



First trimester fetal aortic Doppler for hemoglobinopathies

Hemoglobinopatiler için ilk trimesterde fetal aortik Doppler

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Abstract

Objective: To evaluate fetal aortic Doppler for the prenatal diagnosis of hemoglobinopathies in the first trimester of pregnancy.

Materials and Methods: Between January and November 2014, a total of 108 patients were enrolled in the study. The couples were carriers of either alpha/beta thalassemia, sickle cell disease or combined carriers of these and were admitted to Çukurova University Faculty of Medicine, Department of Obstetrics and Gynecology Prenatal Diagnosis Center. One hour before the chorionic villus sampling (CVS), patients were evaluated using fetal aortic Doppler. Pulsatility index, peak systolic velocity, and heart rate were noted.

Results: There were no statistically significant differences in Doppler indices between different groups of CVS results when compared with the healthy controls.

Conclusion: Fetal aortic Doppler investigation was found to be ineffective for the prenatal diagnosis of hemoglobinopathies.

Keywords: Hemoglobinopathies, thalassemia, sickle cell anemia, prenatal diagnosis, Doppler ultrasonography

Öz

Amaç: Gebeliğin ilk üç ayında hemoglobinopatilerin prenatal tanısı için fetal aortik Doppler incelemesinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Ocak 2014 ve Kasım 2014 tarihleri arasında 108 hasta çalışmaya dahil edildi. Çiftler, Çukurova Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum Bölümü Prenatal Tanı Merkezi'ne alfa/beta talassemi, orak hücre anemisi veya bunların kombinasyonu olup başvuran kişilerden oluşmaktadır. Kordonik villüs örneklemesinden (KVÖ) bir saat önce fetal aortik Doppler incelemesi yapıldı. Pulsatilit indeksi, tepe sistolik hız, kalp hızı not edildi.

Bulgular: Farklı KVÖ sonucu grupları sağlıklı kontrollerle karşılaştırıldıklarında Doppler indeksleri açısından istatistiksel olarak anlamlı fark bulunmamıştır.

Sonuç: Fetal aortik Doppler incelemesi hemoglobinopatilerin prenatal tanısında etkin bulunmamıştır.

Anahtar Kelimeler: Hemoglobinopatiler, talassemi, orak hücreli anemi, prenatal tanı, Doppler ultrasonografi

Introduction

A hemoglobinopathy is a genetic defect that results in abnormal structure of one of the globin chains of the hemoglobin molecule. There are various types of defects known today. They are single gene disorders and generally inherited in an autosomal recessive pattern. The most common are beta/alpha-thalassemia and sickle cell disease.

These diseases have different prevalences around the world. Beta-thalassemia is particularly prevalent among Mediterranean people, whereas alpha-thalassemia is common in sub-Saharan Africa, the Mediterranean Basin, the Middle East, South Asia, and Southeast Asia. Sickle cell disease is found most prevalently in sub-Saharan Africa, tribal regions of India, and the Middle-East⁽¹⁾. Therefore, these hemoglobinopathies are important health problems for Turkey because of its geographic location. The heterozygote carriers of these diseases show some degrees

of anemia, whereas homozygote mutation carriers exhibit significant health problems. They require intermittent blood transfusions and have many complications such as increased incidence of infections, cholelithiasis (gallstones), cholecystitis, and growth problems during childhood. Carriers also face complications related to treatment such as excess iron load and related complications in thalassemias and other complications of frequent blood transfusions. Bone marrow transplantation is the only known cure for these diseases.

Prenatal diagnosis is very important for carrier couples. Early prenatal diagnosis may provide the chance of early termination for these couples during the first trimester. Current approaches for prenatal diagnosis include invasive [chorionic villus sampling (CVS), amniocentesis or cordocentesis] and non-invasive diagnosis with fetal DNA in maternal plasma⁽²⁻⁴⁾.

