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The Effect of Fetal Intrauterine Blood Transfusion on Ductal Arterial Blood Flow Velocity and Cardiac Output Changes in Fetuses with Rhesus Alloimmunization-Related Anemia

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Abstract

Background: Echocardiographic indicators may be useful as an alternative method to determine the appropriate time for intervention, especially when Doppler assessment of the middle cerebral artery peak systolic velocity (MCA-PSV) is inconclusive. In this study, arterial duct blood flow and cardiac output of fetuses with Rh alloimmunization-related anemia were compared before and after intrauterine transfusion (IUT).

Methods: Fifty intrauterine blood transfusions were performed on anemic fetuses in this study. All cases were attributed to Rh alloimmunization, detected with MCA-PSV Doppler ultrasound imaging assessments. They all underwent intrauterine blood transfusion and echocardiographic assessments a day before and 24 *hr* after the procedure. The measured cardiac indices included the velocity time integral (VTI) of the aortic and pulmonary valves, the ductus arteriosus acceleration-to-ejection time (AT/ET) ratio, and the MCA-PSV. Analyses were performed in SPSS v24 ($p<0.05$) using paired t-tests for mean differences and Pearson's correlation for associations between continuous variables.

Results: Aortic and pulmonary valve VTI, MCA-PSV, and ductus arteriosus AT/ET ratio significantly decreased after intrauterine blood transfusion ($p<0.001$). The aortic valve VTI and ductus arteriosus accurately detected anemia, with areas under the receiver operating characteristic (ROC) curve of 99.9% and 98.9%, respectively.

Conclusion: All the measured parameters showed significant changes after intrauterine blood transfusion. Aortic valve VTI and ductus arteriosus AT/ET ratio seem to have the highest accuracy in detecting anemia. Echocardiographic assessment can be beneficial in defining the appropriate time for intrauterine blood transfusion in cases where MCA-PSV is inefficient.

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Introduction

Intrauterine transfusion (IUT) may be indicated in cases of severe fetal anemia. The main indication for intrauterine transfusion is anemia caused by red blood cell alloimmunization. During alloimmunization, maternal IgG antibodies against red blood cells pass into fetal circu-

lation through the placenta and may cause hemolysis (1, 2). Over the past decades, the survival rate after IUT has exceeded 80% for red blood cell alloimmunization in specialized centers worldwide (3). In one study, the mean gestational age at the first transfusion was 26 weeks, and 26% of the fetuses were hydropic at that time. The average number of transfusions per fetus was three. The most important preoperative risk factor for neurodevelopmental disorder was the presence of hydrops. Prevention of fetal hydrops with timely diagnosis and treatment may improve long-term outcomes (4-6).

Evaluation of bilirubin in amniotic fluid started in 1961 and was used for detecting fetal anemia caused by immune system incompatibilities (7). Today, this method has been replaced by serial Doppler ultrasound imaging of the middle cerebral artery (8, 9). Doppler measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) can be started at 16 to 18 weeks of gestation. Its reliability decreases after the 35th week of pregnancy, and also with multiple transfusions (10, 11). Every intrauterine transfusion has the risk of hemorrhage from the umbilical cord, hematoma formation in fetal vessels, bradycardia, and fetal death. The risk of cordocentesis complications is 3.1% and the risk of fetal loss is 1.6%. In addition to risks for the fetus, maternal risks, including infection, may also occur (12, 13). Therefore, accurate identification of anemic fetuses allows timely intervention and helps avoid unnecessary procedures in those not truly at risk.

There is an inverse relationship between fetal hematocrit and MCA-PSV (14, 15). Studies have shown that in non-hydropic fetuses with RBC alloimmunization, MCA-PSV above 1.5 MoM can detect all cases of moderate to severe anemia, with a false-positive rate of 12% (8). Cardiac output decreases following IUT, which may be transient. Estimating cardiac output is a suitable method for determining the hemodynamic function of the heart although this index increases in conditions such as fetal anemia or arteriovenous fistula (10). Severe anemia significantly affects blood circulation. These changes include enlargement of the heart chambers, increased Doppler blood flow in MCA, and alterations in the mitral valve and left ventricle at the level of the aorta. Also, umbilical artery pulsation index (UAPI) decreases (16).

