



Research Article | Araştırma Makalesi

EVALUATION OF THE PATIENTS FOLLOWED IN NEWBORN INTENSIVE CARE UNIT FOR CARDIAC DISEASES

YENİDOĞAN YOĞUN BAKIM ÜNİTESİNDE TAKİP EDİLEN HASTALARIN KALP HASTALIKLARI AÇISINDAN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Congenital Heart Diseases (CHD) constitute 0.8-1.2% of all live births. Echocardiography is frequently preferred in the diagnosis and follow-up of diseases because it is practical and noninvasive. We aimed to evaluate the frequency, types and clinical findings of congenital heart diseases in newborn babies admitted to the neonatal intensive care unit for different reasons.

Methods: The study was conducted among the patients hospitalized in neonatal intensive care unit between January 2022 and January 2023. The patients were grouped according to their gestational week, presence of murmur, cyanosis, respiratory distress, genetic anomaly, Down syndrome, maternal diabetes. The family history of consanguinity, presence of congenital heart disease were also recorded.

Results: Two hundred thirty seven patients were included in our study. Boys constituted 59% of all patients. 60.34% of the patients were between 37-42 gestational weeks. Mean age of the patients was 4.54 ± 8.90 days. Among acyanotic CHD, 30.2% had atrial septal defect (ASD), 23.6% had patent ductus arteriosus, 18.4% had ventricular septal defect (VSD). Among the cyanotic CHD, transposition of great arteries, hypoplastic left heart syndrome, truncus arteriosus were present. Reasons for requesting pediatric cardiology consultation were, in order of frequency, respiratory distress, cyanosis and murmur. 3.8% of the patients consisted of babies of diabetic mothers, and the other 3.8% were the patients with Down syndrome and genetic/congenital anomalies. The frequency of familial consanguinity was 4.2%.

Conclusion: Early detection of congenital heart diseases by using echocardiography will contribute to early interventional procedures and reduce the mortality in babies who are hospitalized in neonatal intensive care unit.

Keywords: Congenital heart disease, echocardiography, newborn

ÖZ

Amaç: Doğumsal Kalp Hastalıkları (DKH) tüm canlı doğumların %0,8-1,2'sini oluşturmaktadır. Ekokardiyografi pratik olması ve invaziv olmaması nedeniyle hastalıkların tanı ve takibinde sıklıkla tercih edilmektedir. Yenidoğan yoğun bakım ünitesine farklı nedenlerle yatan bebeklerde doğuştan kalp hastalıklarının sıklığını, tiplerini ve klinik bulgularını değerlendirmeyi amaçladık.

Yöntem: Çalışma Ocak 2022-Ocak 2023 tarihleri arasında yenidoğan yoğun bakım ünitesinde yatan hastalar arasında yapıldı. Hastalar gebelik haftası, üfürüm varlığı, siyanoz, solunum sıkıntısı, genetik anomali, Down sendromu, maternal diyabet varlığına göre gruplandırıldı. Ailede akrabalık öyküsü, doğumsal kalp hastalığı varlığı kaydedildi.

Bulgular: Çalışmamıza 237 hasta dahil edildi. Erkekler tüm hastaların %59'unu oluşturmaktaydı. Hastaların %60,34'ü 37-42 gestasyon hafta arasındaydı. Hastaların yaş ortalaması $4,54 \pm 8,90$ gündü. Asiyantotik DKH arasında %30,2'sinde atriyal septal defekt (ASD), %23,6'sında patent duktus arteriozus (PDA), %18,4'ünde ventriküler septal defekt (VSD) vardı. Siyanozlu hastalar arasında büyük arter transpozisyonu, hipoplastik sol kalp sendromu, trunkus arteriozus mevcuttu. Kardiyoloji konsültasyonu istenme nedenleri sıklık sırasına göre solunum sıkıntısı, siyanoz ve üfürümdü. Hastaların %3,8'ini diyabetik anne bebekleri, %3,8'ini ise Down sendromlu ve genetik/konjenital anomalili hastalar oluşturdu. Akraba evliliği sıklığı %4,2 idi.

