardiac function assessed directly with echocardiography and indirectly with laboratory data including ANP, hs-CRP and Alk-P. The combined assessment of inflammatory and cardiac biochemical markers can be useful for identifying young children at increased risk of low cardiac

**Table 2.** Shortening fraction (%), ejection fraction (%) and serum biomarker levels in case subgroups and control group

subgroup	SF (%)	EF (%)	ANP (ng/ml)	hs-CRP (µg/ml)	Alk-P (IU/L)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DCM	18.9 ± 6.4	38.8 ± 10.9	1213.5 ± 426.0	17.9 ± 15.2	500.1 ± 315.2
ESRD	20.9 ± 3.0	42.3 ± 6.0	297.6 ± 158.5	12.3 ± 14.5	561.4 ± 424.2
Fontan	30.1 ± 4.5	57.6 ± 6.8	180.4 ± 184.1	8.8 ± 10.0	463.3 ± 304.5
Normal	35.6 ± 3.2	66.0 ± 4.4	18.0 ± 16.6	2.5 ± 3.0	447.2 ± 207.2

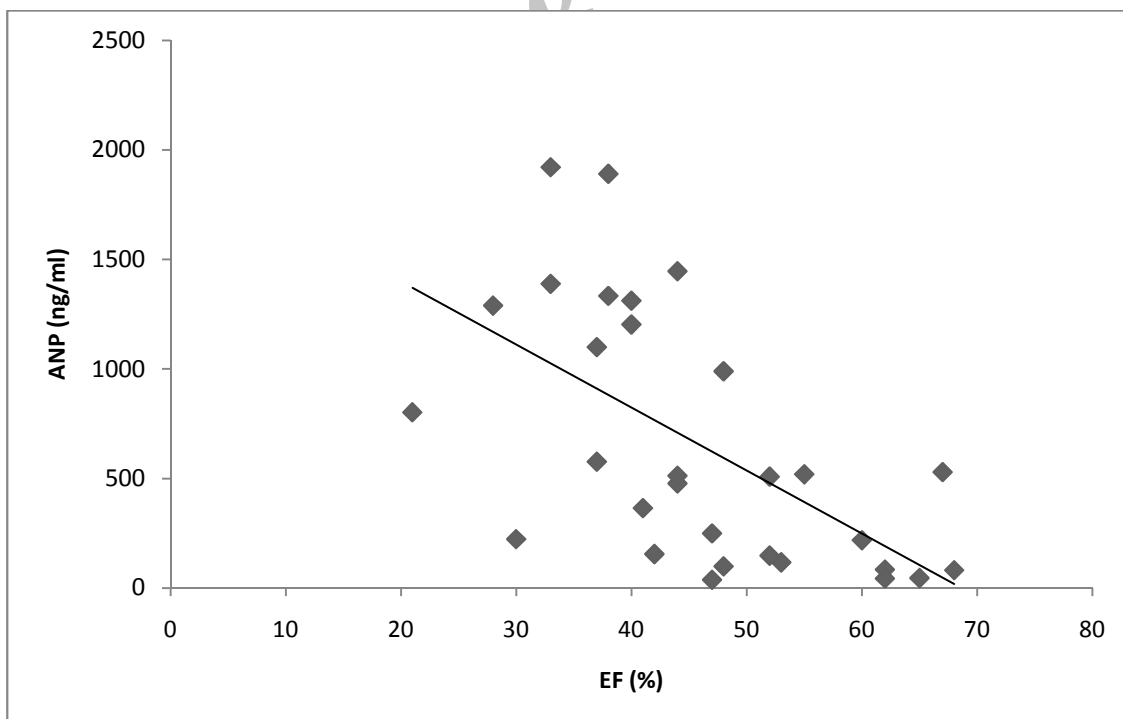
SF: Shortening Fraction; EF: Ejection Fraction; ANP: Atrial Natriuretic Peptide; hs-CRP: high-sensitivity C-Reactive Protein; Alk-P: Alkaline Phosphatase; DCM: Dilated cardiomyopathy; ESRD: end-stage renal disease



**Figure 1.** Correlation of shortening fraction to serum concentration of atrial natriuretic peptide in case group

output syndrome.<sup>13</sup> ANP is specific cardiac biomarker but hs-CRP and Alk-P are nonspecific cardiac biomarkers. The role of natriuretic

peptides as cardiac hormones is clear given their close relationship to cardiac structure and function, reflecting left ventricle wall stress