As an echocardiographic finding after birth, myocardial hypertrophy has been described in infants suffering from immune hemolysis, but has not been evaluated before birth (17). In previous studies, there was no significant relationship between standard echocardiographic findings, including heart rate, cardiothoracic ratio, contractility, and the severity of fetal anemia. However, the combination of cardiac output and hematocrit has been a useful criterion for transfusion in preterm infants (18). In a study of 24 fetuses with anemia due to alloimmunization, Doppler echocardiography showed no significant differences between severe and non-severe anemia in terms of heart rate, cardiothoracic ratio, or contractility (19). There was an increase in the left ventricular dimensions and MCA-PSV, and a decrease in UAPI in the severe anemia group. The combination of MCA-PSV and UAPI demonstrated high diagnostic performance for detecting severe anemia, with a sensitivity of 86%, specificity of 91%, positive predictive value of 80%, negative predictive value of 94%, and a positive likelihood ratio of 9.6 (19).

Sirivat et al. studied cardiac blood flow characteristics, filling time fraction and acceleration time/ejection time (AT/ET) ratio, in anemic fetuses to evaluate the effectiveness of these parameters in identifying the affected fetuses (20). Fetuses at risk of hemoglobin Bart's disease were prospectively selected for echocardiography before diagnostic cordocentesis between 17 to 22 weeks of gestation (20). They reported that the values of the filling time fraction were significantly lower, while the AT/ET ratio was significantly higher in the affected group (20).

As an indicator of severe anemia, the sensitivity of the MCA Doppler index decreases after multiple transfusions. Few studies have investigated echocardiographic indicators of fetal anemia severity. Echocardiography, as a non-invasive method, may also be helpful in determining the severity of fetal anemia. Therefore, this study was designed to evaluate changes in specific echocardiographic parameters following IUT and to assess their diagnostic correlation with the pre-transfusion state of anemia, compared to the standard MCA-PSV measurement.

Methods

Participants: This observational, descriptive-analytical study included 50 IUT procedures performed in the 24th to 35th week of pregnancy on anemic fetuses in 2021-2022. All Rh-negative pregnant women with positive antibody screening tests and confirmed Rh alloimmunization, who

were candidates for intrauterine transfusion based on fetal MCA-PSV Doppler evaluation, were included in the study. Written informed consent to participate in the study was obtained from all participants.

The inclusion criteria for this study were pregnant women aged 18 years or older with a singleton pregnancy, a gestational age between 24 and 35 weeks with a normal anatomical survey, and the ability to understand the study's purpose, potential risks and benefits, and the voluntary nature of participation. The exclusion criteria included multiple pregnancies, major chromosomal or structural fetal abnormalities, intrauterine growth restriction, hydrops fetalis, and unwillingness to cooperate at any stage of the study. Gestational age was calculated based on accurate crown-rump length measured during ultrasound screening between 11 to 14 weeks of gestation. The medical and perinatal history of the participant was recorded. Intrauterine transfusion was performed for all anemic fetuses with an MCA-PSV >1.5 MoM on Doppler assessment. In this study, a fetus was considered anemic if the hemoglobin level measured by cordocentesis prior to the procedure was less than 10 *g/dL*. If the fetal hemoglobin level exceeded 10 *g/dL* after the blood transfusion, the fetus was classified as non-anemic.

