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# In response to "Role of Fetal Blood Sampling in the Prenatal Diagnosis of Thalassemia"

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**Dear Editor,**

We appreciate the letter's author's considerate, illuminating ideas and recommendations for our article. We appreciate the chance to address the concerns highlighted and clarify our research.[1] As the authors stated, hemoglobinopathies are one of the leading health problems in Turkey.[2] Within the scope of the national hemoglobinopathy prevention program, in provinces with a high prevalence since the 2000s; It is applied in the form of prenatal screening programs and prenatal diagnosis.[3,4] Firstly, we should note that since the mid-1990s, prenatal diagnosis of hemoglobinopathies has been performed at the molecular level by Chorionic villus sampling (CVS), and in some cases cordocentesis, in collaboration with the Medical Faculty, Department of Obstetrics and Gynecology (Our laboratory does not diagnose thalassemia with amniocentesis sampling. Another department in our hospital uses amniocentesis to diagnose trisomies). Families that applied for prenatal diagnosis of hemoglobinopathies at our gynecology department are consulted to our medical biochemistry molecular laboratory unit for molecular analysis. high performance liquid chromatography (HPLC) is used to determine hemoglobin (Hb) fractions in samples to seperate hemoglobins, and molecular diagnostic methods are used for determining the mutation profiles of the parents. We use Amplification Refractory Mutation System, RE-Analysis and multiplex polymerase chain reaction (PCR) to detect the most frequently observed mutations. At a scientific level, we try to resolve cases with suspected thalassemia but whose mutations cannot be detected by classical molecular methods by performing gene expression analysis in addition to qPCR and Sanger sequencing. The genotype of the CVS sample collected by the perinatologist is analyzed and the results are shared with the relevant departments in addition to providing genetic counseling and information to the families. Second, as the authors pointed out, we drew attention in our publication to fetal blood collection, which was often used in laboratories prior to the oldest prenatal diagnostic methods.[5] In reality, we attempted to demonstrate that these strategies can still be employed extremely effectively when necessary. Especially analysis of fetal HbS using automated HPLC system has been used as another alternative method when DNA analysis is not routinely performed and mutations of the parents are unknown.[6] In 129 cord blood samples, hematological data from fetuses with a wide range of mutations were analyzed. Cord blood samples taken by perinatologists from fetuses of couples whose mutations had not yet been determined in the laboratory (mutation profiles have changed with the effect of Syrian immigrants in our region for the last 10 years, the frequency of frequently observed mutations has decreased, and the mutation profile has changed). CVS was administered to the fetuses of couples harboring the most frequent mutations in our region. As the authors indicated, cord blood collection was not used for all prenatal diagnostic samples. Approximately 5000 samples with prenatal diagnosis were collected during the study period. Only 129 of them had cordocentesis, and the rest had their molecular investigations done through CVS sampling. Another aspect that the authors wished to emphasize why was the Hb levels low in beta thalassemia major fetuses. We agree with the authors that the beta gene is inactive throughout pregnancy and that its levels should not be low.[7] We know that in the presence of an alpha mutation, low data is feasible.[8] Although we were aware of the authors' legitimate comments and questions on this issue, we tried to examine the samples using gene expression and Multiplex ligation-dependent probe amplification analyses that we did later. However, we could not reach a decision to solve the problem because the methods of solving the cases did not provide sufficient data. There was no way to collect samples from these cases again after that time. From this point of view,

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perhaps it would have been better to exclude these cases. I would like to add to the authors question regarding why cord blood sample was done when improved procedures were available. We all agree that technology and molecular DNA methods have evolved significantly in recent years.[9] Why did we submit a study uses cord blood sampling, why not another method? In the availability of fully automated systems and techniques, such as cation-exchange HPLC, have led a large proportion of laboratories, replacing cellulose acetate electrophoresis as a first-line screening method mentioned Ryan et al.[4] in the guideline for screening hemoglobinopathies. The issue we intended to emphasize was that HbA levels and other hematological parameters detection in cord blood are the key relevant signs in cases of thalassemia major. The primary reason for presenting these statistics in this study is to provide information that can guide molecular laboratories with limited opportunity. In the event that the molecular DNA methods are insufficient (the most advanced methods may not be available in every laboratory), or if the mutation of one of the couples cannot be determined, fetal hematological data obtained from the cord blood sample can be used to evaluate the fetus' genotype.

## REFERENCES


1. Dündar Yenilmez E, Tuli A. Cord Blood Hematological Parameters of Fetuses Detected Different Thalassemia Genotypes in the Second Trimester of Pregnancy. *Balkan Med J.*2023;40:279-86. **[CrossRef]**
2. Yuregir G, Arpaci A, Aksoy K, Tuli A, Dikmen N, Ozgunen F, et al. Population at risk for hemoglobinopathies in Cukurova, Turkiye: need for prenatal diagnosis. 1995. **[CrossRef]**
3. Cao A, Galanello R, Rosatelli MC. 8 Prenatal diagnosis and screening of the haemoglobinopathies. *Baillieres Clin Haematol.* 1998;11:215-238. **[CrossRef]**
4. Ryan K, Bain B, Worthington D, et al. Significant haemoglobinopathies: guidelines for screening and diagnosis. *Br J Haematol.* 2010;149:35-49. **[CrossRef]**
5. Srisupundit K, Wanapirak C, Sirichotiyakul S, et al. Fetal red blood cell hematology at mid-pregnancy among fetuses at risk of homozygous β-thalassemia disease. *J Pediatr Hematol Oncol.* 2013;35:628-630. **[CrossRef]**
6. Srivorakun H, Fucharoen G, Sae-Ung N, Sanchaisuriya K, Ratanasiri T, Fucharoen S. Analysis of fetal blood using capillary electrophoresis system: a simple method for prenatal diagnosis of severe thalassemia diseases. *Eur J Haematol.* 2009;83:57-65. **[CrossRef]**
7. Thein SL. 6 β-Thalassaemia. *Baillieres Clin Haematol.* 1993;6:151-175. **[CrossRef]**
8. Srisupundit K, Piyamongkol W, Tongsong T. Comparison of red blood cell hematology among normal, α-thalassemia-1 trait, and hemoglobin Bart's fetuses at mid-pregnancy. *Am J Hematol.* 2008;83:908-910. **[CrossRef]**
9. Hashiloni-Dolev Y, Nov-Klaiman T, Raz A. Pandora's pregnancy: NIPT, CMA, and genome sequencing-A new era for prenatal genetic testing. *Prenat Diagn.* 2019;39:859-865. **[CrossRef]**