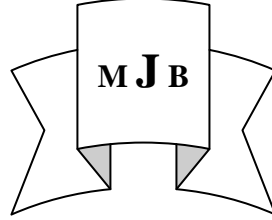


The Value of Kleihauer Test in the Detection of Fœtomaternal Transfusion in Iraqi Patients

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Abstract

Back ground: Rh isoimmunization represents an important problem that contributes to foetal morbidity resulting in haemolytic disease of newborns (HDN) and abortions in successive pregnancies. The major treatment strategy relies upon prevention of sensitization by administration of Anti-D immunoglobulin to Rh negative mothers giving birth to Rh positive babies within 72 hours after birth. This is usually controlled by counting the number of foetal red cells in the maternal circulation by Kleihauer test which is routinely conducted to all Rh negative mothers.

Objectives: This study is designed for detection of random cases of foetomaternal transfusion during delivery in Iraqi patients irrespective of mother's Rh status, in order to understand fully the probability of isoimmunization after uneventful deliveries.

Material and method: Blood samples were collected from mothers just delivered, immediately smeared making thin blood film and subjected to Kleihauer test.

Results: Out of 86 maternal blood samples collected one hour after delivery, 25 (28.9%) cases revealed significant foetomaternal transfusion, ranging from minor (1-3 ml) transfusion representing 16 cases out of 86 (18.6%), moderate (4-10 ml) transfusion representing 4 cases (4.6%) and major (>10 ml) transfusion representing 5 cases (5.8%). Massive foetomaternal transfusion (>30 ml) was not detected in all cases.

Conclusions: Foetomaternal transfusion with different degrees is a frequent event during IU Anti-D immunoglobulin 72 hours after birth is adequate in elimination of almost all foetal red cells in Rh negative mothers giving birth to Rh positive babies.

أخلاصه

الخلفية: المتلازمة المناعية للعامل الريصي تمثل مشكلة مهمة تسهم في مرضيه الجنين الناتجة من مرض انحلال الدم لحديثي الولادة و الاجهاض المتكرر. العلاج الاستراتيجي الرئيسي يكمن بمنع الحساسية عن طريق إعطاء مضاد للمناعة الكلوبيني نوع د إلى الامهات اللاتي يحملن العامل الريصي السالب ويمنح ولادات لأطفال يحملون العامل الريصي الموجب خلال ٧٢ ساعة بعد الولادة. وهذه عادة تنظم بصوره روتينيه بعدد الكريات الحمراء الجنينيه في الدورة الدمويه للام بواسطه اختبار كليور والتي تعمل بصوره روتينيه الى جميع الامهات اللاتي يحملن العامل الريصي السالب.

الهدف: هذه الدراسه خطت لتقدير الحالات العشوائيه لانتقال الدم من الجنين الى الأم خلال الولادة في المرضى العراقيين بصرف النظر عن حاله العامل الريصي للام. لكي نفهم بشكل تام احتماليه الحساسيه المتعاده بعد وضع هادئ.

الأدوات وطريقه البحث: نماذج الدم جمعت من الأمهات بعد الولادة بقليل وحالا صنعت مسحه دمويه رقيقه خضعت الى اختبار كليور. **النتائج:** اظهر ٨٦ نموذج دم جمعت بعد ساعه من الولادة ، ان ٢٥ حاله (٢٨.٩%) أبدت انتقال معنوي بين الجنين والأم ، يتراوح من انتقال بسيط (١ - ٣ مل) تمثل ١٦ حاله (١٨.٦%) ، الى انتقال متوسط (٤ - ١٠ مل) يمثل ٤ حالات (٤.٦%) وانتقال كبير (أكثر من ١٠ مل) يمثل ٥ حالات (٥.٨%). انتقال ضخم بين الأم والجنين (أكثر من ٣٠ مل) لم يكتشف.

