Acoustic radiation force impulse elastosonography of placenta in maternal red blood cell alloimmunization: a preliminary and descriptive study

Orkun Cetin¹, Erbil Karaman¹, Harun Arslan², Ibrahim Akbudak², Recep Yıldızhan¹, Ali Kolusarı¹

¹Department of Obstetrics and Gynecology, ²Department of Radiology, Yuzuncu Yil University, Faculty of Medicine, Van, Turkey

Abstract

Aims: Maternal red blood cell alloimmunization is an important cause of fetal morbidity and mortality in the perinatal period, despite well-organized prophylaxis programs. The objective of the study was to evaluate placental elasticity by using Acoustic Radiation Force Impulse (ARFI) in Rhesus (Rh) alloimmunized pregnant women with hydropic and nonhydropic fetuses and to compare those with healthy pregnant women. Material and methods: This case-control and descriptive study comprised twenty-eight healthy pregnant women, 14 Rh alloimmunized pregnant women with nonhydropic fetuses, and 16 Rh alloimmunized pregnant women with hydropic fetuses in the third trimester of pregnancy. Placental elasticity measurements were performed by ARFI elastosonography at the day of delivery. The maternal characteristics and neonatal outcomes of the patients were also noted. Results: The highest mean placental ARFI scores were observed in Rh alloimmunized pregnant women with hydropic fetuses (1.13 m/s) (p=0.001). Healthy controls and Rh alloimmunized pregnant women with nonhydropic fetuses had similar mean placenta ARFI scores (0.84 m/s, 0.88 m/s, respectively) (p<0.05). Conclusions: Based on the present findings, the placenta becomes stiffer in Rh alloimmunized pregnancies complicated with hydrops fetalis. The increased placental ARFI scores may be a supplemental marker for adverse pregnancy outcomes, additional to Doppler evaluation of middle cerebral artery. This data should be confirmed with a large sample size and prospective studies by using serial measurements of ARFI elastosonography in maternal red blood cell alloimmunization.

Keywords: maternal red blood cell alloimmunization, placental elasticity, ARFI.

Introduction

Maternal red blood cell alloimmunization is an important cause of morbidity and mortality in the antepartum and neonatal periods with the frequency found to range from 0.4% to 2.7% worldwide [1-5]. Maternal red blood cell alloimmunization causes fetal-neonatal anemia with the mechanism of transplacental passage of maternal hemolytic antibodies that produce fetal red blood cell destruction. This eventually leads to anemia, hyperbilirubinemia, extra-medullary hematopoiesis and generalized body edema which is called hydrops fetalis [6]. There are three main types of red blood cell alloimmunization, based on the antigen(s) involved: Rhesus (Rh), minor red cell antigens (i.e. Kell, Duffy, Kidd antigens), and ABO [5]. The rate of alloimmunization has been found to be decreased sharply from 16% to ~2% in the past few years with the routine use of postpartum anti-D Immunoglobulin (Ig) for Rh D-negative women [7].

The ancient measurements such as placental thickness and abdominal circumference could not predict the development of fetal anemia [8]. Currently, the main diagnostic tool for prediction of fetal anemia is middle cer-
Acoustic radiation force impulse elastosonography of placenta in maternal red blood cell alloimmunization

Orkun Cetin et al

Echocardiography Doppler evaluation. This is a non-invasive and feasible technique without any potential risks to the mother and fetus. It is accepted as worldwide in the decision of fetal anemia and intrauterine transfusion treatment [5].

Human placenta has crucial functions in fetal oxygenation, endocrinological contributions and nutrition [9]. The placental thickening has been linked with various pathologies such as congenital infections, maternal diabetes and fetal hydrops [9]. Also, fetal anemia, fetal heart failure and chromosomal abnormalities such as trisomies have been reported to be associated with placental thickening [10,11]. Placental features may have important clues for maternal and fetal risks because the placenta is usually the first organ of manifestation of pregnancy complications [12]. The placental changes that are seen in maternal red blood cell alloimmunization result from edema and compensatory hypertrophy [12]. However, there is a limited number of studies that have evaluated the placental tissue in maternal red blood cell alloimmunization.

Elastosonography is a non-invasive diagnostic tool that evaluates tissue stiffness. It has been used for various diseases as well as in placenta pathology [13-19]. Acoustic radiation force impulse (ARFI) elastography is relatively a new method which uses a short acoustic push pulse in the target tissue, which causes a tissue displacement of approximately 1-20 um. Placental evaluation with ARFI elastosonography has been studied in hypertensive disorders and fetal growth restricted pregnancies and a stiffer placenta in both groups was reported [13]. To date, no study has been found which has evaluated the placental tissue stiffness in maternal red blood cell alloimmunization.

