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Comparison of Pro-BNP levels and myocardial performance index before and after iron treatment in children with congenital cyanotic heart disease with iron deficiency anemia

Demir eksikliği anemisi olan doğumsal siyanotik kalp hastalıklı çocuklarda demir tedavisi öncesi ve sonrası pro-bnp düzeyleri ve miyokardiyal performans indekslerinin karşılaştırılması

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Abstract

Introduction: Our aim was to compare NT-proBNP levels and cardiac functions after iron therapy in children with congenital cyanotic heart disease who had iron deficiency anemia.

Methods: We included 40 children with pre-established cyanotic congenital heart disease and accompanying iron deficiency anemia, between the age of 6 months and 17 years, who were admitted to the outpatient clinic of Çukurova University Faculty of Medicine, Department of Pediatric Cardiology between September 2015 and March 2016. We recorded demographic data and performed following investigations: complete blood count, peripheral blood smear, reticulocyte count, measurement of serum iron levels, total iron-binding capacity, ferritin levels, transferrin saturation and NT-proBNP levels, and echocardiographic examination.

Results: There was a statistically significant increase in following laboratory parameters after iron therapy: hemoglobin, hematocrit, MCV, MCHC, serum iron and ferritin, transferrin saturation and oxygen saturation. During the follow-up period, RDW and NT-proBNP levels were significantly decreased. In left ventricular tissue doppler; there was a significant difference in MPI measurements before and after treatment. There was no significant difference in other echocardiography findings.

Discussion and Conclusion: Iron therapy has improved cardiac functions in children with cyanotic congenital heart disease, and NT-proBNP levels can be used to evaluate the efficacy of treatment in the follow-up period

Keywords: Cyanotic congenital heart disease; echocardiography; iron deficiency anemia; NT-proBNP.

Özet

Amaç: Demir eksikliği anemisi olan doğumsal siyanotik kalp hastalıklı çocuklarda demir tedavisi sonrası NT-proBNP düzeyleri ve kardiyak fonksiyonların karşılaştırılmasıdır.

Gereç ve Yöntem: Eylül 2015–Mart 2016 tarihleri arasında Çukurova Üniversitesi Tıp Fakültesi Çocuk Kardiyoloji Bilim Dalı polikliniğine başvuran, siyanotik doğuştan kalp hastalığı tanılı, demir eksikliği anemisi saptanan altı ay ile 17 yaşları arasındaki 40 çocuk hasta çalışmaya alınmıştır. Çeşitli sebeplerden dolayı 26 hasta ile sonuçlar değerlendirilmiştir. Hastaların ilk geliş ve üçüncü ay kontrollerinde; demografik verileri, tam kan sayımı, periferik yayma, retikülosit, serum demir, total demir bağlama kapasitesi, ferritin düzeyleri, transferrin satürasyonlarıu, NT-proBNP düzeyleri ve Eko bulguları değerlendirilmiştir.

Bulgular: Hastaların demir tedavisi öncesi ve sonrası laboratuvar değerlerinde; hemoglobin, hematokrit, MCV, MCHC, demir, ferritin, transferin saturasyonu, oksijen saturasyonu değerleri tedavi sonrası istatistiksel olarak anlamlı derece artmıştır. RDW ve NT-proBNP düzeyleri yüksekliği kontrol sonuçlarında anlamlı olarak düşmüştür. Sol ventrikül doku dopplerinde; MPİ ölçümlerinde tedavi öncesi ve sonrasında anlamlı farklılık saptanmıştır. Diğer EKO bulgularında anlamlı farklılık saptanmamıştır.

Sonuç: Demir tedavisi sonrası siyanotik doğuştan kalp hastalıklı çocuklarda kardiyak fonksiyonlarda iyileşme saptanmış olup NT-proBNP takipte, tedavinin değerlendirilmesinde kullanılabilir.

Anahtar Sözcükler: Siyanotik doğuştan kalp hastalığı; ekokardiyografi; demir eksikliği anemisi; NT-proBNP.

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The most common hematologic disease in childhood is iron deficiency anemia (IDA) characterized by insufficient hemoglobin synthesis due to iron deficiency.[1] This is even more important because the blood circulation factors are affected in children with heart disease who have IDA, and the hemodynamics of normal circulation may vary, particularly in some heart diseases (e.g., complex cyanotic congenital heart diseases).

