

## The relation of plasma level of atrial and brain natriuretic peptide to left ventricular function in subjects with congenital heart disease

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### الخلاصة

**الهدف:** قياس مستوى هورموني البيبتيد الاذيني والقلبي المدر للصوديوم في بلازما الأشخاص الذين لديهم عيوب خلقية في القلب ومعرفة مدى علاقة هذين الهورمونين بكفاءة البطين الأيسر.

**الطريقة:** أجريت هذه الدراسة على ٦٢ شخصا (٢٨ ذكرا و ٣٤ أنثى) يعانون من عيوب خلقية في القلب من مراجعي وحدة وظائف القلب (الأيكو) في مستشفى ابن سينا التعليمي في الموصل/ العراق. للفترة من الأول من تشرين الأول ٢٠٠٤ ولغاية الثلاثون من أيلول ٢٠٠٥. وبأعمار تتراوح بين السنة ٢٠ سنة. كما ضمت الدراسة ٢٠ شخصا من الأصحاء كعينة ضابطة تراوحت أعمارهم من السنتين ولغاية ٢٠ سنة.

**النتائج:** أظهرت الدراسة وجود زيادة ذات أهمية إحصائية في تركيز هورموني البيبتيد الاذيني والقلبي المدر للصوديوم في بلازما الأشخاص الذين لديهم عيوب خلقية في القلب والمصحوبة بازرقاق بالمقارنة مع الأشخاص الذين لديهم عيوب خلقية في القلب وليست لديهم ازرقاق (ب<٠.٠٠٠١، و ب<٠.٠٠٠٢) والعينة الضابطة (ب<٠.٠٠٠١، و ب<٠.٠٠٠١) وأظهرت الدراسة أيضا وجود نقصان معنوي في معدل الإفراغ التجزيئي للبطين الأيسر لدى الأشخاص الذين لديهم عيوب خلقية في القلب والمصحوبة بازرقاق بالمقارنة مع الأشخاص الذين لديهم عيوب خلقية في القلب وليست لديهم ازرقاق (ب<٠.٠٠٠١) والعينة الضابطة (ب<٠.٠٠٠١).

كما أظهرت الدراسة وجود علاقة معنوية سالبة بين تركيز هورمون البيبتيد القلبي المدر للصوديوم ومعدل الإفراغ التجزيئي للبطين الأيسر لدى الأشخاص الذين لديهم عيوب خلقية في القلب والمصحوبة بازرقاق (ر=-٠.٨٠، ب<٠.٠١) والأشخاص الذين لديهم عيوب خلقية في القلب وليست لديهم ازرقاق (ر=-٠.٧٠، ب<٠.٠١) والمجموعة العمرية الأولى (ر=-٠.٨، ب<٠.٠١) والثانية (ر=-٠.٧٥، ب<٠.٠١) والثالثة (ر=-٠.٦٩، ب<٠.٠٢) وعلاقة معنوية سالبة بين تركيز هورمون البيبتيد الاذيني المدر للصوديوم ومعدل الإفراغ التجزيئي للبطين الأيسر لدى الأشخاص الذين لديهم عيوب خلقية في القلب والمصحوبة بازرقاق (ر=-٠.٣٨، ب<٠.٠٢).  
**الاستنتاجات:** أظهرت نتائج الدراسة وجود ارتفاع ملحوظ في مستوى هورموني البيبتيد الاذيني والقلبي المدر للصوديوم في بلازما الأشخاص الذين لديهم عيوب خلقية في القلب وان هنالك علاقة معنوية سالبة قوية بين مستوى هذين الهورمونين في البلازما ومعدل الإفراغ التجزيئي للبطين الأيسر لدى هؤلاء الأشخاص. كما يبدو من الدراسة ان هورمون البيبتيد القلبي المدر للصوديوم أكثر دقة من هورمون البيبتيد الاذيني المدر للصوديوم في تحديد مثل هذه الحالات.

### Abstract

**Aim:** To assess Atrial and Brain natriuretic peptide levels in subjects with congenital heart disease, and their role in evaluation left ventricular function among these subjects.