الاستنتاج: الانتقال الجنيني للام مع مختلف الدرجات هو حدث متكرر خلال الوضع. الانتقال الضخم بين الجنين والأم غير شائع. أعطاه بصوره منتظمه ٥٠٠ وحده عالميه من مضاد الكلوبولين المناعي نوع د ٧٢ ساعة بعد الولادة لازاله معظم الخلايا الدمويه الجنينيه لدى الأم التي تمنح ولاده لطفل يحمل عامل ريصي موجب.

Introduction

Anti-D antibodies do not develop without exposure of a D-negative individual to D-positive red cells by transfusion or entrance of fetal blood into the maternal circulation. [1] About 85 per cent of all white people are Rh positive and 15 per cent, Rh negative. In American blacks, the percentage of Rh-positives is about 95, whereas in African blacks, it is virtually 100 per cent .[2]

Sensitization of Rh(D) negative mothers by Rh(D) positive fetuses has been greatly reduced by the prophylactic administration of anti-D (Rh0) immunoglobulin. [3] In the United Kingdom (and in our country as well), 500 IU of anti-D (Rh0) immunoglobulin is routinely given post partum to all Rh (D) negative mothers who have given birth to Rh (D) positive babies. This is considered adequate for most deliveries except when there has been a fetomaternal bleed of more than 4 ml.[4]

Massive transplacental hemorrhage is now recognized as one of the causes of anemia of the newborn. The suggestion of Weiner in 1948 that trans-placental hemorrhage from the fetus might be responsible for nonhemolytic anemia in the newborn was confirmed in 1954 by Chown who demonstrated fetal erythrocytes in the circulation of the mother of an anemic baby [5]. The passage of fetal erythrocytes into the maternal circulation may occur as early as the 3rd month of pregnancy, and it

has been suggested that 50% of all pregnancies show evidence of fetal erythrocytes at some stage[6], as well as during obstetric procedures; amniocentesis, external cephalic version[7], etc...

Although in the majority of these pregnancies the fetal blood loss is small and clinically insignificant, massive transplacental hemorrhage in excess of 30 ml has been observed in 0.3 to 0.7% of all pregnancies[5]. Since trans-placental hemorrhage was first described, several cases of neonatal anemia resulting from massive transplacental hemorrhage have been reported[5]. In most of the babies the anemia was evident at birth and in some the hemorrhage was associated with pallor and shock, indicative of acute blood loss.

Detection of fetal blood in maternal circulation[8,9]:

Fetal blood in the maternal circulation was detected by the acid elution technique described previously. After the percentage of fetal erythrocytes in the maternal blood smear had been estimated, the volume of fetal blood loss was calculated by assuming a maternal blood volume of approximately 70 ml/kg body weight at the time of delivery.

Acid elution test of Kleihauer:

Principle: Air-dried and fixed blood films are treated with citric acid-phosphate buffer of low pH. Hb A is dissolved out of the red cells; Hb F remains and is stained with eosin. As it is shown in **figure 1**.