**Figure 2.** Correlation of ejection fraction to serum concentration of atrial natriuretic peptide in case group

and subclinical myocardial dysfunction.<sup>14</sup> Most of the studies evaluated the level of both ANP and BNP.<sup>15,16</sup> We chose to measure ANP as our marker for cardiac filling state. Due to budget limitation in this study, only the level of ANP was evaluated. It would have made more sense to use both BNP and ANP in this study. The ANPs rapidly reflect changes in filling pressures while the B-type peptides respond much slower.<sup>17</sup> To our knowledge, this is the first study which investigated whether there is an equation between echocardiographic parameters of SF and EF and serum biomarkers (ANP). Our study demonstrated that mean ANP was significantly higher in dilated cardiomyopathy (DCM) patients probably due to stretching of the myocardium. The second subgroup which had high ANP levels was end-stage renal disease patients with low contractility. Patients with Fontan operation also had high ANP level due to volume overload in atrium. Plasma natriuretic peptide levels measured preoperatively and postoperatively can be a prognostic indicator in the management of the pediatric patient after surgical intervention for congenital heart repair.<sup>18</sup> High levels of ANP measured early after Fontan operation can be used as a marker for the successful establishment of Fontan circulation in patients with complex congenital heart defects.<sup>19</sup> Stewart et al.<sup>20</sup> investigated ANP levels in 15 patients undergoing the Fontan procedure and compared them with control levels in nine patients undergoing cardiac surgery for lesions not associated with atrial hypertension. There were no significant differences in preoperative ANP levels ( $57 \pm 15$  pg/ml for patients undergoing the Fontan procedure vs.  $43 \pm 8$  pg/ml for control patients). There was no significant change in ANP during surgery or in the postoperative period in control patients. In contrast, ANP increased significantly to  $333 \pm 70$  pg/ml ( $p < 0.0025$ ) after establishment of right atrial-pulmonary artery continuity with the Fontan procedure and was related to right atrial pressure, which increased from 5 mmHg to 14 mmHg after the Fontan procedure ( $p < 0.001$ ).<sup>20</sup> In our study, patients with Fontan operation

(mean ANP 180.4 ng/ml) had higher ANP level than normal group (mean ANP 18 ng/ml). Chello et al.<sup>21</sup> investigated the effectiveness of atrial and brain natriuretic peptides (ANP and BNP, respectively) as indicators of recovery of left ventricular (LV) function after coronary surgery. They measured the concentrations of these peptides in 31 patients with poor LV function (EF  $< 35\%$ ) undergoing coronary artery bypass, and evaluated their correlation with the echocardiographic indexes of LV function. Preoperatively, the plasma levels of both ANP and BNP were markedly higher in coronary patients compared to normal control subjects, and strongly correlated with both EF (BNP:  $r = -0.8$ ,  $p < 0.001$ ; ANP:  $r = -0.6$ ,  $p < 0.001$ ). Plasma levels of ANP and BNP might be used in routine clinical practice as a support to echocardiography in detecting recovery of the LV function after coronary surgery.<sup>21</sup> In our study, hs-CRP was another marker that increased mostly in DCM and in lesser degree in ESRD and Fontan operation groups. Hs-CRP is an inflammatory factor that participates in development of ventricular dysfunction. Chin et al.<sup>22</sup> recently noted severely reduced total alkaline phosphatase (TALP) levels in two children with early-onset protein losing enteropathy after Fontan operations, both of whom had low cardiac output. Although Alk-P is a marker of osteoblastic function that may decrease in low perfusion, our study showed no significant difference between the subgroups and normal group. In addition, our study demonstrated a probable equation between ANP serum concentration and SF and also EF, but a small number of subjects were participated in the case group.

### Study Limitations

The study would be more valuable if more parameters such as cardiac index and other serum biomarker such as BNP were measured. Because of the budget limitation, other serum biomarkers were evaluated. Some of the subgroups only contained small number of cases which was due to our selection criteria. The study would have more power with a larger

number of participants in each subgroup.

### Conclusion

As low cardiac contractility is associated with high morbidity and mortality, early diagnosis of these states can be helpful in patients. If echocardiography is not available in some centers, laboratory data may be helpful in this situation. We concluded that determination of the plasma ANP and hs-CRP level can be help-

ful for decisions related to early diagnosis and treatment of patient with low cardiac output.

### Acknowledgments

This study was supported in part by a grant from Isfahan University of Medical Sciences (research no. 389123). Laboratory tests were performed by Physiology Research Center. We acknowledge all the investigators and patients for their commitment to the study.

### Conflict of Interests

Authors have no conflict of interests.

### Authors' Contributions

MRK planned the study, collected the data and finalized the manuscript. MRS and ARN supervised the project. SHJ supervised the project and help in the laboratory analysis. All authors read and approved the content of the manuscript.

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