**Methods:** Sixty two subjects with congenital heart disease (28 males, 34 females) aged 1-20 years, were studied in the Echocardiography unit at Ibn-Sena teaching hospital in Mosul, during the period of 1 year, from 1st October 2004 to 30th September 2005.

The study also included 20 apparently healthy volunteers (9 males and 11 females) aged 2-20 years as control group. for every subject participate in the study Plasma concentration of ANP and BNP, and left ventricular ejection fraction were determined

**Results:** The mean of plasma level of ANP and BNP were significantly higher in cyanotic subjects with congenital heart disease compared with non-cyanotic ( $P \leq 0.002$ ;  $P \leq 0.0001$ ) and control subjects ( $P \leq 0.001$ ;  $P \leq 0.0001$ ). The mean of LVEF% was significantly lower in cyanotic subjects compared with non-cyanotic subjects ( $P \leq 0.0001$ ) and control group ( $P \leq 0.0001$ ), but there was no significant difference in the

mean of LVEF% between non-cyanotic subjects and control group. The result of study showed a significant negative correlation between the mean of BNP and mean of LVEF% in cyanotic subjects ( $r=-0.8$ ;  $p\leq 0.01$ ), non-cyanotic subjects ( $r=-0.7$ ;  $p\leq 0.01$ ), groups I ( $r=-0.8$ ;  $p\leq 0.01$ ), groups II ( $r=-0.75$ ;  $p\leq 0.01$ ) and groups III ( $r=-0.69$ ;  $p\leq 0.02$ ). There was also a significant negative correlation between the mean of ANP and mean of LVEF% in groups I ( $r=-0.78$ ;  $p\leq 0.02$ ).

**Conclusion:** the result of this study revealed that increased plasma level of ANP and BNP are common in subjects with congenital heart disease and it is strongly correlated with the ventricular function. However, the plasma level of BNP appear to be superior to ANP as efficient, non-invasive cardiac markers for facilitating diagnosis of various pattern of congenital heart disease.

### **Introduction**

The Natriuretic peptides family include three peptides that are structurally similar and share the same amino acid ring but are genetically distinct. The members of this family are atrial natriuretic peptide (ANP), brain natriuretic peptides (BNP), and C-type natriuretic peptide (CNP). They play a very important role in the regulation of extracellular fluid and electrolyte balance as well as blood pressure and affect the internal environment of the body by their actions of natriuresis, diuresis, vasodilation, inhibition of sympathetic system, and complex interaction with cytokines and vasopressin<sup>(1,2)</sup>. These effects are mediated by binding of the peptides to specific receptors on the cell membrane. These receptors have been characterized, purified and cloned from cell of the vasculature, kidney, adrenal gland and brain. Myocyte stretch is thought to be the primary stimulus for natriuretic peptide secretion<sup>(3)</sup> in addition to, hypoxia, hyperosmolality, and vasoconstriction<sup>(4)</sup>. The development of ANP and BNP during embryogenesis have been extensively studied in mouse models and human embryos and have been postulated to play an important role in the cardiac development<sup>(5)</sup>. The measurement of plasma natriuretic peptide is increasingly used to aid diagnosis, assess prognosis, and conduct risk stratification in adults with various cardiovascular disease<sup>(6,7)</sup>. In several studies, BNP has turned out to be superior to ANP in terms of sensitivity as well as specificity<sup>(8)</sup>. The mRNA of BNP contains four repetitive AUUUA sequences, which lead to its destabilisation and enable the synthesis of BNP in bursts<sup>(9,10)</sup>. BNP therefore reflects hemodynamic changes more accurately than ANP. There is now accumulating evidence that reduced oxygen tension acts directly on cardiac myocytes or through a feedback loop involving oxytocin or another mediator to increase natriuretic peptide secretion<sup>(11)</sup>. Congenital heart diseases exhibit a complex interactions of factors that may stimulate natriuretic peptide secretion involving myocyte stretch, volume and pressure load, actions on cell growth and proliferation of fibroblasts, and vascular smooth muscle cells<sup>(12,13)</sup>. A number of studies have been performed in children with congenital heart disease and most of these studies have focused upon plasma levels of the BNP in infants and children<sup>(14,15)</sup>. The purpose of this study is to assess ANP and BNP levels in subjects with congenital cardiac disease, and their role in evaluation left ventricular function among these subjects.