Alterations in fetal blood parameters in hemoglobinopathies may cause changes in fetal Doppler parameters. Therefore,

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prenatal diagnosis using Doppler ultrasound may be another noninvasive method for the carriers. In this study we aimed to evaluate the indices of fetal aortic Doppler for the non-invasive diagnosis of hemoglobinopathies.

Materials and Methods

One hundred eight patients were enrolled in this prospective study between January 2014 and November 2014. All patients and their husbands were carriers of either alpha/beta thalassemia, sickle cell disease or combined carriers of these. They were admitted to Çukurova University Faculty of Medicine, Department of Obstetrics and Gynecology Prenatal Diagnosis Center for the prenatal diagnosis of hemoglobinopathies. They all had CVS during 11 and 14 weeks of their pregnancy. Gestational age was based on crown-lump length (CRL). Ethical Committee Approval was obtained from Çukurova University Clinical Research Ethics Committee (Report no: 28/14) and all subjects gave informed consent to participate in the study. One hour before the CVS, patients were examined using a GE Voluson 730 Pro-ultrasound machine with a convex volumetric transabdominal (RAB 4-8 MHz) probe by a single experienced operator. In order to achieve an insonation angle of <30 degrees, fetuses were examined in a position where the fetal aorta was perpendicular to the probe (Figure 1). The magnification of the image was such that the fetus occupied the whole screen. The range gate was set at 2 mm. Pulsatility index, peak systolic velocity (PSV), and heart rate (HR) were noted.

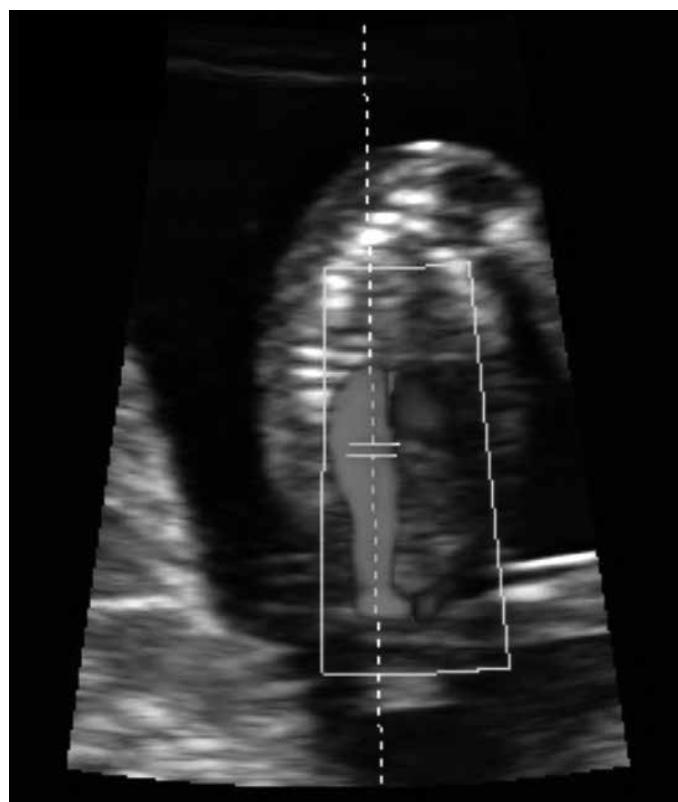


Figure 1. First trimester fetal aortic Doppler

After the ultrasound examination, CVS was performed. Fetal karyotype was also determined for all fetuses. Patients were divided into groups by the results of the hemoglobinopathy status. Therefore, the operator was blinded to the patients' groups.

Student's t-test was used for normally distributed data, and the Mann-Whitney U test was used to compare non-normally distributed data. The p value of <0.05 was considered as statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 15 (SPSS, Inc., Chicago, IL).