Sonuç: Doğumsal kalp hastalıklarının ekokardiyografi kullanılarak erken tespiti, yenidoğan yoğun bakım ünitesinde yatan bebeklerde erken girişimsel işlemlere katkı sağlayacak ve mortaliteyi azaltacaktır.

Anahtar Kelimeler: Doğumsal kalp hastalığı, ekokardiyografi, yenidoğan

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Introduction

Congenital heart diseases (CHD) are structural or functional anomalies of the heart that occur during pregnancy. It is the most common congenital anomaly in the neonatal period.¹ Congenital heart diseases have the prevalence of 0.8-1.2% among all live births. It is around 2% among premature babies.¹ Etiology includes genetic and employment factors.² These diseases can be presented as respiratory disorders, cyanosis, and murmur, but also can be asymptomatic.^{3,4} Therefore, echocardiography plays an important role in the diagnosis. Early diagnosis is important because early diagnosis and treatment will affect morbidity and mortality if CHD is detected.^{5,6} The aim of the study is to examine the newborns in the neonatal intensive care unit of our hospital who were evaluated by echocardiography.

Methods

Among the patients hospitalized in the neonatal intensive care unit between January 2022 and January 2023, those who underwent echocardiographic examination were evaluated retrospectively. The patients were analyzed from the archive files. The patient's anamnesis, physical examination findings, reasons for cardiology consultation, echocardiographic diagnoses, presence of additional findings, parental consanguinity, presence of CHD in the family, hospitalization diagnoses, and duration of hospitalization were recorded. Patent ductus arteriosus (PDA) detected during the first 3 days and patent foramen ovale were considered normal and were excluded from the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all the patients and their parents.

Statistical Analysis

After the data was obtained from the research, SPSS 29 (Statistical Program in Social Sciences) was used for statistical analysis. Descriptive characteristics were evaluated as mean \pm standard deviation, median (min-max). The values were expressed as numbers and as percentage. Chi-square test was used to compare the data. Statistical significance level was accepted as $p < 0.05$ for all tests.

Results

60.34% of the patients were between 37-42 weeks of gestation (GW). It was followed by 34-37 GW with a frequency of 24.05%. There were patients with 21-25 GW with a rate of 0.84%. The ratio of men to women was 140/97 (Table 1). Mean age of the patients was 4.54 ± 8.90

days. The mean duration of hospital stay was 14.93 ± 19.39 days.

Table 1. Demographic findings of the patients

		n (%)
Gestation week	21-25	1 (0.84)
	26-30	12 (5.06)
	30-34	20 (9.28)
	34-37	45 (24.05)
	37-42	156 (60.34)
	>42	3 (0.42)
Gender	M/F	140/97

M: Male, F: Female

Respiratory problems related to respiratory system diseases were the most common reason for requesting pediatric cardiology consultation. The other reasons for evaluation were presence of murmur and cyanosis. The rest of evaluation requirements include prematurity, jaundice, diabetic mother's babies, presence of congenital anomaly or suspected genetic anomaly, and having a family history of congenital heart disease (Table 2).

Table 2. Reasons for requesting cardiac consultation

	n (%)
Murmur	16 (6.75)
Cyanosis	19 (8.02)
Respiratory Distress	106 (44.73)
Congenital / Genetic disorders	7 (2.95)
Arrhythmia	2 (0.9)
Prematurity	13 (5.4)
Familial consanguinity / CHD in family	10/5 (4.2/2.1)
Jaundice	15 (6.4)
Down Syndrome	2 (0.84)
Diabetic mother's baby	9 (3.8)

CHD: Congenital heart disease

Jaundice, transient tachypnea of the newborn (TTN), respiratory distress syndrome (RDS), pneumonia were the most prevalent disorders among the patients who were hospitalized (Table 3). RDS was the most common diagnosis in preterm patients, too.