Normal hemoglobin levels indicate relative anemia in patients with congenital cyanotic heart disease (CCHD). In patients with CCHD with a hemoglobin level of 15 g/dL, IDA cannot be easily recognized and there may be an increase in clinical findings due to insufficient oxygenation. Although less cyanotic, these patients are usually more symptomatic. Due to iron deficiency, microspherocytic erythrocytes with higher deformability and rigidity are produced instead of bi-concave resistant erythrocytes. A decrease in hemoglobin concentration causes further increase in blood viscosity. In order to transport oxygen to the tissues, there is a right shift of the oxygen-hemoglobin curve, but with a decrease in pulmonary affinity for oxygen.[2] Severe iron deficiency can produce ventricular dysfunction and overt heart failure.

Brain (B-type) natriuretic peptide (BNP) is sensitive to changes in ventricular function. It is also a ventricular hormone that is a specific determinant of these changes. An effective treatment for heart failure is known to decrease serum BNP levels. The reduction in ventricular load with a suitable treatment also reduces wall tension and BNP levels. As a result, BNP is an important neurohumoral marker for the diagnosis and treatment of acute heart failure and for the evaluation of treatment success.[3–5]

In our study, we aimed to prospectively collect the data of children with CCHD who have IDA and to make a comparative evaluation for iron parameters, cardiac functions and BNP levels before and after 3 months of therapy.

This study is expected to shed light on the importance of NT-proBNP levels in the management and follow-up of IDA, which may cause mortality and morbidity in patients with CCHD, to determine the effect of iron therapy on cardiac functions in children with CCHD who have IDA, to identify the role of iron therapy in the follow-up of these patients, and to lead to a reduction in the complications and associated mortality and morbidity of iron deficiency.

Materials and Method

This prospective study was carried out to investigate the effect of iron therapy on cardiac functions in children with CCHD who had no clinical heart failure, between the age of 6 months to 17 years, who have IDA and were admitted to Çukurova University Faculty of Medicine, Department of Pediatric Cardiology, between September 2015 and March 2016. Forty children who met the inclusion criteria were enrolled in the study.

The patients were excluded from the study if they had bleeding symptoms leading to anemia, received blood transfusion in the previous week, received iron therapy in the last three months and had an infection in the last month. In addition, patients who were diagnosed with CCHD, but were scheduled for operation during the evaluation period (less than three months), and who did not want to participate in the study were excluded from the study.

We excluded a total of fourteen patients, including five patients who did not participate in regular follow-ups, four patients who did not use regular medication, two patients who could not continue treatment due to side effects, one patient who died during follow-up, and two patients who had undergone emergent operation and received blood transfusions during follow-up. Therefore, 26 patients were included in the study and their results were evaluated.

Ethical approval was obtained from the Ethics Committee of Çukurova University Faculty of Medicine on 06 March 2015. The parents of all patients were informed about the purpose of the study and their verbal and written consent was obtained.

The data including age, gender, weight, height, pulse rate, oxygen saturation measured by pulse oximetry and blood pressure were recorded by a pediatric cardiology nurse at the first admission and at the third month follow-up. In addition, a detailed physical examination was performed.

Transthoracic echocardiography was performed using a Philips EPIQ 7 ultrasound system (Philips Medical Systems, Andover, MA, USA) with multifrequency transducers (3–5 MHz). Initially, routine diagnostic imaging was performed. Myocardial performance index (MPI) by tissue Doppler imaging, was calculated as the sum of isovolumic contraction time (ICT) and isovolumic relaxation time (IRT) divided by ventricular ejection time (ET).

The patients with IDA, bivalent oral iron preparations (5 mg/kg/day in two equally divided doses) were commenced. Parents were instructed to administer iron treatment on an empty stomach half an hour before meals and were informed about the use, maintenance and side effects of iron preparations. Laboratory and clinical examinations were repeated after 3 months.

Statistical analysis

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0. McNemar-Bowker test was used for the pre- and post-evaluation of categorical measurement data. The Kolmogorov–Smirnov probability test was used to determine the normal distribution of numerical data. Wilcoxon Signed Rank test was used to compare two dependent numerical data with non-normal distribution. The Kruskal-Wallis test was used for general comparison of non-normal distributed numerical data among more than two groups. The level of statistical significance was set at 0.05 in all tests.