### **Materials and methods**

Sixty two subjects with congenital heart disease (28 males, 34 females), aged 1-20 years were studied in the Echocardiography unit at Ibn-Sena teaching hospital in Mosul, during the period of 1 year, from 1st October 2004 to 30th September 2005. Twenty

seven had cyanotic congenital cardiac defects (17 ventricular septal defect, 6 with tetralogy of Fallot, and 4 with tetralogy of Fallot and pulmonary atresia,). The other 35 had non-cyanotic congenital cardiac defects (14 with atrial septal defect, 15 with ventricular septal defect, 4 with aortic stenosis, and 2 subject with pulmonary stenosis).

The subjects with congenital heart disease was further divided into 3 groups according to their age, group I included 36 subjects (11 males, 25 females) their age range from 1-5 years, twenty three of them were cyanotic, group II included 16 subjects (9 males, 7 females) their age range from 6-10 years, four of them were cyanotic and group III included 10 subjects (8 males, 2 females) their age range from 11-20 years. Twenty apparently healthy volunteers (9 males and 11 females) aged 2-20 years included in the study as control group.

Blood samples were collected by venipuncture into tubes containing EDTA as an anticoagulant. Immediately after the collection of samples plasma were separated by centrifugation (at 4°C for 10 min), then the aliquots of plasma were kept frozen at -20°C until ANP and BNP were analyzed. Plasma ANP and BNP concentration was determined by Enzyme Linked Immunosorbent Assay (ELISA) utilizing kits provided by DRG International Inc., USA (Cat. No.: Eia-1524) (16).

Transthoracic echocardiogram was performed for every subject participate in the study by expert echocardiologist. Utilizing parasternal long axis view a two dimension M-mode echocardiogram were obtained to calculate left ventricle ejection fraction (LVEF%).

The data were classified by using mean  $\pm$  standard deviation (SD) for the categorical variables. The one way analysis of variance (ANOVA) model was used to evaluate the difference in the means of ANP and BNP levels and LVEF % of different groups. Pearson correlation coefficient model was utilized to investigate the correlations between the LVEF% and ANP and BNP levels. Statistical analysis was performed with SPSS 15 for Windows (SPSS Corporation, Chicago, Illinois).

## **Results**

The mean plasma ANP, BNP levels, and LVEF% in cyanotic, and non-cyanotic subjects with congenital heart disease and control group are shown in table(1).

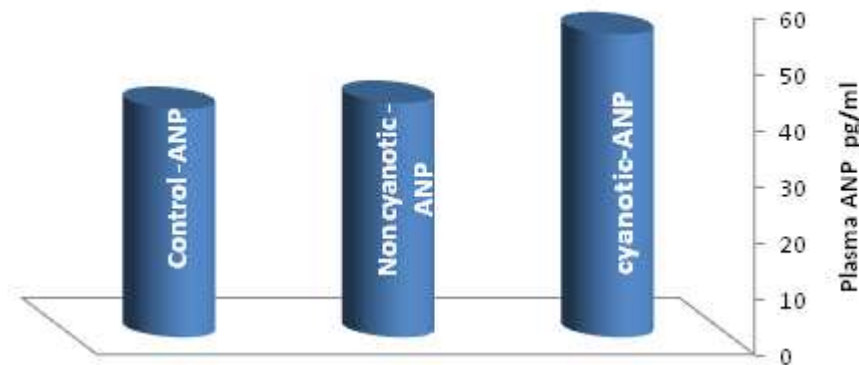
**Table (1): Mean  $\pm$  SD of Atrial , and Brain natriuretic peptide, and left ventricular ejection fraction in cyanotic and non - cyanotic subjects with congenital heart disease and control group.**

Parameter	cyanotic subjects (n=27)	non-cyanotic subjects (n=35)	Control group (n=20)
BNP pg/ml	130.5 $\pm$ 12.4*	120.4 $\pm$ 10.1**	55.1 $\pm$ 12.3
ANP pg/ml	54.0 $\pm$ 10.7*	41.8 $\pm$ 4.4	40.7 $\pm$ 7.6
LVEF%	50.7 $\pm$ 4.2*	56.9 $\pm$ 4.6	59.4 $\pm$ 3.2