Atrial septal defect (ASD) was the most common acyanotic CHD among all patients with a ratio of 30.2%, followed by PDA with a ratio of 23.6% and ventricular septal defect (VSD) with a ratio of 18.4%. ASD secundum, PDA, and VSD were also the most common CHD among preterm patients. Cyanotic CHDs include hypoplastic left

heart syndrome (HLHS), transposition of great arteries (TGA) and truncus arteriosus in our study.

Table 3. Hospitalization diagnoses at admission of inpatients

Diagnoses of the patients	n (%)
Jaundice	39 (16.46)
Transient Tachypnea of Newborn	31 (13.08)
Respiratory Distress Syndrome	28 (11.81)
Pneumonia (Congenital/Neonatal)	24 (10.13)
Sepsis	21 (8.86)
Lower Tract Respiratory Infections	17 (7.17)
Prematurity	15 (6.33)
Meconium Aspiration Syndrome	8 (3.38)
Hypoglycemia	7 (2.96)
Congenital Heart Disease	5 (2.11)
Convulsion	5 (2.11)
Omphalitis	4 (1.69)
Upper Respiratory Tract Infection	4 (1.69)
Enteral Nutrition Problems	4 (1.69)
Conjunctivitis	3 (1.27)
Thrombocytopenia	1 (0.42)
Metabolic Disease	3 (1.27)
Gastroenteritis	2 (0.84)
Congenital Adrenal Hyperplasia	2 (0.84)
Polycythemia	2 (0.84)
Abscess	1 (0.42)
Substance Use of Mother	1 (0.42)
Asphyxia	1 (0.42)
Anomaly of Eye	1 (0.42)
Hypertremic Dehydration	1 (0.42)
Atresia of Ileum	1 (0.42)
Ischemic Trombosis	1 (0.42)
Urinary Tract Infection	1 (0.42)
Congenital Toxoplasmosis	1 (0.42)
Macrosomia	1 (0.42)
Pneumothorax	1 (0.42)
Cleft palate	1 (0.42)

Considering the weeks of gestation, the most common acyanotic diseases included VSD, PDA and ASD secundum and were most frequently prevalent at 37-42 gestation week. At 37-42 GW, coarctation of the aorta was detected in 1 patient and TGA in 1 patient. HLHS was detected in 1 patient between 34-37 GW. Truncus arteriosus was detected in 1 patient between 30-34 GW (Table 4).

The study included 24 acyanotic preterm and 41 acyanotic term patients, while there was 2 cyanotic preterm and 1 cyanotic term patients. Congenital heart diseases were detected in a total of 26 preterm patients and 42 term patients among the patients included in the study.

Discussion

Early diagnosis and management of critical CHD is important to avoid heart failure, cardiovascular collapse and sudden death, therefore echocardiographic evaluation has become an important method for evaluation.⁷ CHD constitutes one third of all congenital anomalies.⁸ In the literature, the frequency of CHD differs between 1.6%-11.7%.⁹⁻¹¹ Karapınarlı et al.⁴, declared that the frequency of CHD among newborn patients was 13.2%. Yalaki et al.³ reported that 31.1% of the patients were preterm and 53.7% were male in their study. Varal et al.¹² also stated similar results. In our study, 32.9% of our patients were preterm and %59 were male. Bulut et al.¹³ stated that the most common reason for consultation was murmur in their study.

Şap et al.¹⁴ stated that, most commonly respiratory diseases, afterwards infectious diseases, gastrointestinal system related diseases and jaundice were the reasons for hospitalization among newborns.¹⁰ Karapınarlı et al.⁴ and Zan et al.¹¹ reported, respiratory distress (tachypnea, retraction, etc.) as the main reason for cardiac evaluation of the patients like in our study. Ertürk et al.⁵ declared that murmur was the most common reason for evaluation with a rate of 50%. The rate of murmur was 27.4% in the study of Karapınarlı et al.⁴, 39.3% in the study of Bulut et al.¹³. The frequency of murmur was lower in our study than the studies mentioned and the most common reason for cardiac evaluation was respiratory distress instead of murmur. Şap et al.¹⁴ and Farrer et al.¹⁵ stated that CHD was also present in the patients without murmur. We supported this finding by showing that congenital heart diseases were reported in many patients who did not have murmur.