One hour before the IUT procedure, mothers received 75 *mg* of meperidine and 25 *mg* of promethazine, both administered intramuscularly. A 20-gauge needle was inserted into the fetal umbilical vein under ultrasound guidance during IUT. Immediately after taking the initial blood sample, atracurium (0.3-0.5 *mg/kg*) was administered intravenously through the inserted needle to induce fetal immobility. The transfusion volume was calculated as 60-90 *ml/kg* of fetal body weight. The transfused blood consisted of washed, irradiated O-negative red blood cells, cross-matched with the mother to ensure immunologic compatibility. Sampling of fetal hemoglobin was done at the beginning, the middle, and the end of the procedure to obtain a target hemoglobin concentration of 14 -16 *g/dL*.

Fetal echocardiography was performed before and 24 *hr* after IUT. Echocardiographic parameters, including aortic velocity time integral (VTI), pulmonary valve velocity time integral (PV VTI), as well as ductus arteriosus AT/ET ratio and MCA-PSV, were measured in all cases by the same pediatric cardiologist specialized in fetal echocardiography, and their changes were subsequently analyzed (21, 22). The project and experimental protocols were approved by the Ethics Committee of Tehran University of Medical Sciences (Ethics Code: IR.TUMS.MEDICINE.REC.1401.181).

Statistical analysis: All analyses were performed using SPSS version 24 software (IBM Corp., USA) at a significant level of $p<0.05$. Continuous variables were expressed as mean±standard deviation. Nominal variables were expressed as frequencies and percentages. The difference in means was determined with the paired t-test. The association between continuous variables was determined with Pearson's correlation. Receiver operating characteristic (ROC) curves were generated for each cardiovascular index. The area under the curve (AUC) was calculated for each index.

Results

The aortic and pulmonary VTI, MCA-PSV, and ductus arteriosus AT/ET ratio were compared before and 24 *hr* after IUT in all 50 anemic fetuses. The mean gestational ages at the time of IUT were 28.16±3.13, with a range of 24 to 35 weeks. The data showed that all three parameters, including aortic and pulmonary VTI and MCA-PSV decreased significantly ($p<0.001$) in 24 *hr* of post IUT (Table 1). By comparing cardiac parameters before and after IUT within the same fetus, this approach controls for potential confounding factors such as gestational age and fetal growth.

Table 1. Comparison of aortic valve VTI, pulmonary valve VTI, ductus arteriosus AT/ET ratio, as well as MCA-PSV before and after 24 *hr* of intrauterine blood transfusion

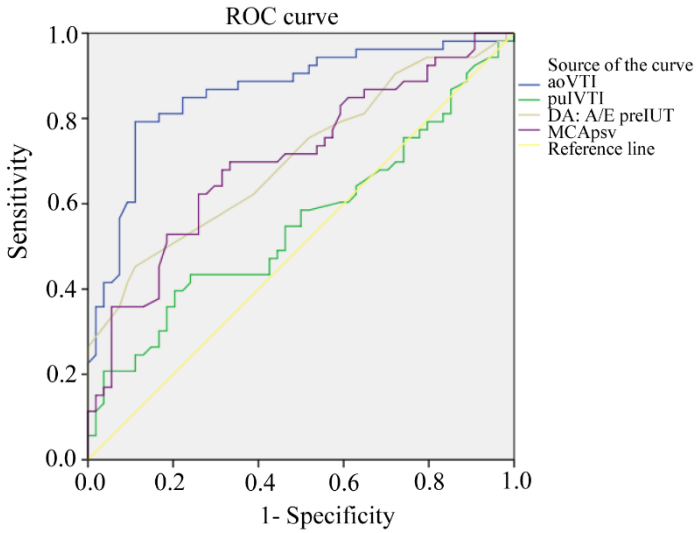
Parameters	Before IUT		24 *hr* after IUT		p-value
	Mean	Standard deviation	Mean	Standard deviation	
Aortic valve VTI	11.92	1.42	5.96	0.9	<0.001
Pulmonary valve VTI	7.74	1.31	6.92	1.02	<0.001
Ductus arteriosus AT/ET	0.4	0.05	0.26	0.04	<0.001
MCA-PSV	53.44	9.56	43.27	11.18	<0.001

