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Haptoglobin phenotypes distribution among Hemolytic Disease of Newborn (HDN)

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Abstract

Background; Hemolytic disease of the newborn (HDN) is a condition in which transplacental passage of maternal antibodies results in immune hemolysis of fetal / neonatal red cells. A Haptoglobin function is to bind free plasma hemoglobin and acute phase reactant protein. The aim of this study was to determine the frequency of Haptoglobin phenotypes among newborns with HDN and to explore the Haptoglobin phenotypes in the fetus with HDN according to their ABO group and Rh system. **Materials and Methods;** A total of 80 patients had HDN were enrolled in this study; 33 (41.25 %) were females and 47 (58.75%) were males; their age ranged (1-8) days. Haptoglobin phenotypes were identified using polyacrylamide gel electrophoresis (PAGE) method. **Discussion and conclusions;** A higher frequency of Haptoglobin 1-1

phenotype was observed among newborns with HDN. Both males and females newborns were showed high percent of Hp1-1 in distribution. The highest hemoglobin concentration was related with Hp1-1 and the lowest concentration was related to Hp2-2. The highest total white blood cells count was related to Hp2-1, and this might described that Hp2 subjects were characterized by a higher immune reactivity. Also we observed the lowest count of RBCs, PCV and indices was related to Hp2-2.The most frequent ABO group in patients was O Rh positive. However, it remains unclear the association between the lowest level of direct and indirect bilirubin and Hp2-2 in our study, perhaps to the small data of Hp2-2 phenotype in the study.

Keywords: Haptoglobin phenotypes (Hp), Hemolytic Disease of The New Born (HDN). Polyacrylamide gel electrophoresis (PAGE)

1.Introduction

Hemolytic disease of the newborn (HDN) is a condition in which the lifespan of the fetal or neonatal RBCs is shortened due to maternal antibodies against red blood cells (RBC) antigens inherited from the father[(1)].Moreover, it accepted also to be as a condition in which transplacental passage of maternal antibodies results in immune hemolysis of fetal/neonatal red cells [(2)]. ABO hemolytic disease occurs almost exclusively in infants of blood group A or B born to O group mothers, because IgG anti A, anti B, occur more commonly in group O than group A or B individual [(3)]. Although the Rh antibody was and still is the most common cause of severe hemolytic disease of the newborn, other alloimmune antibodies belonging to Kell (K and k),

Duffy (Fya), Kidd (Jka and Jkb), and MNSs (M, N, S, and s) systems do cause severe hemolytic disease of the newborn [4].These antibodies are produced when fetal erythrocytes, which express an RBC antigen not expressed in the mother, gain access to the maternal circulation [5].Rh immunization occurs almost exclusively in porous D negative women , who have had at least one prior pregnancy, which stated that D positive fetal red cells cross the placenta in to the D negative mother during pregnancy and at time of delivery, and that this exposure to a foreign antigen leads to the production of anti Rh antibodies[6].

Haptoglobin (Hp) is a α2–sialoglycoprotein with hemoglobin (Hb) binding capacity[7].The well-known biological function of Hp is capture of Hb to prevent both iron loss and kidney damage during hemolysis [8].Hp is also a positive acute phase protein and is characterized by a molecular heterogeneity with three major phenotypes: Hp 1-1 Hp 2-2 and the heterozygous Hp 2-1, which are the expression of two alleles (HP1 and HP2) on chromosome 16q22.1 . Several researches have evaluated the correlation between Hp types in different diseases and cancers, such as leukemia, rheumatoid arthritis, cardiovascular diseases, malaria, diabetes, inflammation, and nephritic syndrome [9, 10, 11, 12].

2.Materials and Methods

A descriptive cross-sectional study was aimed to determine the frequency of haptoglobin phenotypes among newborns with HDN. The diagnosis and clinical evaluation of HDN were performed by the pediatric consults at the maternal hospitals in Khartoum state, Sudan. Eighty newborns were recruited in the study after informed consent from their parents. Thirty three (41.25%) were females and 47(58.75%) were males. An EDTA venous blood was collected from each patient for identifying haptoglobin phenotypes; blood groups (ABO and Rh) and Complete Blood Count, while the serum was used for bilirubin estimation (direct and indirect).

Haptoglobin phenotypes have been performed by discontinuous polyacrylamide gel electrophoresis (PAGE) , according to Davis and Orenstein (1968) method as modified by Linke et al. (1984) (non-reducing) was applied using the Mini-V 8.10 (BRL, Life Technologies Inc, Gaithersburg, USA)[15,16] .Data collected in this study were analyzed using Statistical Package for Social Sciences (SPSS). Significance results were obtained when the P.value≤ 0.05.

3.Results

Haptoglobin phenotypes have different structural features on PAGE. In Hp1-1 phenotype, only one thick band was seen somewhere closer and cathodic to the free hemoglobin band. In Hp2-1 phenotype, there was a band close to the free hemoglobin band, which corresponded to the Hp band in Hp1-1 type and multiple fine bands that were more cathodic as in Hp2-2, but with greater distance between them. In Hp2-2 phenotype, apart from the free hemoglobin band, there were multiple cathodic bands which were fine and closer to one another; as shown, the fastest migrating band among the multiple bands appeared fainter than its preceding band. (Fig1).