Results

The mean age of the patients was 28.34 ± 5.2 years (range, 17-40 years). The mean gestational age was 12 ± 0.8 weeks (range, 11-14 weeks) and the mean CRL of the fetuses was 59.90 ± 9.6 mm (range, 41-85 mm). There was no statistically significant differences between the groups regarding the mean age of the women and CRL of fetuses based on CVS results. Eight women were Rh negative but none were isoimmunized. Hemoglobinopathy status of the couples is given in Table 1. All CVS procedures were completed without complication. The karyotypes of all fetuses were normal. Hemoglobinopathy results and the Doppler indices of the fetuses are summarized in Table 2. There were no statistically significant differences in Doppler indices between the different groups of CVS results compared with the healthy (Hemoglobin AA) controls. Comparisons were made in subcategories of gestational weeks (11, 12, 13, and 14 weeks), because Doppler indices normally differ with advancing gestational age. Furthermore, there was no statistically significant difference between subgroups as for fetal HR (mean fetal HR = 165.01 ± 3.1 beat/min).

All patients whose fetuses were diagnosed as having sickle cell disease and beta-thalassemia major decided to terminate pregnancy, all were terminated without complication using sublingual misoprostol.

Discussion

Fetal arterial and venous Doppler evaluations are used effectively in different trimesters. For instance, fetal ductus venosus Doppler

Table 1. Hemoglobinopathy status of the couples

Hemoglobinopathy	Number of couples
BT carriers	21
SCD carriers	55
SCD*	1
AT carriers	2
Combined (AT + SCD) carriers	9
Combined (BT + SCD) carriers	20
Total	108

BT: Beta thalassemia, SCD: Sickle cell disease, AT: Alpha thalassemia, *: 1 patient had SCD (Hemoglobin SS) and her husband was SCD carrier.

Table 2. Chorionic villus sampling results and doppler indices of fetuses

CVS Result*	Gestational week	Number of fetuses	PI	PSV
HBAA (Healthy)	Total	22	2.92±0.35	41.87±8.56
	11	3	3.06±0.22	35.80±3.02
	12	12	3.05±0.38	39.73±6.87
	13	6	2.62±0.14	49.29±10.07
	14	1	2.7	41.3
HBAS	Total	34	8.85±15.03	34.98±14.85
	11	7	2.94±0.18	40.91±4.28
	12	21	8.06±13.10	34.38±14.6
	13	4	12.69±20.34	32.09±20.51
	14	2	30.16±39.40	26.27±34.65
HBSS	Total	21	9.16±13.31	33.70±16.64
	11	7	2.98±0.15	37.88±4.80
	12	8	19.34±17.68	22.07±21.05
	13	5	2.65±0.22	44.51±10.22
	14	1	2.40	43.44
BT Minor	Total	13	7.82±18.34	38.06±13.09
	11	3	24.76±38.19	24.95±18.94
	12	8	2.82±0.25	43.01±9.43
	13	1	2.51	36.45
	14	1	2.34	39.41
BT Major	Total	5	2.86±0.26	38.02±5.18
	11	2	3.08±0.11	38.72±3.66
	12	3	2.72±0.22	37.55±6.79
	13	0	-	-
	14	0	-	-
AT silent carrier	Total	4	14.03±22.64	31.83±19.79
	11	0	-	-
	12	2	2.89±0.15	41.69±3.07
	13	2	25.17±32.27	21.97±27.87
	14	0	-	-
S/BT	Total	3	2.76±0.57	43.09±8.57
	11	1	2.86	49.14
	12	1	3.27	33.28
	13	1	2.15	46.85
	14	0	-	-
S/AT	Total	4	10.87±16.01	32.53±20.46
	11	0	-	-
	12	2	2.79±0.16	40.93±4.30
	13	2	18.96±22.53	24.12±30.90
	14	0	-	-

Table 2 continue. Chorionic villus sampling results and doppler indices of fetuses

CVS Result*	Gestational week	Number of fetuses	PI	PSV
BT/AT	Total	2	2.59±0.49	46.47±7.30
	11	1	2.93	41.30
	12	0	-	-
	13	1	2.24	51.63
	14	0	-	-
Total		108		