VTI: Velocity Time Integral, IUT: Intrauterine Blood Transfusion, AT/ET: Acceleration Time/Ejection Time, MCA-PSV: Middle Cerebral Artery Peak Systolic Velocity

Table 2. Correlation between aortic valve VTI, pulmonary valve VTI, ductus arteriosus AT/ET ratio, as well as MCA-PSV before and after 24 *hr* of intrauterine blood transfusion

	Aortic valve VTI		MCA-PSV	
	r	p	r	p
Aortic valve VTI	1	-	0.255	0.074
Pulmonary valve VTI	0.412	0.003	0.072	0.617
Ductus arteriosus AT/ET ratio	0.617	<0.001	0.300	0.034
Aortic valve VTI-24	0.212	0.139	-0.039	0.786
Pulmonary valve VTI-24	0.163	0.257	0.11	0.447
Ductus arteriosus AT/ET-24	-0.041	0.776	0.049	0.735
MCA-PSV-24	0.207	0.150	0.521	<0.001

VTI: Velocity Time Integral, IUT: Intrauterine Blood Transfusion, AT/ET: Acceleration Time/Ejection Time, MCA-PSV: Middle Cerebral Artery Peak Systolic Velocity



Diagonal segments are produced by ties

Test result varizble(s)	Area	Std. error [a]	Asymptotic sig. [b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
aoVTI	0.862	0.037	0.000	0.789	0.934
pulVTI	0.555	0.056	0.325	0.445	0.666
DA:A/E preIUT	0.706	0.050	0.000	0.608	0.804
MCApsv	0.704	0.050	0.000	0.605	0.803

The test result variable(s): aoVTI, pulVTI, DA: A/E preIUT, MCApsv has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. a. Under the nonparametric assumption. b. Null hypothesis: true area=0.5

Figure 1. Receiver operating characteristics curve for assessing the diagnostic accuracy of cardiovascular indices in detecting fetal anemia

Table 2 shows the correlations between pre- and post-IUT echocardiographic parameters. A significant positive correlation was observed between aortic valve VTI and pulmonary valve VTI (r= 0.412, p=0.003), between pulmonary valve VTI and pulmonary valve VTI at 24 *hr* post-IUT (r= 0.539, p<0.001), and between MCA-PSV and MCA-PSV at 24 *hr* post-IUT (r=0.521, p<0.001).

Diagnostic characteristics for anemia: Receiver operating characteristic curves were used to determine the diagnostic performance of aortic and pulmonary valve VTI, ductus arteriosus AT/ET ratio, and MCA-PSV in detecting anemia (Figure 1). The aortic valve VTI showed the highest diagnostic accuracy for detecting anemia, with an area under the curve (AUC) of 99.9%. Pulmonary

valve VTI had the lowest accuracy, with an AUC of 68.4%. The ductus arteriosus index (98.8%) and MCA-PSV (74.9%) were also important for identifying anemia. Analysis of the AUCs demonstrated that the diagnostic performance of cardiovascular parameters ranked the highest in the following order: aortic valve VTI (0.862±0.037), MCA-PSV (0.704±0.05), and ductus arteriosus index (0.706±0.05).

Discussion

Studies have shown that severe fetal anemia significantly changes fetal blood circulation. Severe fetal anemia leads to the enlargement of the cardiac chambers and increases Doppler-measured velocities at the mitral valve, left ventricular outflow tract, and aortic valve. Also, severe anemia is associated with lower UAPI. Increased Doppler velocities and decreased UAPI are likely attributable to elevated cardiac output, systemic vasodilation, and decreased blood viscosity. However, several studies have reported that standard parameters of echocardiography, such as heart rate, cardiothoracic ratio, and contractility indices do not significantly change in severe anemia and appear to be of limited value in assessing its severity (23). In cases of severe anemia, the mean blood flow velocity in MCA increases (24, 25). Although MCA-PSV is an established indicator for detecting the severity of fetal anemia, its sensitivity decreases after several fetal transfusions (12).