The most common CHD detected in the literature was VSD with a rate of 25-30%, followed by ASD.^{4,5} Şimşek et al.⁹ also detected VSD most frequently, followed by ASD and PDA. We detected ASD secundum more frequently, followed by PDA and afterwards VSD. Among acyanotic CHD, Karapınarlı et al.⁴ declared that the most common CHD was ASD, followed by PS, afterwards PDA and VSD like our study. The frequency of acyanotic CHD is 96.2%, and the rate of consultation due to cyanosis is 6.6% in the literature.⁴ In our study, the frequency of congenital

cyanotic CHD was found to be 1.26 %. Shah et al.¹⁶ stated the rate of acyanotic CHD to be 69%, and that of cyanotic 31% similar with the study of Aydoğdu et al.¹⁷ The frequency of cyanotic CHD was 3.8%, and HLHS had the highest ratio in the study of Karapınarlı et al.⁴ In the studies of Ekici et al.¹⁸, and Gürakan et al.¹⁹ transposition of great arteries was declared more frequently than other cyanotic diseases. In the study of Şimşek et al.⁹, the frequency of cyanotic CHD was 9.4%, while in our study, the frequency of cyanotic CHD was 1.26%. This difference can be attributed to the lack of a cardiovascular surgery

clinic in our hospital and referring the patients requiring surgery to other institutions. Also the patient population in the intensive care unit was more likely to have respiratory distress, jaundice, prematurity and metabolic or genetic disorders. Therefore, the patients with antenatal or postnatal diagnosis of CHD requiring intervention or surgery were transported to other hospitals. Cyanotic CHD included HLHS, TGA, Truncus arteriosus without a predominance of TGA.

Table 4. Distribution of the cardiac diseases according to gestational week

Gestational week	21-25 (n)	25-30 (n)	30-34 (n)	34-37 (n)	37-42 (n)	>42 (n)
VSD	0	1	3	2	12	0
PDA	1	1	0	7	10	1
PS	0	1	0	0	0	0
ASD secundum	0	3	1	6	13	1
HLHS	0	0	0	1	0	0
TGA	0	0	0	0	1	0
Truncus arteriosus	0	0	1	0	0	0
HCMP	0	0	0	1	0	0
PPS	0	0	0	1	0	1
PHT	0	0	1	0	0	0
AC	0	0	0	0	1	0
AA dilatation	0	0	1	0	0	0
Coronary artery fistula	0	0	0	0	1	0
BAV	0	0	0	0	1	0
Septal Hypertrophy	0	0	0	1	0	0

AA: Ascending aorta, AC: Aortic coarctation, ASD: Atrial septal defect, BAV: Bicuspid aortic valve, HCMP: Hypertrophic cardiomyopathy, HLHS: Hypoplastic left heart syndrome, PDA: Patent ductus arteriosus, PPS: Peripheral pulmonary stenosis, PS: Pulmonary stenosis, TGA: Transposition of great arteries, VSD: Ventricular septal defect

Mortality rates in cases with congenital heart disease vary according to different hospitals. The most common causes of death were recorded as heart failure, sepsis and pneumonia.^{11,13} Karapınarlı et al.⁴ stated that the most common causes of death were sepsis, cardiac causes, disseminated intravascular coagulation, and metabolic crisis. The mortality rates in the study of Tokel et al.²⁰ was 23.6%. The high mortality rates in the study of Tokel et al.²⁰ were attributed to the fact that pediatric cardiac operations and interventions were performed with a high frequency in their hospital. Karapınarlı et al.⁴ reported that the mortality rates of CHD in their study was 6.8%. Cardiac causes included septic myocarditis, arrhythmia, pulmonary hypertension, heart failure. Prematurity, chromosomal or extracardiac anomalies,

and complications related to ventilatory treatment also played important role in the mortality. In our study, the mortality rate was 4.4%. The reasons for mortality were sepsis, heart failure and the secondary problems related with prematurity. The relatively low rate of mortality in our study can be associated with the absence of a cardiovascular surgery clinic, therefore referring the patients with antenatally and postnatally diagnosed important CHD to other hospitals.

