

Measurement of Fetomaternal Hemorrhage among Sudanese Females

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Abstract

Background: Measurement fetomaternal haemorrhage (FMH) is an important process before the administration of prophylactic anti-D in Rhesus (D) negative women who delivered Rhesus (D) positive baby. This process not always done, and instead standard single dose (500 i.u) of anti-D IgG is administrated to all Rhesus (D) negative women delivered Rhesus (D) positive baby; this standard dose based on the fact that FMH is usually not exceed 4 ml.

Objective: This study aimed to measure the volume of post-partum FMH among Sudanese females using Kleihauer test, and camper the volume in vaginal delivery and caesarean section delivery.

Materials and methods: It is a descriptive cross-sectional study, in which a total of 76 Sudanese post-partum women were enrolled. 19 of them delivered by cesarean section and 57 delivered vaginally. Blood samples were collected from all participants and FMH was measured by Kleihauer-Betke test.

Results: The mean of FMH was 6.2 milliliter (ml). The mean of FMH in normal vaginal delivery was greater than cesarean section but the difference was not statistical significant (Mean±SD: 6.58±4.37 ml,

and 4.98 ± 2.68 ml respectively, $P.value > 0.05$). FMH ≤ 4.0 ml was observed in 34.2% (26/76) and excessive volumes of FMH of more than 4.0 ml was observed in 65.8% (50/76).

Conclusion: FMH is greater than 4 ml in a large proportion of Sudanese women. No statistically significant difference was found in the volume of FMH in women delivered by cesarean section and those delivered vaginally.

Keywords: Fetomaternal hemorrhage; Kleihauer test; Sudanese women

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Introduction

Fetomaternal hemorrhage (FMH) refers to the entry of fetal blood into the maternal circulation before or during delivery [1]. Small bleeds of less than 1ml of blood occur in 96% of all pregnancies, and larger losses of approximately 30 ml occur in up to 0.3% of all pregnancies. Although situations are recognized when it is more likely that a large FMH may occur, these often occur without significant signs or symptoms in either the mother or fetus [2].

Passage of fetal red cells that contain antigens not present in the maternal circulation leads to sensitization and formation of antibodies against these foreign antigens ; these antibodies can pass the placenta in the following pregnancy and cause hemolysis in the fetal circulation. The hemolytic process

may result in anemia or hyperbilirubinemia or both; thereby affecting fetal / neonatal morbidity and mortality; this condition known as hemolytic disease of newborn [3, 4].

Before the discovery of the Rhesus immunoglobulin, HDFN due to anti D was a significant cause of prenatal mortality. Administration of Rhesus immunoglobulin to Rhesus (D) negative women during pregnancy and shortly after the birth of D positive infants has reduced the incidence of Rh D hemolytic disease. The use of prophylaxis anti-D IgG has drastically reduced the cases of Rh-induced HDN; nevertheless, this pathology continues to be relevant in 0.4 of 1,000 births (0.04%), this can be related- at least in part- to the lack of efficacy of immunoprophylaxis because of the administration of an insufficient dose of anti-D IgG that is not congruent with the volume of the foetal-maternal haemorrhage [5].

Therefore, it is important to assess the volume of FMH to determine the dose of anti-D immunoglobulin required to prevent sensitization. The British Committee for Standards in Haematology (BCSH 2006a) guidelines for the use of anti-D immunoglobulin for Rhesus prophylaxis state that, at least 500 iu of anti-D immunoglobulin must be given to every (D) negative woman with no preformed anti-D within 72 hours of delivery of a D positive baby. This dose will be sufficient to prevent sensitization from a bleed of up to 4 ml fetal red cells. FMH greater than 4 ml is rare but unpredictable; 1% of women have a FMH of greater than 4 ml and up to 0.3% greater than 15 ml and may not be protected by a 500 iu and 1500 iu dose of anti-D immunoglobulin, respectively. Therefore, a suitable dose(s) of anti-D immunoglobulin that prevent maternal alloimmunization depends on accurately assessed FMH [2].

In order to examine whether the single standard dose of prophylaxis anti-D is enough to prevent alloimmunization among Sudanese women or not, we conduct his study which aimed to quantify the volume of FMH among Sudanese women, and compare the volume in those delivered vaginally and those delivered by cesarean section.

Materials and methods

Study population and sample collection

Post-partum blood samples were collected in ethylene diamine tetra acetic acid (EDTA) blood tube, from 76 Sudanese women delivered at Alsheikh Ali Fadul and Al- Saudi Hospitals, Omdurman, Sudan, during April 2014. 19 of them were delivered by cesarean section and 57 delivered vaginally.