Placental structural abnormalities may occur in maternal red blood cell alloimmunization. This condition may affect placental elasticity. Therefore, we aimed to evaluate placental elasticity by using ARFI in Rh alloimmunized pregnant women with hydropic and nonhydropic fetuses and to compare those with healthy pregnant Women.

Materials and methods

This case-control and descriptive study was carried out at Yuzuncu Yil University Medical Faculty, Department of Obstetrics & Gynecology and Department of Radiology from January 2015 till July 2016. The University’s Local Ethics Committee approved the study and informed consent was obtained from all participants. We identified 39 singleton pregnancies with maternal red cell anti-D alloimmunization referred to our hospital. The gestational age was determined according to the last menstrual period or to the crown-rump length on the first trimester ultrasonographic examination. The maternal antibody titers of alloimmunized patients ranged from 1:16 to 1:256. Patients were managed exclusively through noninvasive assessment (Doppler evaluation of the middle cerebral artery) and/or rising antibodies. Intrauterine transfusion was decided in immature (<34 weeks) fetuses when the measurement of middle cerebral artery peak systolic velocity was up to 1.55 multiples of the median (MoM) and in case of sonographic signs (as hydrops). The hydrops were described as the excess liquid in two or more fetal spaces [20]. The target of our management strategy was to deliver the fetuses at 34th week of gestation in adequate fetal well-being. Five patients who underwent intrauterine transfusion and four patients with posterior placental location were excluded from the study. Patients with accompanying diseases, multiple gestations and congenital abnormalities were also excluded. The study population consisted of 28 healthy pregnant women (Group 1), 14 Rh alloimmunized pregnant women with nonhydropic fetuses (Group 2) and 16 Rh alloimmunized pregnant women with hydropic fetuses (Group 3) in the third trimester of pregnancy. The healthy pregnant women (Group 1) consisted of clinically normal pregnancies with normal fetal sonography and normal perinatal outcomes. Group 3 comprised of Rh alloimmunized pregnant women who had not received any antenatal care visits. They were all in lower socioeconomic status and located in a rural part of Eastern Turkey. They were referred to our clinic in a severe state of hydrops fetalis. and intrauterine transfusion was recommended. But, no one gave the informed consent for the invasive procedure and they were followed with conservative management. The timing of delivery was decided on a case-by-case basis. The clinical characteristics of the patients, as maternal age, gravidity, parity and body mass index (BMI) were noted. Data concerning obstetric and neonatal outcomes as, gestational age at delivery, birthweight, and Apgar scores were also collected.

We used 4-9 MHz 9L4 linear transducer with the Virtual Touch IQ option (Siemens ACUSON S2000™, Siemens Healthcare, Erlangen, Germany) for ARFI elastosonography measurements. The standardization of the ARFI elastosonography technique was obtained by examining 5 healthy pregnant women before starting the study. The radiologist who performed the ARFI had at least 10-year experience in gray-scale ultrasound and 5 years in elastosonography. The measurements of placenta were performed in the supine position while the patients held their breath. The placenta was evaluated and divided into three equal parts as fetal edge (inner 1/3 of
placenta (ARFI-fetal), maternal edge (outer 1/3 of placenta) (ARFI-maternal) and the central part (central 1/3 of placenta) (ARFI-central). Three measurements were taken in each of the three regions of the placenta and the mean of these three measurements were obtained as the value of shear wave velocity in m/s for each region (fig 1, fig 2). The mean of these 9 measurements were also calculated and accepted as the mean placental elasticity value (ARFI-mean). All ARFI measurements of the patients were performed at the day of delivery.

**Statistical analysis**

Descriptive statistics for studied variables (characteristics) were presented as Median, Minimum and Maximum values. The Kruskal-Wallis Test was used to compare continuous variables among the three groups. The Mann-Whitney U test was used to compare continuous variables between the two groups. The Duncan’s test was performed to determine which group differed significantly from the other groups. A Chi-Square test was used to examine the association between the categorical variables. The Spearman’s correlation analysis was used to define the relationship between the variables. Statistical significance levels were considered as 5%. The SPSS (Version 20.0 – IBM Corp. Released 2011. IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.) statistical program was used for all statistical computations.

**Results**

The clinical characteristics and perinatal outcomes of the patients are presented in Table I.

The placental ARFI elastosonography scores of the patients are detailed in Table II. The highest placental ARFI-maternal, ARFI-central and ARFI-fetal elastosonography scores were measured in Group 3 (p=0.001, p=0.002, and p=0.004, respectively). The highest placental ARFI-mean elastosonography scores were measured in Group 3 (p=0.001). Group 1 and Group 2 had similar placental ARFI-mean elastosonography scores (p>0.05). The middle cerebral artery peak systolic velocity MoM values were 0.95±0.31 and 1.54±0.26 in Group 2 and Group 3, respectively. There was a significant difference between the groups (p=0.001). Also, we found a significant positive correlation between the placental ARFI-mean elastosonography scores and the middle cerebral artery peak systolic velocity.