Development of CHD is related with some environmental and maternal factors.²¹ Asymmetric septal hypertrophy, VSD, ASD, PDA can be present in the babies of diabetic mothers. Consanguinity in the family and family history of CHD also increase the risk for CHD.^{4,5} Consanguinity in the family was found at a rate of 15.3% in the literature.⁵

Ertürk et al.⁵ declared familial consanguinity with a rate of 15.3%. Down syndrome is highly associated with CHD with a ratio of 50%.²¹ Reinhold et al.²² stated that Down syndrome had a ratio of 1.4% among the patients with CHD. Atrioventricular canal defect, VSD, ASD are frequently detected in patients with Down syndrome.^{4,5} In the studies conducted, presence of CHD in the family was found at a rate of 3.6-4.3%.⁵ In our study, the ratio of diabetic mother's babies was 3.8%, familial CHD was 2.1%, familial consanguinity was 4.2%, and Down syndrome was 0.8%. 4 of the patients with family consanguinity include ASD or VSD. Congenital heart diseases are quite common in newborns. Acyanotic congenital heart diseases are more frequent than the others. Cardiac evaluation is requested more frequently in the patients with respiratory problems, while the rate of CHD is higher in the patients who were evaluated for cyanosis and murmur. Diagnosis of CHD in the neonatal period is more difficult due to hemodynamic differences, but early diagnosis and treatment are important, therefore evaluation with echocardiography has become the gold standard method for this purpose.

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Health Sciences University, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (decision number: 2023-05-11, date: 06.03.2023) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all the patients and their parents.

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

All the authors equally contributed to this work.

