

Original Article

Negative Predictive Value of the Chorionic Villous Sampling (CVS) in Diagnosis of Thalassemia in Genetic Laboratory of Dastgheib Hospital, Shiraz, Iran, 2012

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Abstract

Background: Chorionic Villous Sampling (CVS) is a diagnostic method for determining genetic disorders. The present study aimed to determine the negative predictive value of the CVS in the diagnosis of major thalassemia in genetic laboratory of Dastgheib Hospital, Shiraz, Iran.

Methods: The present research was an evaluation diagnostic test conducted on 372 records of embryos examined through CVS in the genetic lab in 2010 and definitely diagnosed by electrophoresis after birth in 2012. The sensitivity and positive predictive value of the test were assessed for minor thalassemia. The negative predictive value and the specificity of this test were determined, as well.

Results: A total of 3 embryos (0.8%) were aborted due to testing. In this study, the sensitivity and specificity were 94.8% and 80.4%, respectively. Also, the negative predictive values for diagnosis of major and minor thalassemia were 100% and 89.2%, respectively. No relationships were found between the gestational age and the test results.

Conclusion: The results of this study showed that CVS genetic testing in genetic laboratory of Dastgheib Hospital was valid and had a high diagnostic value. Thus, minor couples can undergo this test with relative safety in order to prevent major thalassemia.

Keywords: Chorionic Villous Sampling (CVS), genetic, laboratory, predictive value, thalassemia

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Introduction

Chorionic Villous Sampling (CVS) is an invasive pre-natal diagnostic method for genetic disorders.¹ Major thalassemia is one of the diseases which can be diagnosed by testing during pregnancy. This test is performed when the couple has minor thalassemia and the baby is likely to be born with major thalassemia. Thalassemia is the most common single-gene disorder with more than 170 different complex genetic mutations.² This disease is prevalent in many provinces of Iran.³

CVS is normally done 10 to 12 weeks after the last menstrual period.⁴ This is earlier than the time amniocentesis is done. Whenever it is necessary to be informed of the fetal condition sooner than 14 weeks, CVS is used preferably which makes it possible to make a timely decision about the fetus.⁵

Genetic counseling and testing should be offered to couples with a family history of genetic disorders, a previously affected fetus or child, or a history of recurrent miscarriage. Studies on CVS complications and major thalassemia screening in Iran have been carried out in Zahedan and Tehran.^{6,7} The sensitivity and specificity of this test have been reported in Greece, France, and Thailand.^{8,9,10} Until now, no research has been done on the sensitivity,

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and diagnostic value of this test in Iran.

The Thalassemia and Hemophilia Genetic and PND Center at Shahid Dastgheib Hospital in south Iran is affiliated with the Hematology Research Center of Shiraz University of Medical Sciences, Shiraz, Iran. The center started genetic counseling and major thalassemia diagnosis using CVS through trans abdominal method in 2001. In 2010, an epidemiological study was conducted by the researcher on patients referring to the center for pre-natal diagnosis of thalassemia and the results were recorded.¹¹ The present study aims to determine the negative predictive value of CVS in the diagnosis of major and minor thalassemia and also assess the sensitivity and specificity of this test.

Materials and Methods

This is an evaluation study to measure the sensitivity and specificity of CVS diagnostic test in pregnant mothers. Sampling was done by census, so as to include all mothers who referred to the genetic laboratory of Dastgheib Hospital in Shiraz to test the fetus for major thalassemia genetic condition in 2010 (372 cases).

These groups were chosen because they were between one or two years old in 2012 and the status of the disease or carriers of the gene were identified. In a previous study, conducted on the same cases, the demographic characteristics of the test subjects were recorded in a questionnaire. In the questionnaire used in the current study, the postnatal information of the participants was collected using their address and phone number.

The number of positive and negative cases (major or minor thalassemia) diagnosed by CVS as well as the number of healthy and unhealthy cases (or thalassemia gene carriers) diagnosed by electrophoresis after birth was registered. To prevent maternal cell

Table 1. CVS and electrophoresis findings in diagnosis of minor thalassemia

| Results | Positive | Negative | Total |
|-------------------|----------|----------|-------|
| Minor thalassemia | 165 | 9 | 174 |
| Healthy | 18 | 74 | 92 |
| Total | 183 | 83 | 266 |

contamination (MCC), samples were checked via a microscopic investigation with almost 90% of the samples diagnosed. The accuracy of the origin of the samples was confirmed by PCR (VNTR and RFLP). The results were recorded in Table 2 and analyzed for positive predictive value, sensitivity and specificity.