\*.significantly differ in cyanotic group from respective values in non-cyanotic and control group

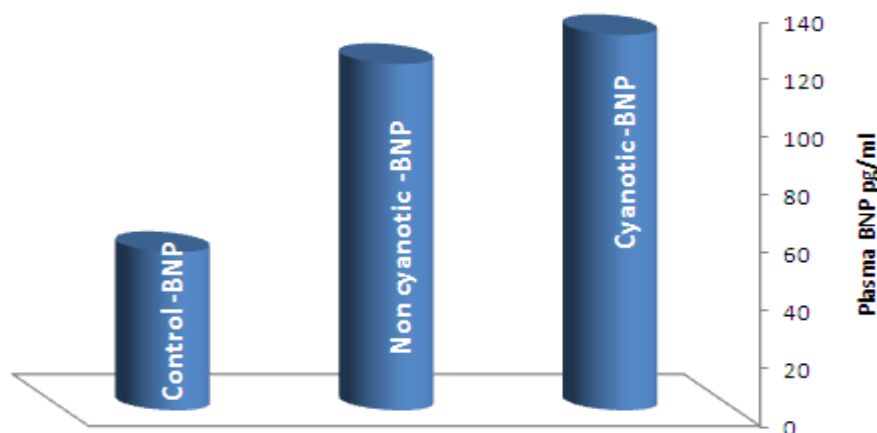
\*\*. significantly differ in non-cyanotic group from respective values in control group

The mean plasma ANP and BNP levels were significantly higher in cyanotic subjects with congenital heart disease compared with non-cyanotic ( $P \leq 0.002$ ;  $P \leq 0.0001$ ) and control subjects ( $P \leq 0.001$ ;  $P \leq 0.0001$ ) as shown in figure (1 and 2).



**Figure (1) : Mean  $\pm$  SD of Atrial natriuretic peptide, in cyanotic and non-cyanotic subjects with congenital heart disease and control group .**

The mean of LVEF% was significantly lower in cyanotic subjects compared with non-cyanotic subjects ( $P \leq 0.0001$ ) and control group ( $P \leq 0.0001$ ), but there was no significant difference in the mean of LVEF% between non-cyanotic subjects and control group. The mean plasma BNP was significantly higher in non-cyanotic subjects with congenital heart disease compared with control group ( $P \leq 0.0001$ ). However, there was no such differences in the mean of ANP between the non-cyanotic subjects and control group.



**Figure (2) : Mean  $\pm$  SD of Brain natriuretic peptide, in cyanotic and non-cyanotic subjects with congenital heart disease and control group.**

The mean plasma ANP, BNP levels, and LVEF% in different age groups of subjects with congenital heart disease and control group are shown in table (2).

**Table (2) : Mean  $\pm$  SD of Atrial , and Brain natriuretic peptide, and left ventricular ejection fraction among different age groups of subjects with congenital heart disease and control group.**

Parameter	Group1 (1-10years) N=36	Group1I (11-20years) N=16	Group1II (21-34years) N=10	Control N=20
BNP pg/ml	130.8 ± 11.2*	113.3 ± 11.4	121.5±8.6**	55.1 ± 12.3
ANP pg/ml	48.5 ± 10.3†	41.1 ± 4.1††	52.0 ±11.1**	40.7 ± 7.6
LVEF%	53.2 ± 5.1†	58.1 ± 4.8††	51.7 ±3.3**	59.4 ± 3.2

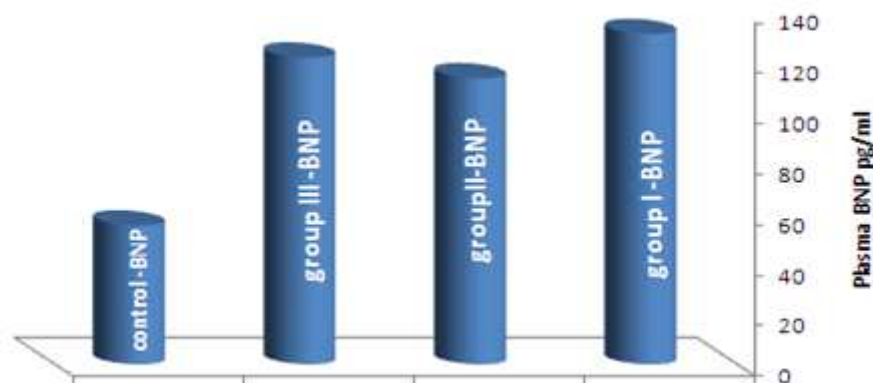
\*. significantly differ in group I from respective values in group II,III, and control group.