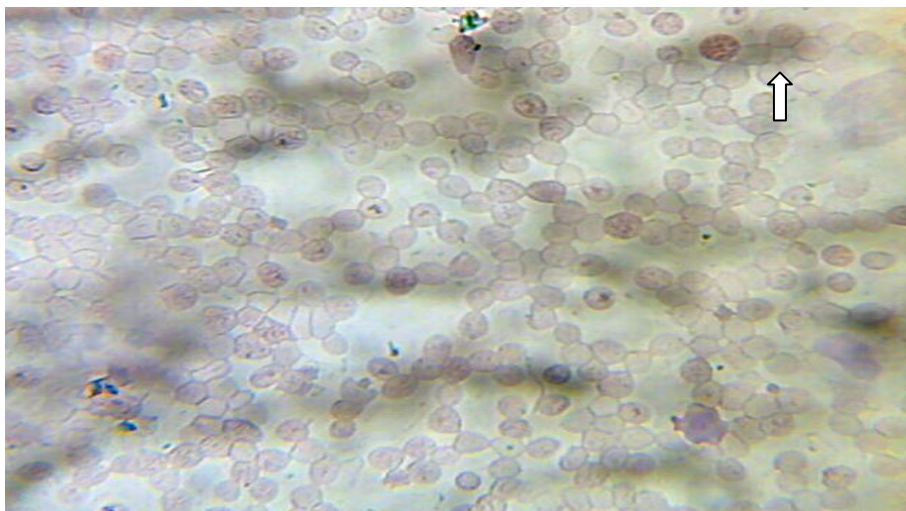


Figure 1 A high power view (x400) of maternal blood showing foetal red cells (arrow) by Kleihauer test

Reagents:

1. Ethyl alcohol, 80%.
2. Citric acid-phosphate buffer, pH 3.2.
- A. Stock solution 1 (0.2 M Na_2HPO_4): Dissolve 36.5 g disodium phosphate in distilled water and dilute to 1000 ml.
- Stock solution 2 (0.1 M citric acid): Dissolve 21 g citric acid ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$) in distilled water and dilute to 1000 ml.
- B. Working solution:

Stock solution 1	24.7 ml.
Stock solution 2	75.3 ml.

 Measure the pH and adjust it at 3.2 if necessary. Warm to 37°C for 15 minutes before use.
3. Alum hæmatoxylin.
4. Eosin 0.1% aqueous solution.

Administration of Anti-D after delivery [2,10]

Anti-D is a concentrated anti-Rh₀ IgG immunoglobulin stored at $2-8^\circ\text{C}$ administered intramuscularly to an Rh₀ mother just after delivery of an Rh₀ positive baby. It is used to suppress the mother's antibody response to Rh₀ (D) positive foetal cells. The patient should not be isoimmunized previously by previous pregnancy or abortion. The baby should give a negative direct Coomb's test result before administration. Positive result may indicate presence of antibodies other than anti-Rh₀. One vial of anti-D (500

IU) neutralizes about 30-35 ml Rh positive blood. If foetal blood loss into the maternal circulation exceeds 35 ml blood, more than one vial is recommended, judged by disappearance of Hb F containing cells 12-24 hours after administration.

Objectives:

This study is designed for detection of random cases of fetomaternal transfusion during delivery in Iraqi patients irrespective of mother's Rh status, in order to understand fully the probability of isoimmunization after uneventful deliveries.

Material and Method

Material:

The 86 blood samples were randomly collected from the delivery department in Babylon Hospital of Maternity And Children during one month period in April 2011.

Method:

Blood samples were collected from mothers just delivered (strictly after 1 hour of delivery), immediately smeared making thin blood films and subjected to Kleihauer test.

Prepare thin blood films of patients and of Hb F-negative (adult blood specimens) as a control. Air dry for 10 min. Fix in 80% ethanol for 5 min. Wash well in water and air dry.

Immerse in buffer in a Coplin jar in 37°C for 5 min. moving the slide up and down. Wash in water and stain with hæmatoxylin for 3 min., wash and stain in eosin for 3 min. Wash, air dry and mount in DPX.

Interpretation: Hb A-containing cells appear as unstained ghosts, while Hb F-containing cells stain red with different shades according to the concentration of Hb F.

The Kleihauer method is exquisitely sensitive, capable of detecting 1 Hb F containing cell/ one million adult cell or about 0.47%.

4 Hb F containing cells / 1 million adult cells = 1% HbF

8 Hb F containing cells / 1 million adult cells = 2% HbF

100 Hb F cells / 100 adult cells = 10% HbF.

The amount of foetal blood that has escaped into the maternal circulation can roughly be calculated using the following formula:

$$\text{ml foetal blood} = \% \text{Hb F cells} \times$$

50

Three patterns of HbF distribution are recognized:

1. Hb A and Hb F present in strictly separate cell populations: seen in fetomaternal hæmorrhage examining the mother's blood and in maternal-foetal hæmorrhage when examining foetal blood.
2. Even distribution of Hb F and Hb A within red cells seen in hereditary persistence of Hb F.
3. Uneven distribution of Hb A and Hb F in red cells seen in thalassæmia (minor and major), S-S disease, Fanconi anæmias and hereditary spherocytosis.