Results

There were 26 patients with CCDH who had IDA included in our study. The age range of the patients was between 6

Table 1. Laboratory findings before and after treatment of iron deficiency anemia

Number (n=26)	Day						
	Day 0			Day 90			
	Mean±SD	Median	Range	Mean±SD	Median	Range	p
Hb (g/dL)	15.2±1.9	15.1	(10.3–18.5)	16.7±1.9	17.1	(12.5–21.3)	<0.001
Htc (%)	47.6±5.3	48.3	(33.6–584)	50.2±5.1	50.3	(38.9–62.2)	<0.001
MCV (fl)	78.9±7.8	79.9	(62.8–94.6)	81.8±5.8	81.5	(69.7–91.1)	0.002
MCHC (g/dL)	31.4±2.0	318.0	(24.9–343.0)	32.9±2.0	33.3	(24.9–35.8)	<0.001
RDW (%)	17.0±2.9	16.2	(12.7–24.5)	15.2±2.0	14.6	(12.9–22.5)	0.001
Iron (ug/dL)	60.0±32.4	54.0	(16.0–139.0)	123.8±66.6	112.5	(29.0–30.0)	<0.001
TIBC (ug/dL)	441.3±75.2	421.5	(338.0–567.0)	362.0±52.3	353.0	(280.0–485.0)	<0.001
Transferrin saturation (%)	13.8±7.8	13.1	(4.3–30.4)	34.7±19.4	31.6	(9.0–86.4)	<0.001
Ferritin (ng/dL)	9.5±2.5	10.5	(3.5–12.0)	60.6±53.1	40.8	(13.0–254.0)	<0.001
Oxygen saturation (%)	81.6±6.1	83.5	(69.0–89.0)	83.9±5.6	85.5	(73.0–93.0)	<0.001
Pro-BNP (pg/ml)	285.0±270.8	204.2	(76.6–1272.0)	236.2±231.2	149.1	(61.5–1090.0)	0.012

Table 2. Myocardial performance index of the ventricles

	Day						
	Day 0			Day 90			
	Mean±SD	Median	Range	Mean±SD	Median	Range	p
RV - IRT (ms)	47.7±14.5	40.0	30.0–77.0	50.1±13.8	42.0	35.0–75.0	0.475
RV - ICT (ms)	48.8±8.8	50.0	26.0–61.0	48.5±8.2	50.0	30.0–63.0	0.692
RV - ET (ms)	255.5±35.0	248.0	214.0–310.0	252.4±40.2	240.0	198.0–320.0	0.529
RV - MPI	0.37±0.06	0.38	0.25–0.49	0.39±0.10	0.38	0.26–0.66	0.651
LV- IRT (ms)	46.4±12.7	43.0	29.0–79.0	45.8±8.9	42.0	35.0–68.0	0.887
LV - ICT (ms)	51.8±11.0	50.0	34.0–82.0	49.0±7.6	48.0	35.0–65.0	0.195
LV - ET (ms)	249.2±40.7	238.0	156.0–330.0	256.9±38.4	245.0	170.0–330.0	0.022
LV - MPI	0.39±0.07	0.38	(0.31–0.63)	0.37±0.06	0.36	(0.30–0.60)	0.004

RV: Right ventricle; LV: Left ventricle; ICT: Isovolumic contraction time; IRT: Isovolumic relaxation time; ET: Ejection time; MPI: Myocardial performance index.

months and 17 years, with a mean age of 52.7±54.6 (median: 34.5) months. Fourteen of the patients were female and 12 were male.

In our study, pre- and post-treatment laboratory values were examined on days 0 and 90. Accordingly, there was a statistically significant increase in hemoglobin, hematocrit, MCV, MCHC, serum iron, serum ferritin, transferrin saturation and oxygen saturation values. A comparison of all laboratory values on days 0 and 90 showed a p value of 0.002 for MCV, but showed a p value of <0.001 for remaining parameters (Table 1).

In our study, mean NT-proBNP levels at first visit and after the treatment were 285.0±270.8 pg/ml and 236.2±231.2 pg/ml, respectively, with a p value of 0.012. The results were statistically significant (Table 1).

Reticulocyte counts were performed on day 0 and at post-treatment 7th day to evaluate the efficacy of iron therapy. There was a statistically significant increase in reticulocyte counts from day 0 to day 7, with a p value of <0.001.

Nineteen of the patients had univentricular physiology (11 patients had right ventricular (RV) dominant, 8 had left ventricular (LV) dominant), and 7 biventricular physiology. None of the patients had evidence of heart failure by physical examination or routine diagnostic imaging by echocardiography. Twelve patients had no atrioventricular (AV) valve regurgitation, 7 had mild and 7 had moderate regurgitation.