\*\*. significantly differ in group III from respective values control group.

† significantly differ in group I from respective values in group II and control group. .

†† significantly differ in group II from respective values in group III.

The mean of plasma BNP was significantly higher in group I (age; 1-5 years) in comparison with group II (age; 6-10 years), group III (age;11-20 years) and control group ( $P \leq 0.0001$ ,  $P \leq 0.002$ , and  $P \leq 0.0001$  respectively). The mean of plasma BNP was significantly higher in group II and group III than that of control group ( $P \leq 0.0001$ , and  $P \leq 0.0001$  respectively) as shown in figure (3).



**Figure (3): Mean ± SD of Brain natriuretic peptide, in group I (1- 5 years) , group II (6-10 years),group III (11-15 years),and control group.**

The mean of plasma ANP was significantly higher in group I in comparison with group II( $P \leq 0.007$ ), and control group ( $P \leq 0.003$ ). The mean of plasma ANP was significantly higher in group III in comparison with group II ( $P \leq 0.003$ ), and control group ( $P \leq 0.002$ ) as shown in figure (4).

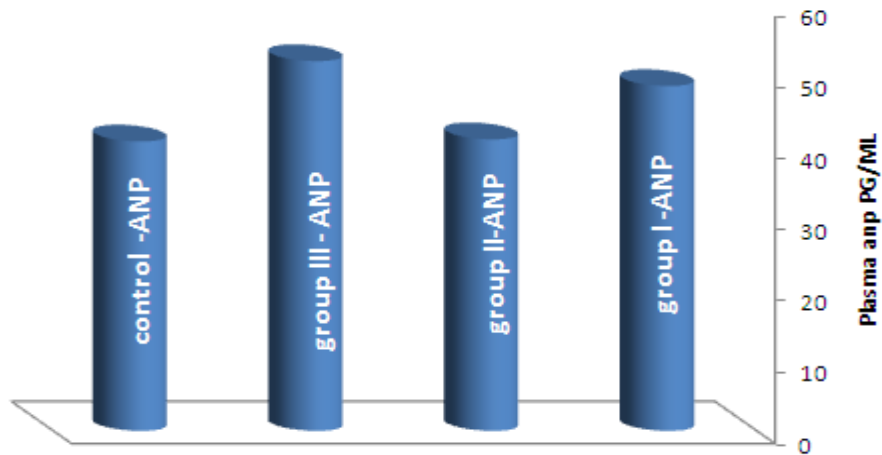


Figure (4): Mean  $\pm$  SD of Atrial natriuretic peptide, in group I (1-5 years), group II (6-10 years), group III (11-20 years), and control group.

The mean of LVEF% was significantly lower in group I compared with group II ( $P \leq 0.0001$ ) and control group ( $P \leq 0.0001$ ), it was also significantly lower in group III compared with group II ( $P \leq 0.001$ ) and control group ( $P \leq 0.0001$ ). Concerning the effect of gender on plasma level of ANP and BMP, the result of the study revealed no significant difference between males and females.

The result of study showed a significant negative correlation between the mean of BNP and mean of LVEF% in cyanotic subjects with congenital heart disease ( $r = -0.8$ ;  $p \leq 0.01$ ), non-cyanotic subjects ( $r = -0.7$ ;  $p \leq 0.01$ ) groups I ( $r = -0.8$ ;  $p \leq 0.01$ ), groups II ( $r = -0.75$ ;  $p \leq 0.01$ ) and groups III ( $r = -0.69$ ;  $p \leq 0.02$ ) as shown in figure(5,6).