* HBAA: Healthy fetuses, HBAS: Sickle cell carrier, BT: Beta thalassemia, HBSS: Sickle cell disease, AT: Alpha thalassemia, S/BT: Sickle cell/Beta thalassemia double heterozygote, S/AT: Sickle cell/Alpha thalassemia double heterozygote, BT/AT: Alpha thalassemia/Beta thalassemia double heterozygote, PI: Pulsatility index, PSV: Peak systolic velocity, CVS: Chorionic villus sampling

is found effective in screening for aneuploidies during the first trimester, whereas it is used for fetal well-being from the mid-trimester. The other most commonly used Doppler evaluations include umbilical, uterine, and middle cerebral arteries; each has a different purpose such as fetal well-being, preeclampsia screening, and detection of fetal anemia. Of these, middle cerebral artery PVS is used for the detection of fetal anemia but it is technically used after the 18th week of gestation⁽⁵⁾.

Fetal anemia can be expected in hemoglobinopathies, especially in homozygous alpha-thalassemia⁽⁶⁾. During the first trimester, fetal aortic Doppler is found to have significantly different indices in fetuses with homozygous alpha-thalassemia⁽⁶⁾. In a recent study by Karateke et al.⁽⁷⁾ ductus venosus Doppler was also found to have significantly different indices during the first trimester in fetuses with beta-thalassemia and sickle cell disease⁽⁷⁾.

To the best of our knowledge, this is the first study to investigate fetal aortic Doppler for the prenatal diagnosis of beta-thalassemia and sickle cell disease during the first trimester. We chose fetal aorta for screening because it can be easily visualized during this period. We were able to visualize the fetal aortic blood flow in all patients with transabdominal probe. No transvaginal evaluation was needed.

We found no significant difference in fetuses with beta-thalassemia and sickle cell disease in arterial Doppler parameters during the first trimester compared with the healthy fetuses. Therefore, current fetal aortic Doppler was not found effective for the prenatal diagnosis of these hemoglobinopathies.

Fetal hemoglobin is a tetramer composed of two copies of each of two different peptide chains. The type of chains determine the type of hemoglobin produced. Adult hemoglobin consists of two beta and two alpha chains; however, during embryonic and fetal stages there are various types of hemoglobins. The first fetal hemoglobins are produced in the yolk sac and are called hemoglobin (Hb) Gower 1 (two zeta chains and two epsilon chains), Hb Gower 2 (two alpha chains and two epsilon chains), and Hb Portland (two zeta chains and two gamma chains)⁽⁸⁾. Erythropoiesis then moves to the liver, where Hb F (two alpha and two gamma chains) is produced. Finally, at around 11 weeks, normal Hb A is produced by fetal bone marrow and progressively increases in fetal blood as the fetus matures⁽⁹⁾. The final adult version of the alpha chain is produced

exclusively by 6 weeks and there are no functional alternative versions thereafter⁽¹⁰⁾. If an alpha gene mutation or deletion occurs, there is no alternate alpha type chain that can substitute to form functional hemoglobin. In contrast, at least two versions of the beta chain (gamma and delta) remain in production throughout fetal life and beyond. In the case of a beta gene mutation or deletion, these two other versions of the beta chain often continue to be produced, which results in Hb A2 or Hb F and substitute for the abnormal or missing hemoglobin⁽¹⁰⁾. The reason that we found no hemodynamic change in fetuses with hemoglobinopathy was probably because of these substitute hemoglobins. In fetuses with hemoglobinopathy, substitute hemoglobins (Hb A2 and Hb F) conceal the disease during fetal life, but the disease manifests after birth. However, fetuses with alpha gene defect may even manifest during fetal life if all three or four genes are affected, because of the lack of substitute chains⁽¹¹⁾. We had no fetuses with alpha thalassemia major (Hb Barts or Hb H disease) in our study.