---

**Table I. Comparison of the clinical characteristics and perinatal outcomes in the separate groups of patients**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n: 28)</th>
<th>Group 2 (n: 14)</th>
<th>Group 3 (n: 16)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>25.5 (18-39)b</td>
<td>29.5 (18-40)a</td>
<td>30.5 (23-39)a</td>
<td>0.023*</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (1-9)b</td>
<td>3 (1-8)a</td>
<td>5 (1-10)a</td>
<td>0.001*</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0-8)b</td>
<td>2 (0-7)</td>
<td>3 (0-9)</td>
<td>0.002*</td>
</tr>
<tr>
<td>BMI</td>
<td>27.8 (23.5-37.5)</td>
<td>28.2 (24.0-36.9)</td>
<td>27.7 (21.7-32.3)</td>
<td>0.542*</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>39 (37-40)</td>
<td>36 (34-38)</td>
<td>31 (24-36)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Birthweight</td>
<td>3150 (2750-4000)</td>
<td>2400 (1400-3500)</td>
<td>1775 (450-3000)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Apgar 1. minute</td>
<td>7 (6-8)</td>
<td>6 (3-7)</td>
<td>1 (1-7)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Apgar 5. minutes</td>
<td>9 (7-10)a</td>
<td>8 (5-9)</td>
<td>5 (2-9)</td>
<td>0.001*</td>
</tr>
<tr>
<td>NICU admission</td>
<td>0</td>
<td>5/14 (35.7%)</td>
<td>16/16 (100%)</td>
<td>0.001f</td>
</tr>
<tr>
<td>Early neonatal death</td>
<td>0</td>
<td>0</td>
<td>14/16 (87.5%)</td>
<td>0.001e</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test was used to compare continuous variables; * Chi-Square test was used to compare categorical variables; a, b, c → Defined significance between each groups (a>b>c); BMI – body mass index; NICU – neonatal intensive care unit.
cerebral artery peak systolic velocity mean-MoM values ($r=0.486; p=0.006$).

**Discussions**

The current study is the first descriptive, case-control study that has examined the placental elasticity by ARFI elastosonography in Rh alloimmunized pregnancies. The results of our study demonstrated that there was a positive correlation between placental elasticity and development of hydrops fetalis in Rh alloimmunized pregnancies. Increased placental stiffness may be a useful predictor for the worse perinatal outcomes, additionally to other non-invasive procedures, such as middle cerebral artery peak systolic velocity in this condition.

The diagnosis and management of maternal red cell alloimmunization has been broadly identified in several studies around the world. During the past decades, Rh hemolytic disease has decreased with the widespread adoption of guidelines for antenatal and postpartum use of anti-D Ig prophylaxis [21]. However, there are limited data available on placental changes in maternal red cell alloimmunization [12]. It is known that placentalmegaly is a possible finding of erythroblastosis fetalis. In early studies, increased placental thickness was used as an early predictor for fetal anemia in maternal red cell alloimmunization [8]. However, increased thickness is non-specific and can be found with other adverse pregnancy complications such as perinatal infections [22]. The thick, heterogeneous (jellylike) appearance of the placenta is also associated with perinatal death, hypertensive diseases, fetal growth restriction and preterm delivery [23]. Three-dimensional Power Doppler analysis in fetal growth restricted pregnancies have evidenced that decreased placental vascularity and impaired budding of the villous circulation contribute to the thick and heterogeneous (jellylike) placenta [24].

Sonography has recently been used to measure tissue elasticity [14]. ARFI is a new technique of elastography which can be applied without any manual compression and also allows detecting histological changes in tissues [13]. It is an ultrasound-based elastography which depends on the usage of a short acoustic push in the target tissue. It induces a tissue displacement of nearly 1-20 μm. This displacement generates a lateral shear wave that propagates through the tissue during recoil, the velocity of which is described as Vs (m/s). The Vs level reflects tissue elasticity. Tissue stiffness increases in fast shear wave speeds and small displacements. Oppositely, slow shear wave speeds and large displacements are seen in softer tissues [13]. ARFI technique was firstly used for the evaluation of liver fibrosis, liver cirrhosis, and inflammatory pancreatic diseases [15,16]. Mateen et al [16] stated that increased Vs values occur as a result of inflammatory cell infiltration and cellular swelling with increased fluid content in the target tissues. In recent studies, elevated Vs values were also detected in acute hepatitis and pancreatitis. However, the reasons of increased elasticity in these parenchymal organs are still unknown [15,16].