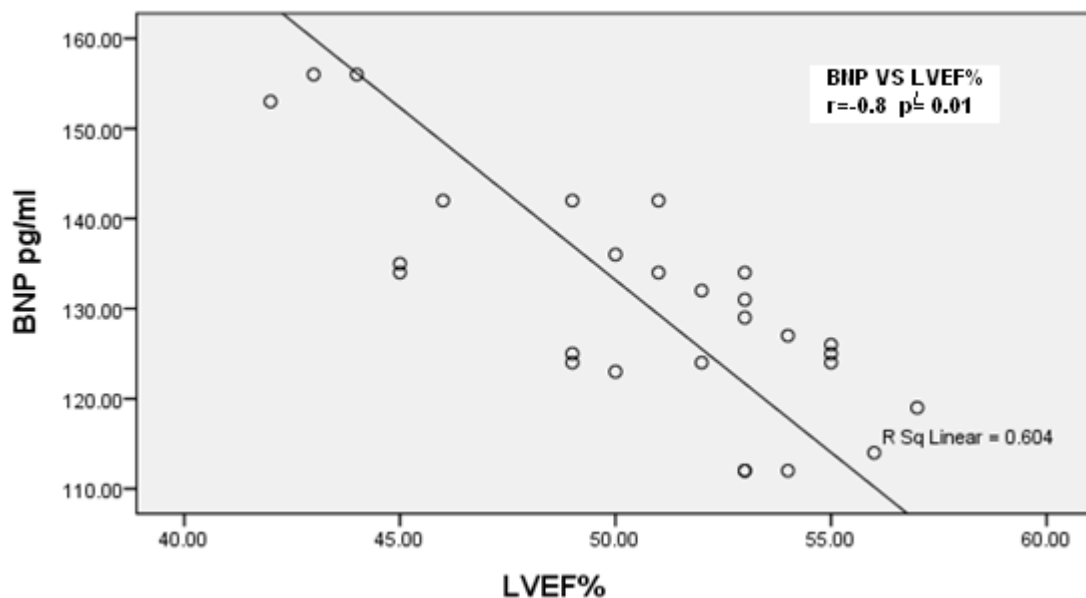


Figure (5): Correlation between Brain natriuretic peptide and left ventricular ejection fraction in cyanotic subjects with congenital heart disease.

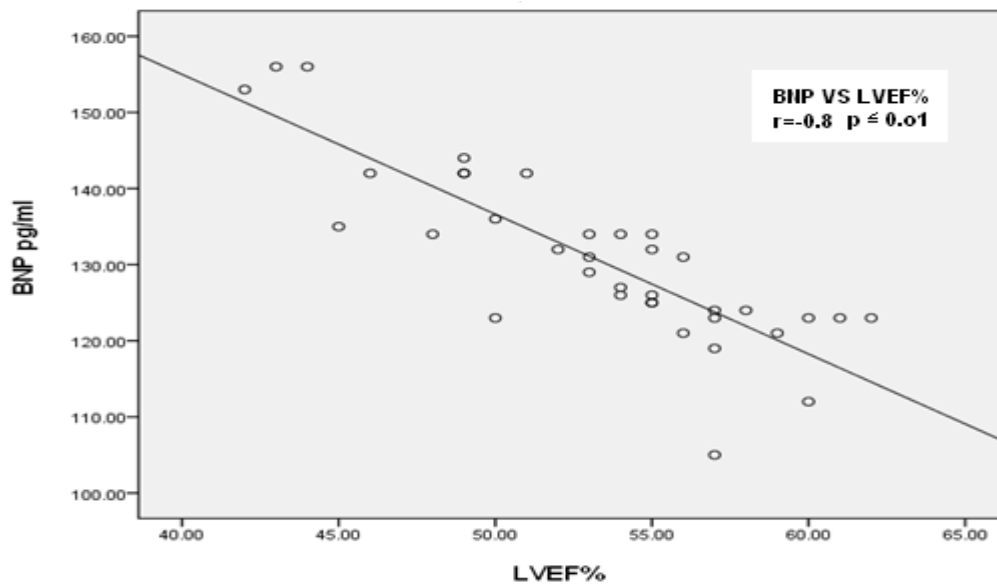


Figure (6): Correlation between Brain natriuretic peptide and left ventricular ejection fraction in group I.