No significant differences were found between the IRT, ICT, ET and MPI parameters of the patients with RV dominance (p>0,05). However, there was a significant difference between ET and MPI values of the left ventricle on days 0 and 90 (p<0.05) (Table 2).

Discussion

Congenital heart diseases (CHD) are the most common cardiac malformations of fetal and neonatal periods, which represent a heterogeneous group of defects with a lesser known

cause. In other words, CHD includes congenital structural or functional abnormalities in the cardiovascular system, which can be defined at birth or later.[6,7] The frequency of congenital heart diseases is known to be approximately 0.5-0.8% of all live births.[8–10]

Iron deficiency anemia is not only a condition affecting the hematological system, but also a clinical condition that may lead to multisystemic disorders.[11–13] It is known that anemia has a negative effect on cardiovascular system and hemodynamics and long-term severe anemia can lead to congestive heart failure.[14]

In our study, there were 26 patients with CCHD who had IDA. The age range of the patients was between 6 months and 17 years, with a mean age of 57.7±54.6 months. These patients were evaluated during routine outpatient visits. Of the patients, 46.1% were male and 53.9% were female.

The literature review showed no gender difference for congenital heart defects. However, there is a known relationship between certain types of diseases and genders. The frequency of severe, especially cyanotic and complex heart defects was higher in males, whereas the frequency of less severe defects was higher in females. A study has shown that the frequency of double-outlet right ventricle, hypoplastic left heart syndrome, transposition of the great vessels and aortic stenosis was almost two times higher, and of pulmonary atresia and tricuspid atresia was approximately one and a half times higher in males than females. The prevalence of less severe defects, including atrial septal defect, PDA and AVSD, was higher in women.[15–18] In our study, nineteen of the patients had univentricular physiology (11 patients had right ventricular (RV) dominant, 8 had left ventricular (LV) dominant), and 7 biventricular physiology. The number of patients was not sufficient to demonstrate this relationship between heart pathologies and gender.

In this study, hemoglobin and hematocrit levels were found to be higher than age-expected values of the patients. This is a natural result of the expected secondary erythrocytosis in cyanotic patients. Serum ferritin levels were measured to determine iron deficiency in our patients. Additionally, serum transferrin saturation was calculated to support the diagnosis.

Onur et al.,[19] in their study on 44 children with CCHD, found iron deficiency in 28 patients by measuring serum ferritin levels and administered oral iron treatment in these patients. However, 16 patients were followed-up without any treatment. Three months later, the laboratory analysis showed a decrease in Hb, Hct and ferritin values in the untreated group. Onur et al. included children aged between 6 and 48 months, which is the period with the highest physiological iron requirement. Therefore, there may be a higher need for iron in this age group due to secondary erythrocytosis. However, although this study included children over two years of age, laboratory studies showed a decrease in iron and ferritin levels even after three months in patients not receiving iron treatment. For all patients, Hb, Htc, RBC, MCV, MCH, MCHC, RDW, SI, TIBC, TS and SF levels were studied at baseline and at the end of 3rd month. In conclusion, the prevalence of iron deficiency was found to be 63.6% in 44 children with CCHD. In patients with iron deficiency, three months of iron therapy resulted in an increase in Hb, Htc, MCV, MCH, MCHC, RDW, SI, TIBC and SF levels. These results were comparable with the results of our study. In patients with adequate iron levels, MCV, MCH, RDW, SI, TIBC and SF levels were normal at baseline, but at the end of the 3-month follow-up, they reached levels consistent with iron deficiency. In this study, it was emphasized that it is possible to diagnose iron deficiency in children with CCHD by complete blood count, or even by measuring only RDW, MCV and MCH levels, and that these patients should be given iron prophylaxis even without iron deficiency.

All of the 26 patients in our study had IDA. In our study, laboratory studies found significantly lower MCHC, TS, MCV values, but significantly higher RDW and TIBC values. Our results were comparable with the results of other study that have addressed this issue.[19] In the present study, lower levels of MCHC and MCV and higher levels of RDW were correlated with lower ferritin levels.

In our study, we measured NT-proBNP levels in order to determine the effect of iron deficiency on myocardium in children with CCHD who had IDA, and tried to determine the role of NT-proBNP in clinical practice. In our study, the mean NT-proBNP levels at first visit and after treatment were 285.0±270.0 (mean 204.2) pg/ml and 236.2±231.2 (mean 149,1) pg/ml, respectively, with a p value of 0.012. There was a statistically significant decrease in NT-proBNP levels.