Results

Out of the 372 fetuses tested by CVS at Shahid Dastgheib Hospital genetic center, 25.5%, 48.7%, 0.8%, 1.3%, and 23.7% were diagnosed as major thalassemia, minor thalassemia, intermediate, homozygous sickle cell, and healthy, respectively.

The mean of the gestational age was 11 weeks, ranging from 7 to 14 weeks. Among the 372 study samples, 3 (0.8%) miscarriages occurred due to the CVS test. One of these aborted embryos was detected with minor thalassemia and the other two were without the carrier gene. Also, among the cases of spontaneous abortion with unknown reasons, 5 cases were minor and 3 were without the carrier gene. These 11 cases of abortion were excluded from positive and negative tests.

The negative predictive values of the CVS test for diagnosis of major and minor thalassemia were 100% and 89.2%, respectively. None of the negative test cases had symptoms of major thalassemia after birth and their electrophoresis test results were negative, as well. However, 9 cases that had been considered as negative through CVS were diagnosed with minor thalassemia by electrophoresis after birth.

All the cases diagnosed with major thalassemia were referred for abortion and, consequently, it was not possible to calculate the positive predictive value and sensitivity for these cases. Thus, the diagnostic results were determined based only on minor thalassemia. Considering lack of specific clinical symptoms, the diagnosis of minor thalassemia requires exclusive laboratory tests. Electrophoresis experiments were conducted after birth and the results were compared with those of CVS performed *in utero*.

In this study, the sensitivity and specificity of the diagnosis of minor thalassemia were 94.8% and 80%, respectively. In addition, the positive and negative predictive values for diagnosis of minor thalassemia were 89.2% and 90%, respectively (Table 2).

In this study, no relationship was observed between the age and sex of the fetus and the positive and negative results of the diagnostic test ($P > 0.05$).

Follow-up of the children after birth showed that only one child (0.3%) had birth defect due to the CVS test.

Discussion

Beta major thalassemia is a severe and fatal anemia and its diagnosis is based on clinical symptoms. This disease appears in early childhood and its laboratory findings are relatively simple.¹² Follow-up of the 83 cases with negative CVS test results showed that none of the children had major thalassemia after birth. This means that the value of a negative diagnostic test for the diagnosis of major thalassemia is equal to 100%. In a study conducted in

the UK in 1999 to detect the chromosomal abnormalities, such as Down syndrome, 0.02% false-negative cases were reported in CVS tests, while this measure was reported as 3% in another study performed in the Netherlands in the same year.^{13,14} In this study, the frequency of false negative cases for diagnosis of major and minor thalassemia was 0% and 5%, respectively. In comparison to other studies, the specificity of the test in diagnosis of minor thalassemia was low; 80.4% in contrast to 100% and 90% which indicates low true negative. In this study, the rate of abortions due to CVS performed in the Genetic Laboratory was 0.8%. In a study conducted in 2009 in Sistan and Baluchistan, the rate of embryo loss was reported as 0.7% which is similar to the results of this study.⁶ Another study conducted in Tehran also showed no significant complications after the procedure.⁷ Moreover, body organs deficiencies have been reported from 0.03% to 0.1% in some studies.⁵

The results of this study showed that the performance of CVS test in Dastgheib Genetic Laboratory of Shiraz was reliable; especially considering the validity and predictive value in the diagnosis of major thalassemia. Thus, couples with minor thalassemia can undergo this test with relative safety to prevent the birth of the children with major thalassemia.

The population genetic screening program, including genetic testing *in utero*, should respect the rights of all the people in the community. The disease type, test selection (the test should be reliable and valid with high sensitivity and specificity), and distribution and dynamics of the schedule acceptable to the target population should be taken into account, as well. Overall, population screening programs for beta-thalassemia and sickle cell carriers lead to the reduction of homozygous cases in the community.

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