However, there was only a significant negative correlation between the mean of ANP and mean of LVEF% in groups I( $r = -0.38$ ;  $p \leq 0.02$ ) as shown in figure (7).

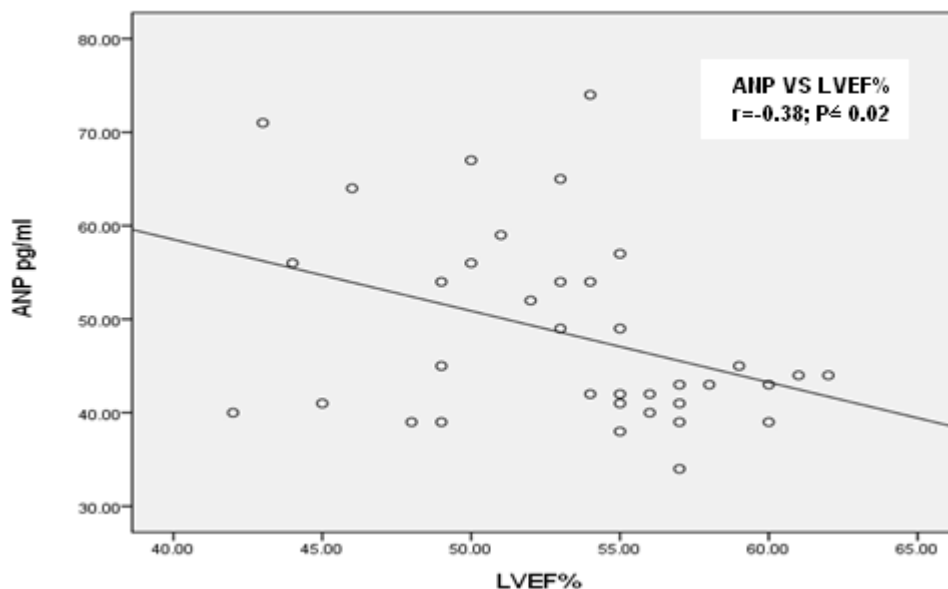


Figure (7): Correlation between Atrial natriuretic peptide and left ventricular ejection fraction in group I.

### Discussion

Congenital heart disease constitute an important group of cardiac diseases in children which carry high incidence of mortality and morbidity<sup>(17)</sup>. Although congenital heart disease represent a big health problem in our country only few studies have been reported in this field. The availability of echocardiogram help to great extent to improve

the frequency and accuracy of diagnosed cases of congenital heart disease. In spite, of high validity of echocardiogram in detecting different pattern of cardiac abnormalities, there is now an increasing interest in using cardiac marker like natriuretic peptides as a simple non invasive diagnostic tool in heart diseases<sup>(19,20)</sup>. Recent studies have reported the usefulness of ANP and BNP as a diagnostic tool in adult patients with various congenital heart diseases<sup>(14,15)</sup>. The result of this study showed a significant increase in both ANP and BNP activity among subjects with congenital heart diseases specially those who had cyanosis. The significantly higher value of these peptides in these subjects probably can not explained on the base of increased myocardial stretch alone. Hypoxia have been considered an important stimulus for natriuretic peptides secretion specially the BNP<sup>(21,22)</sup>. Whether reduced oxygen tension acts directly on cardiac myocytes or through a feedback loop involving another mediator is unclear. The result of this study is in accordance with other studies concerning the plasma level of BNP in subjects with congenital heart disease<sup>(23)</sup>. The link between left ventricular dysfunction and ANP and BNP activity observed in many studies<sup>(24)</sup>. The result of this study showed a strong negative correlation between plasma level of BNP and LVEF% in cyanotic and non-cyanotic subjects with congenital heart disease. The study showed a negative correlation between the plasma level of ANP and LVEF% only in cyanotic subjects with congenital heart disease. In conclusions the result of this study reviled that increased plasma level of atrial and brain natriuretic peptides are common in subjects with congenital heart disease and it s strongly correlated with the ventricular function. However, the plasma level of BNP appear to be superior to ANP as efficient, non-invasive cardiac markers for facilitating diagnosis of various pattern of congenital heart disease.

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