There have been limited studies concerning ARFI investigating placental elasticity [17-19]. Sugitani et al [13] measured the placental elasticity by using ARFI in an ex vivo study and they found higher ARFI scores in intrauterine growth restricted and hypertensive pregnancies. Recent in vivo studies demonstrated that the placenta becomes stiffer in preeclamptic pregnancies [17-19]. Placental infarction, inflammation of trophoblastic villi and vasculitis are major findings which occur in intrauterine growth restricted and hypertensive pregnancies [25]. Villous congestion and inefficient oxygen delivery are also seen in these pregnancies [13]. The authors stated that as a result of these placental histological changes, the placenta may become stiffer in these pregnancies [13].

Elastosonography techniques have similar safety considerations to conventional ultrasonography imaging modes. Although the ARFI technique is based on tissue displacement with generating pulse bursts, Herman et al reported that any temperature increase generated by the ARFI with pulse bursts might still be within the safe limit determined by the US Food and Drug Administration [26]. In a recent study Sugitani et al showed that ARFI elastosonography poses no potential thermal or chemical risk to placenta and fetus [13]. In our study the fetus was

### Table II. A comparison of placental ARFI elastosonography scores in the patients’ groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>ARFI- maternal (m/s)</th>
<th>ARFI- central (m/s)</th>
<th>ARFI- fetal (m/s)</th>
<th>ARFI- mean (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n: 28)</td>
<td>0.81 (0.61-1.19)</td>
<td>0.80 (0.62-1.2)</td>
<td>0.88 (0.60-1.16)</td>
<td>0.84 (0.63-0.98)</td>
</tr>
<tr>
<td>Group 2 (n: 14)</td>
<td>0.90 (0.62-1.49)</td>
<td>0.81 (0.58-1.28)</td>
<td>0.78 (0.53-1.14)</td>
<td>0.88 (0.62-1.16)</td>
</tr>
<tr>
<td>Group 3 (n: 16)</td>
<td>1.17 (0.71-1.98)</td>
<td>1.05 (0.65-2.32)</td>
<td>1.1 (0.69-1.86)</td>
<td>1.13 (0.58-1.87)</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test was used to compare continuous variables; a, b, c→ Defined significance between each groups (a>b>c); ARFI, acoustic radiation force impulse.*
not within the direction of waveform paths of the ARFI elastosonography.

It is well known that the placental thickening in maternal red blood cell alloimmunization is a result of placental oedema and compensatory hypertrophy [12]. We hypothesized that this placental oedema, which is an early predictor for fetal anemia, may affect placental elasticity. If we could demonstrate this placental change by ARFI elastosonography measurements, we could use this technique as a novel marker for adverse perinatal outcomes, additional to middle cerebral artery peak systolic velocity in maternal red blood cell alloimmunization. The current study found that there was a positive correlation between placental stiffness and the development of fetal hydrops. All placental ARFI scores were higher in Rh alloimmunized pregnancies with hydropic fetuses than those in non-hydropic fetuses. We also found that there were similar ARFI scores in Rh alloimmunized pregnancies with non-hydropic fetuses and healthy controls.

Currently, the Doppler evaluation of the middle cerebral artery peak systolic velocity is the referent technique for antenatal detection of fetal anemia in maternal red blood cell alloimmunization [5]. As a result of this study, we can state that increased placental ARFI scores may be a supplemental, useful, and non-invasive marker for adverse pregnancy outcomes, additional to middle cerebral artery peak systolic velocity. This data should be confirmed with a large sample size and prospective studies by using serial measurements of ARFI elastosonography in maternal red blood cell alloimmunization. We hope that future advances in non-invasive techniques for managing fetuses at risk of maternal red blood cell alloimmunization would make a beneficial impact on perinatal morbidity and mortality.

The strength of our study was that all patients were in their third trimester in order to eliminate the possible bias related to the changes in placental growth through second trimester to third trimester. On the other hand, our study has several limitations, namely our sample size was small. Another limitation may be the non-homogeneity of patients with regards to the maternal age, gravidity, parity, and gestational week at birth as confounding variables. There is limited data regarding ARFI scores changes during different gestational weeks. The studies involving ARFI examinations included the third trimester of gestation in the literature [13,19]. We did not evaluate the histopathologic findings of the placentas. All of the ARFI examinations were performed at the day of delivery as a single measurement; we did not examine placental elasticity by serial ARFI evaluations which might give more comprehensive data. We only focused on placental morphological changes in this study.

**Conclusions**

To the best of our knowledge, this is the first study that has evaluated the placental ARFI measurements in maternal red cell alloimmunization. Our study showed that the placenta becomes stiffer in Rh alloimmunized pregnancies complicated with hydrops fetalis, evaluated by the ARFI elastosonography technique. Further studies are required in order to apply this measurement in the follow up examinations of Rh alloimmunized pregnancies. Furthermore, the serial measurements of placental ARFI elastosonography may give additional data regarding the prognosis of maternal red blood cell alloimmunization.

**Conflict of interest:** none

**References**