Given the importance of accurately determining the timing of IUT, this study was designed to evaluate whether measurable cardiac function parameters can help in assessing anemia severity and guiding the optimal timing for IUT. According to our results, echocardiographic parameters, especially aortic valve VTI and ductus arteriosus AT/ET ratio, were significantly different between pre- and post-IUT. Additionally, the ROC curve analysis confirmed that the aortic valve VTI and the ductus arteriosus index, with AUC of 99.9% and 98.8%, respectively, demonstrated the highest accuracy for detecting fetal anemia. This means that the accuracy of these parameters is higher than MCA Doppler (74.9%). This is a valuable finding for determining fetal anemia, especially as echocardiography can be helpful in cases in which MCA-PSV cannot be used due to multiple transfusions or false positive results. In the present study, dynamic Doppler changes were evaluated through intra-fetal comparisons before and after IUT, representing rapid hemodynamic alterations rather than sustained changes related to fetal weight or growth. Therefore, by evaluating cardiac parameter changes through pre- and post-IUT comparisons within the same fetus, this approach controls for confounding variables, including gestational age and fetal growth. Additionally, fetuses with placental or cardiac insufficiency, as well as structural anomalies, were excluded from the study to minimize potential confounding effects on the analyses.

To investigate the relationship between severe anemia and cardiac changes, the most significant echocardiographic finding was myocardial hypertrophy in all ventricular walls (25). In a study by Bigras et al., fetuses with more severe anemia exhibited an enlarged left ventricle. However, in cases of severe anemia, there was no significant difference in the cardiothoracic ratio (19). In Jeong et al.'s study, the systolic peak longitudinal, radial, and circumferential strain in the lateral wall of the left ventricle was lower in anemic fetuses compared to control group before IUT. The UAPI normally decreases with increasing gestational age (26). In cases of severe anemia, a significant decrease was observed in this index. Additionally, the combination of MCA-PSV and UAPI demonstrated high diagnostic performance, with a sensitivity of 86%, specificity of 91%, positive predictive value of 80%, negative predictive value of 94%, and a positive likelihood ratio of 9.6 for detecting severe anemia (19). In another study by Sirivat et al., fetal cardiac filling time fraction and the AT/ET ratio were significantly lower in fetuses with severe anemia and hemoglobin Bart's disease (20). Moreover, they reported that the filling time fraction values were superior to the AT/ET ratio in identifying fetuses affected by hemoglobin Bart's disease (20).

To our knowledge, two new aspects were investigated in our study that were not discussed in previous studies the measurement of aortic and pulmonary valves VTI and their relationship with severe fetal anemia. The results showed a significant change in the mean aortic and pulmonary valve VTI before and after IUT, suggesting that improvement in fetal hemoglobin status is associated with a decrease in these velocities. The limitations of this study include the small sample size and the absence of gestational age–specific reference ranges for the measured cardiac parameters.

Conclusion

The present results revealed that the VTIs of aortic and pulmonary valves, MCA-PSV, and ductus arteriosus AT/ET ratio showed significant changes after intrauterine blood transfusion. This study highlights the aortic valve VTI and the AT/ET ratio as highly sensitive and specific parameters, demonstrating their strong association with severe anemia and underscoring their clinical importance. The use of echocardiographic features in determining anemia can help in the appropriate timing of IUT, especially in cases where MCA-PSV cannot help determine fetal anemia.

It seems that more studies with greater sample sizes are needed to define reference ranges for these key indices according to different gestational ages. Fetal anemia can likely be assessed more accurately through the combined use of these parameters.

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Conflict of Interest

The authors declare that they have no competing interests.

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