Results

Of the 86 patients, 25 patients showed foetal red cells in the maternal blood in a percentage of more than 1:10000 foetal: maternal cells. The percentage of cases, foetomaternal transfusion in regard to the volume transfused is illustrated in the **table (1)**.

Table 1 Number of cases, volume of foetomaternal transfusion & percentage of cases.

Number of cases	Volume (ml.)	Percent
61	0 ml	70.9 %
9	1 ml	10.4 %
3	2 ml	3.48 %
4	3 ml	4.65 %
1	4 ml	1.16 %
2	5 ml	2.32 %
0	6 ml	0 %
0	7 ml	0 %
0	8 ml	0 %
0	9 ml	0 %
1	10 ml	1.16 %
1	11 ml	1.16 %
3	12 ml	3.48 %
1	13 ml	1.16 %
0	>14 ml	0 %

In general look, the above table (1) shows that most cases are uncomplicated by foetomaternal transfusion (70.9%), while around 29% of the cases show different degrees of transfusion, ranging from minor (1-3 ml) transfusion representing 16 cases out of 86 (18.6%), moderate (4-10 ml) transfusion representing 4 cases (4.6%) and major (> 10 ml) transfusion representing 5 cases (5.8%). Massive foetomaternal transfusion (>30 ml) was no detected in this study.

Discussion

Foetomaternal transfusion represents a major problem in cases of Rh incompatibility, this event is shown to happen frequently during uneventful, unattended deliveries as well as miscarriages (abortions), amniocentesis and foetal manipulation such as external cephalic version. It represents the major cause of Rh isoimmunization and hence an

important cause of infant and foetal morbidity and mortality. On the other hand it may be encountered as a case of neonatal anaemia after massive foetomaternal transfusion. The reverse may occur resulting in haemolytic disease in newborn babies (HDN) after successive pregnancies and appreciable maternal-foetal transfusion.

This study demonstrates the high percentage of cases of foetomaternal transfusions in the usual uneventful deliveries (29%) which necessitates the use of anti-D immunoglobulin. It also demonstrates the adequacy of the measures taken by obstetricians and gynaecologist to prevent future isoimmunization, i.e. the adequacy of 500 IU of anti-D immunoglobulin administered to all Rh₀ (Rh-negative) mothers giving birth to Rh positive babies. This dose usually eliminates all foetal red cells effectively without the need of supplementary doses, as there

are no cases of massive foetomaternal transfusions seen in this study.

One argument to the high positive results of Kleihauer test is that the possibility of thalassaemic patients which are not screened in this study, and included by chance within the patient population. As it is well known, thalassaemic patients show increased levels of Hb F, which usually appears by Kleihauer test as red cells taking different shades of colours due to the presence of mixtures of Hb A and Hb F. In few thalassaemic patients few, red cells show predominance of Hb F which appears similar to foetal red cells. This fact may attribute to a considerable source of errors in performing Kleihauer test, especially in our country, to be clarified by further investigations.

Other methods of detection of foetal red cells has been investigated by many authors, including immunofluorescence, flowcytometry and α -foetoprotein level, all has shown no superiority to Kleihauer test [11 ,12,13,14].

Recommendations

1. The introduction of the sensitive Kleihauer test as an essential tool for the detection and management of foetomaternal transfusion.
2. The introduction of Kleihauer test in the detection and management of haemolytic disease of newborns (HDN).
3. A further study of Kleihauer test on normal Iraqi population, to demonstrate the prevalence of Hb F percent.

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