Kleihauer test

Thin blood films were made on glass slides from maternal specimens; after allowed to air dry, blood films were fixed in 80% methyl alcohol for 3 minutes, and dried in incubator. After that, the slides rinsed for 4 minutes in coplin jar containing buffer (pH 3.3). The slides were then washed off with tap water, and stained with Leishman stain as counter stain. After drying the fetal cells and adult cells were counted in five low power microscopic fields, and the volume of FMH was calculated using the following formula:

$$\text{FMH (ml)} = 1800 \times \frac{\text{fetal cells}}{\text{adult cells}} \times \frac{4}{3}.$$

Statistical analysis

Data of this study was analyzed by statistical package for social sciences (SPSS). Quantitative variables were expressed as mean $\pm$ SD. Means of FMH in cesarean section and normal vaginal delivery were compared by independent 2 sample t-test.

Ethical considerations

This study was approved by faculty of medical laboratory sciences, Al Neelain University, and informed consent was obtained from each participant before sample collection.

Results and discussion

Fetal blood likely enters the maternal circulation during all pregnancies, without apparent clinical significance in most cases [6,7]. The volume lost is typically small, with less than 0.025 ml of fetal red cells observed in 75% of cases, less than 0.5 ml in 96%, and less than 15 ml in more than 99% [7].

In this study, we quantify the volume of FMH in Sudanese women and compare the volume in normal vaginal delivery and cesarean section, to verify whether the use of single standard dose (500 i.u) of anti-D is enough to prevent the formation of Rhesus (D) IgG in Rhesus (D) negative Sudanese women following delivery of Rhesus (D) positive baby or not.

A total of 76 Sudanese women were enrolled in this study, 57(76%) were delivered normally and 19(24%) were delivered by cesarean section. FMH was quantified by acid elution test (Figure 1).

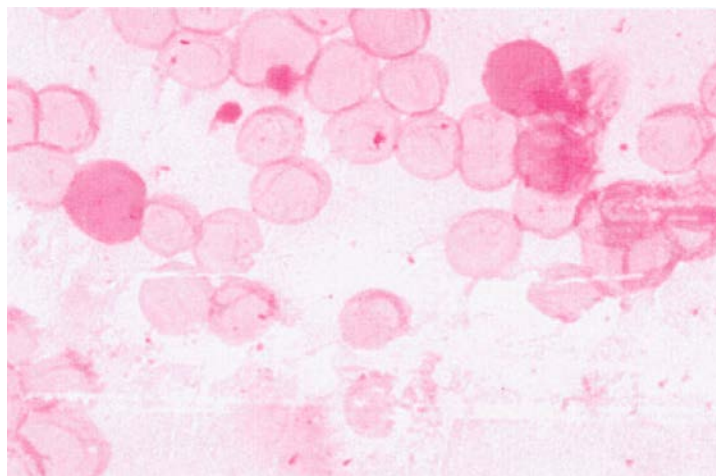


Figure (1): Acid elution test (Kleihauer test): dark cells are Hb-F containing cells (fetal cells); empty (ghost) cells are mother's cells from which Hb-F is eluted.

The mean of the FMH among Sudanese women was 6.2 ml. The mean of FMH in normal vaginal delivery was greater than cesarean section but the difference was not statistically significant (Mean $\pm$ SD: 6.58 $\pm$ 4.37 ml, and 4.98 $\pm$ 2.68 ml respectively, *P*.value: > 0.05).

The result of the present study concludes that there no significant difference in FMH between normal vaginal delivery and delivery by cesarean section. This finding is supported by that of Uriel *et al* (2010) who concluded that, no statistical significance correlation was found between the volume of FMH and type of delivery [8].

FMH ≤ 4.0 ml was observed in 34.2% (26/76) and an excessive volume of FMH of more than 4.0 ml was observed in 65.8% (50/76). This finding indicated that, the prophylactic single dose of 500 i.u could not be enough to prevent alloimmunization in a large proportion of Sudanese females, because this dose is only sufficient to prevent sensitization from a bleed of up to 4 ml fetal red cells [2].

Conclusion

Mean of FMH in Sudanese women is 6.2 ml. excessive FMH greater than 4 ml was observed in a large proportion of Sudanese women. No statistically significant difference was found in the volume of FMH in women delivered by cesarean section and those delivered vaginally.

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