Financial Disclosure

None

References

- Bernstein D. Congenital heart disease. In: Behrman RE, Kliegman RM, Jenson HB, editors. *Nelson text book of pediatrics*. 18th ed. Philadelphia: Saunders; 2007:1878-1942.
- Kliegman RM. In: Kliegman RM, Stanton B, St. Geme J, Schor N, editors. *Nelson Textbook of Pediatrics*. 20th ed. Philadelphia: Elsevier 2016;2182-7.
- Yalaki Z, Çiftçi N, Kara S, Arıkan Fİ, Dallar YB. Yenidoğan Yoğun Bakım Ünitesinde İzlenen Bebeklerde Saptanan Konjenital Kalp Hastalıklarının Retrospektif Değerlendirilmesi. *Turkish J Pediatr Dis*. 2017;3:179-182. doi:10.12956/tjpd.2017.268
- Karapınarlı A, Kibar Gül AE, Ünal S, et al. Retrospective Evaluation of Congenital Heart Disease Cases Diagnosed in Neonatal Service and Intensive Care Unit. *Turkish J Pediatr Dis*. 2022;16:191-199. doi:10.12956/tchd.902641
- Ertürk EY, Küçüködük Ş, Baysal K, Ayyıldız P, Yılmaz A, Oğur G. A Retrospective Evaluation of the Patients with Congenital Heart Disease in Neonatal Intensive Care Unit. *J Curr Pediatr*. 2016;14:67-73. doi:10.4274/jcp.02486.
- Pinto Júnior VC, Branco KM, Cavalcante RC, et al. Epidemiology of congenital heart disease in Brazil. *Rev Bras Cir Cardiovasc*. 2015;30:219-224. doi:10.5935/1678-9741.20150018
- Mertens L, Friedberg MK. The gold standard for noninvasive imaging in congenital heart disease: echocardiography. *Curr Opin Cardiol*. 2009;24:119-124. doi:10.1097/HCO.0b013e328323d86f
- Bernier PL, Stefanescu A, Samoukovic G, Tchervenkov CI. The challenge of congenital heart disease worldwide: epidemiologic and demographic facts. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu*. 2010;13:26-34. doi:10.1053/j.pcsu.2010.02.005
- Şimşek A, Baysal B. Yenidoğan Yoğun Bakım Ünitesi'nde Doğumsal Kalp Hastalığı Sıklık ve Dağılımı: Tek Merkez Deneyimi. *Ortadoğu Medical Journal*. 2019;11:446-449. doi:10.21601/ortadogutipdergisi.525745
- Güven H, Bakiler AR, Kozan M, Aydınlioğlu H, Helvacı M, Dorak C. Yenidoğan servislerinde konjenital kalp hastalıkları. *Çocuk Sağlığı ve Hastalıkları Dergisi*. 2006;49:8-11.
- Zan S, Yapıcıoğlu H, Erdem S, et al. Çukurova Üniversitesi Tıp Fakültesi Hastanesi Yenidoğan Yoğun Bakım Ünitelerinde son beş yılda izlenen konjenital kalp hastalarının retrospektif incelenmesi. *Çocuk Sağlığı ve Hastalıkları Dergisi*. 2015;58:7-16.
- Varal İG, Köksal N, Özkan H. Yenidoğan Yoğun Bakım Ünitelerinde İzlenen Konjenital Kalp Hastalıkları: Sıklığı, Risk Faktörleri ve Prognoz. *J Curr Pediatr*. 2015;13:15964. doi:10.4274/jcp.18291
- Bulut G, Ballı Ş, Atlıhan F, Meşe T, Çalkavur Ş, Olukman Ö. Yenidoğan servisinde izlenen doğumsal kalp hastalığı olanların retrospektif değerlendirilmesi. *İzmir Dr Behçet Uz Çocuk Hast Dergisi*. 2012;2:141-7. doi:10.5222/buchd.2012.141
- Şap F, Baysal T, Karataş Z, Altın H, Alp H, Karaarslan S. Yenidoğan döneminde duyulan üfürümün doğuştan kalp hastalığını saptamadaki önemi. *Yeni Tıp Dergisi*. 2012;29:80-3.
- Farrer KF, Rennie JM. Neonatal murmurs: are senior house officers good enough? *Arch Dis Child Fetal Neonatal Ed*. 2003;88:147-51. doi:10.1136/fn.88.2.F147
- Shah GS, Singh MK, Pandey TR, Kalakheti BK, Bhandari GP. Incidence of congenital heart disease in tertiary care hospital. *Kathmandu Univ Med J*. 2008;6:33-36.
- Ekici F, Ünal S, Dablan S, Alpan N, Şaylan Çevik B, Vidinlisan S. Yenidoğan yoğun bakım ünitesindeki 119 bebeğin klinik ve ekokardiyografik değerlendirilmesi. *Turkish J. Pediatr Dis*. 2010;4:22-29.
- Gürakan B. Konjenital kalp hastalıklarının değerlendirilmesi. içinde: Yurdakök M, Erdem G, (eds). *Türk Neonatoloji Derneği Neonatoloji Kitabı*, 1. baskı. Ankara: Alp Ofset; 2004:503-512.
- Gül Ö, Gürses D, Doğan M. Down sendromlu çocukların klinik ve ekokardiyografik değerlendirilmesi. *Turkish J. Pediatr Dis*. 2017;11:116-119. doi:10.12956/tjpd.2016.263
- Tokel K SA, Mercan S, Varan B, Gürakan B, Aşlamacı S. Doğumsal kalp hastalıklı yenidoğanların değerlendirilmesi. *Türk Pediatri Arşivi*. 2001;36:138-143.

21. Brennan P, Young ID. Congenital heart malformations: etiology and associations. *Semin Neonatol.* 2001;6:17-25.doi: 10.1053/siny.2000.0032
22. Reinhold-Richter L, Fischer A, Schneider-Obermeyer J. Congenital heart defects. Frequency at autopsy. *Zentralbl Allg Pathol.* 1987;133:253-261.