Literature review showed a limited number of studies on NT-proBNP as a marker of myocardial involvement in children with anemia. Nybo et al.,[20] in their study including 6238 adult patients, and Willis et al.,[21] in their study including 209 adult patients with no evidence of heart failure, reported that NT-proBNP levels in patients with anemia were higher than those without anemia. They emphasized that, despite lower concentrations of Hb in females, NT-proBNP levels in females were not as high as in male patients. Arati et al.[22] found a negative correlation between hemoglobin levels and serum NT-proBNP levels in 809 adult patients without heart failure. Mika et al.[23] found a negative correlation between plasma BNP levels and Hb levels in 1036 healthy adults.

A study conducted in 2010 in Inonu University, Faculty of Medicine divided the patients with iron deficiency anemia into three groups as mild, moderate and severe, based on the Ross classification for heart failure, and made a comparison between these groups. There was a statistically significant negative correlation between pre-treatment NT-proBNP and Hb levels in the patient group (p=0.004). The comparison of pre-treatment and post-treatment NT-proBNP levels in the patient group showed a decrease in NT-proBNP levels inversely with the increase in Hb levels after treatment (p=0.0001).[24]

The results of all these studies are consistent with our results and support the decrease in NT-proBNP levels with increasing

Hb concentrations after treatment. Although our patients had no clinical evidence of congestive heart failure, it was thought that NT-proBNP levels decreased as a result of treatment of the negative effects of iron deficiency on myocardium.

Echocardiography is the most important diagnostic modality for ventricular dysfunction in heart failure. It is not technically correct to evaluate the systolic functions of heart with standard echocardiographic methods in CHD patients with single ventricular physiology. However, MPI by tissue Doppler imaging can be used in these patients because it is not affected by the geometric shape of the ventricle since it is the ratio of time intervals. It evaluates both systolic and diastolic functions of the ventricles. Because the age ranges of the patients included in the study were different and the anatomical heart structures were very complex, the Simpson method could not be used for measurements. This is one of the limitations of the study. In our study, there was no significant difference between 0th day and 90th day in right ventricular measurements. In left ventricular parameters; there was a significant difference between the 0th and 90th days of MPI measurements ($p<0.05$). MPI is a good indicator of the assessment of cardiac function and is an important and easy to determine parameter that provides information for clinical course and prognosis. Although there was no statistically significant difference in right ventricular physiology in our study, with iron therapy MPI was better in patients with left ventricular physiology.

Groenning et al.[25] found that serum NT-proBNP measurement in adult patients with heart failure was a stronger marker than the classical echocardiographic parameters for demonstrating left ventricular dysfunction. In our study, no statistically significant correlation was found between echocardiographic parameters and NT-proBNP levels. The higher serum NT-proBNP levels of patients before treatment was considered to be the biochemical reflection of changes in the histological level that we could not detect clinically and with echocardiography. It can be concluded that NT-proBNP measurement can detect early myocardial involvement in which echocardiographic parameters are not affected.

In conclusion, our study evaluating children with CCHD who have IDA suggests that iron therapy improves cardiac symptoms and that IDA should not be missed in patients with CCHD. Evaluation of children with CCHD who have IDA should be performed considering that their laboratory findings may differ from children without heart disease. In our study, the relatively low number of patients could be considered as the limitation of our study. Measurement of NT-proBNP levels, which show a significant decrease after iron therapy, can be used more frequently in follow-up. However, more extensive and prospective studies are needed to make more accurate judgments.

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of Çukurova University Faculty of Medicine on 06 March 2015 with Decision No: 40/15).

Informed Consent: The parents of all patients were informed about the purpose of the study and their verbal and written consent was obtained.

Conflict of interest: There are no relevant conflicts of interest to disclose.