The main limitation of our study is the small number of patients in some subgroups of patients, such as alpha-thalassemia and combined heterozygotes of hemoglobinopathies.

Conclusion

Currently, invasive prenatal diagnostic tests such as CVS, amniocentesis or cordocentesis are the most widely used invasive tests for hemoglobinopathies⁽¹²⁾. Non-invasive diagnostic tests using fetal DNA in maternal plasma shows promising results; however, they are expensive and not yet widely available around the world. Therefore, non-invasive prenatal diagnostic tests like Doppler ultrasound investigations should still be continued.

Ethics

Ethics Committee Approval: The study were approved by Çukurova University Clinical Researchs Ethical Committee, *Informed Consent:* Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Cihan Çetin, *Concept:* Cihan Çetin, Selim Büyükkurt, Mete Sucu, Cansun Demir, *Design:* Cihan Çetin, Selim Büyükkurt, Mehmet Özsürmeli, Cansun Demir, *Data*

Collection or Processing: Cihan Çetin, Ebru Dündar Yenilmez,
Analysis or Interpretation: Cihan Çetin, Mehmet Özşürmeli,
Literature Search: Cihan Çetin, Ebru Dündar Yenilmez, Mehmet Özşürmeli, Mete Sucu, *Writing:* Cihan Çetin, Selim Büyükkurt, Mete Sucu.

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References

1. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ 2001;79:704-12.
2. Yenilmez ED, Tuli A, Evrûke IC. Noninvasive prenatal diagnosis experience in the Çukurova region of southern Turkey: detecting paternal mutations of sickle cell anemia and beta-thalassemia in cell-free fetal DNA using high-resolution melting analysis. Prenat Diagn 2013;33:1054-62.
3. D'Souza E, Sawant PM, Nadkarni AH, Gorakshakar A, Ghosh K, Colah RB. Detection of fetal mutations causing hemoglobinopathies by non-invasive prenatal diagnosis from maternal plasma. J Postgrad Med 2013;59:15-20.
4. Xiong L, Barrett AN, Hua R, Tan TZ, Ho SS, Chan JK, et al. Non-invasive prenatal diagnostic testing for beta-thalassaemia using cell-free fetal DNA and next generation sequencing. Prenat Diagn 2015;35:258-65.
5. Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, et al. Noninvasive diagnosis by doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative group for doppler assessment of the blood velocity in anemic fetuses. N Engl J Med 2000;342:9-14.
6. Lam YH, Tang MH, Lee CP, Tse HY. Cardiac blood flow studies in fetuses with homozygous alpha-thalassemia-1 at 12-13 weeks of gestation. Ultrasound Obstet Gynecol 1999;13:48-51.
7. Karateke A, Silfeler DB, Güngören A, Kurt RK, Okyay AG, Dokuyucu R, et al. Can the ductus venosus doppler predict the hemoglobinopathies? Int J Clin Exp Med 2015;8:4405-9.
8. He Z, Russell JE. Expression, purification, and characterization of human hemoglobins gower-1 ($\zeta(2)\epsilon(2)$), gower-2 ($\alpha(2)\epsilon(2)$), and portland-2 ($\zeta(2)\beta(2)$) assembled in complex transgenic-knockout mice. Blood 2001;97:1099-105.
9. Pataryas HA, Stamatoyannopoulos G. Hemoglobins in human fetuses: evidence for adult hemoglobin production after the 11th gestational week. Blood 1972;39:688-96.
10. Cunningham FG, Williams JW. Fetal growth and development; Williams obstetrics. New York: McGraw-Hill Professional; 2005. p. 104-5.
11. Piel FB, Weatherall DJ. The alpha-thalassemias. N Engl J Med 2014;371:1908-16.
12. Mümüşoğlu S, Sayal B, Beksaç S. Talasemi ve hemoglobinopatilerde prenatal tanı yöntemleri. Turkiye Klinikleri J Hem Onc Special Topics 2010;3:40-3.