References

1. Andrews N, Ullrich CK, Fleming MD. Disorders of iron metabolism and sideroblastic anemia. In: Nathan and Oski's Hematology of Infancy and Childhood. 7th ed. Philadelphia: Saunders, 2008:521–570.
2. Perloff JK, Roseve MH. Adults with cyanotic congenital heart disease: Hematologic management. Ann Intern Med. 1988;109:406–413.
3. Gershwin K. Davis, Fiona Bamforth, Amrita Sarpal, Frank Dicke, Yacov Rabi, Martha E. Lyon: B- type natriuretic peptide in pediatrics. Clinical Biochemistry 39. 2006;600–605.
4. Amıram Nır, MD, and Nadera Nasser, MD. Clinical Value of NT-ProBNP and BNP in Pediatric Cardiology Journal of Cardiac Failure. 2005;11:5.
5. Hoffman JIE, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39:(12)1890–1900.
6. Candan I, Oral D. Cardiology. Ankara: Antıp Inc.- Baran Offset, 2002.
7. Capozzi G, Caputo S, Pizzuti R, Martina L, Santoro M, Santoro G. Congenital heart disease in live-born children: incidence, distribution, and yearly changes in the Campania Region. J Cardiovasc. Med. 2008;9(4):368–374.
8. Bernstein D. Epidemiology and genetic basis of congenital heart disease. Nelson Textbook of Pediatrics. 17th Ed, Philadelphia: Saunders, 2004.
9. Flanagan MF, Yeager SB, Weindling SN. Cardiac disease. Neonatology Pathophysiology & Management of the Newborn,5th ed., Philadelphia: Lippincott, Williams&Wilkins, 1999.
10. Goel M, Shome DK, Singh ZN, Bhattachariee J, Khalil A. Hemostatic changes in children with cyanotic and acyanotic congenital heart disease. İndian Heart J, 2000; 52(5):559–63.
11. Prasad AN, Prasad C. Iron deficiency; non-hematological manifestations, Prog Food Nutr Sci 1991;15:225–283.
12. Gümrük F, Altay Ç. Iron metabolism and iron deficiency anemia. In: Özalp I (ed). KATKI Journal of Pediatrics 1995;3(16):265–87.
13. Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. J Nutr 2001;131(2):568–80.
14. Celkan T, Apak H, Özkan A, Bal Ş, Erener T, Çelik M, Yüksel L, Yıldız I. Prevention and treatment of iron deficiency anemia. Turkish Archive of Pediatrics. 2000;35(4):226–231.
15. Gürkan B. Evaluation of congenital heart diseases. In: Yurdakök M, Erdem G (eds). Turkish Neonatology Society, The Book of Neonatology, 1st edition. Ankara: Alp Offset; 2004.
16. Morris CD. Lessons from epidemiology for the care of women with congenital heart disease. Prog Pediat. Cardiol 19 2004;1: p.5–13.
17. Rosenthal G. Prevalence of congenital heart disease. İn: Garson A, Bricker JT, Fisher DJ, Neish SR (eds). The Science and Practice of Pediatric Cardiology, 2nd ed. Vol II. Baltimore: Williams and Wilkins, 1998.
18. Samanek M. Boy/girl ratio in children born with different forms of cardiac malformation: a population-based study. Pediatr Cardiol 15. 1994;53–57.
19. Onur CB, Sipahi T, Tavil B, Karademir S, Yoney A. Diagnosing iron deficiency in cyanotic heart disease. Indian J Pediat.

2003;70(1):29–31.
20. Nybo M, Benn M, Mogelvang R, Jensen JS, Schnohr P, Rehfeld JF, Goetze JP Impact of hemoglobin on plasma pro-B-type natriuretic peptide concentrations in the general population. Clin Chem. 2007 Nov; 53(11):1921–7. Epub 2007 Sep 14.
21. Willis MS, Lee ES, Grenache DG. Effect of anemia on plasma concentrations of NT-proBNP. Clin chim acta 2005;358:175–81.
22. Arati SD, Kirsten BD, Michael G. Association Between Anemia and N-Terminal Pro-B-Type Natriuretic peptide (NT-proBNP): Findings from the Heart and Soul Study. European Journal of Heart Failure 9 2007;886–891.
23. Mika M, Takeshi T, Yoshiro N, Masaaki LK. Anemia as a factor that elevates plasma brain natriuretic peptide concentration in apparently healthy subjects. Int Heart J 2008;49:577–586.
24. Tekin Nas, S. Clinical significance of serum nt-probnp levels in the diagnosis of heart failure and follow-up of treatment in children with iron deficiency anemia. Inonu University, Faculty of Medicine 2010:1–141.
25. Groenning BA, Nilson JC, Sondergaard L. Detection of left ventricular enlargement and impaired systolic function with plasma N-terminal pro brain natriuretic peptite concentrations. Am heart J 2002;143:923–9.