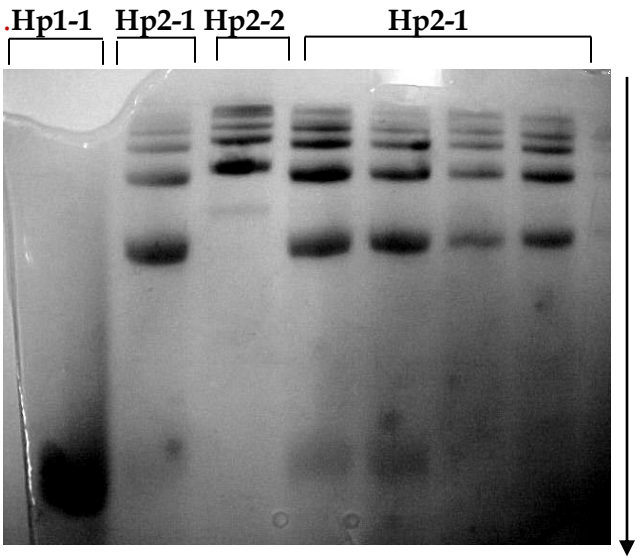

Figure 1: Hp phenotypes on PAGE

3.1Hp and Age

The mean age of the 80 newborns was 2.3 days. The frequencies of Hp1-1, Hp2-2 and Hp2-1 were 53(66.25%),3(3.75%) and 24(30%) respectively. No statistical correlation was found between the newborns age and phenotypes distributions (P.value=0.915).

3.2Haptoglobin and Gender

Of the 80 HDN patients; 47(58.75%) were males and 33(41.25**%**) were females**.** The frequencies of Hp 1-1, 2-1 and 2-2 in males were 63.8%, 31.9% and 4.3% respectively, while in females the frequencies of Hp 1-1, Hp2-1 and Hp2-2 were 69.7%, 27.3% and 1% respectively. No statistical correlation was found between Hp distribution and sex (P.value=0.915).

3.3Hp phenotypes and Hemoglobin concentration (Hb)

The means of Hb concentration in newborns with Hp1-1, Hp2-1 and Hp 2-2 phenotypes were 14.9g/dl, 14.7g/dl and 12.6g/dl respectively. No statistical different was found between those means of RBCs count,(P.value =0.302).

3.4Hp and white blood cells count (WBCs)

The means of WBCs counts in newborns with Hp1-1, Hp2-1 and Hp2-2 phenotypes were 9.5×10^3cu/mm,12.9×10^3cu/mm and 7.8×10^3cu/mm respectively. A statistical different was found between those means of WBCs count (P.value=0.027).

3.5Hp and red blood cells count (RBCs)

The means of RBCs counts in newborns with Hp1-1, Hp2-1and Hp2-2 phenotypes were 4.3×10^6cu/mm, 4.5×10^6cu/mm and 3.9×10^6cu/mm. No statistical different was found between those means of RBCs count,(P.value =0.302).

3.6Hp and Platelets count

The means of platelets count in newborns with Hp 1-1, Hp2-1 and Hp2-2 phenotypes were 226×10^3cu/mm, 224×10^3cu/mm and 263×10^3cu/mm respectively .No statistical different was found between means of Platelets count,(P.value=0.739).

3.7Hp and PCV

The means of PCV in newborns with Hp1-1, Hp2-1, and Hp2-2 phenotypes were 44.9%, 44.9% and 39.3% respectively. No statistical different was found between means of PCV, (P.value=0.460).

3.8Hp and MCV

The means of MCV in newborns with Hp1-1 , Hp2-1 and Hp2-2 phenotypes were 102.8fl, 102.2fl and 101.3fl respectively. No statistical different was found between MCV means with (P.value =0.914).

3.9Hp and MCH

The means of MCH in newborns with Hp1-1, Hp2-1and Hp2-2 phenotypes were (35.2p.g), (34.2p.g) and (35p.g) respectively .No statistical different was found between them,(P.value =0.144).

3.10Hp and MCHC

The means of MCHC in newborns with Hp1-1, Hp2-1and Hp2-2 phenotypes were 33.9 g/dl, 33.1g/dl and 34.1g/dl respectively.

3.11Hp and Direct Bilirubin

The means of direct bilirubin in newborns with Hp1-1, Hp2-1and Hp2-2 phenotypes were 1.2mg/dl, 1.9mg/dl and 0.9mg/dl. No correlation was found (P.value=0.424).

3.12Hp and Indirect bilirubin

The means of indirect bilirubin in newborns with Hp1-1, Hp2-1, and Hp2-2 phenotypes were 7.4mg/dl, 7.9mg/dl and 6.0mg/dl. No correlation was found concerning indirect bilirubin and Hp phenotype distribution (P.value=0.742).

3.13The Hp and positive Rh group newborns

The distribution of Hp1-1, Hp2-1 and Hp2-2 phenotypes in Rh positive patients were (70%), (20%) and (10%) respectively. No correlation was found between Hp and positive Rh group (P.value=0.429).

3.14The Hp and negative Rh group newborns

The distribution of Hp1-1, Hp2-1 and Hp2-2 phenotypes in Rh negative patients were (65.7%), (31.4%) and (2.9%) respectively. No correlation was found between Hp and negative Rh group (P.value=0.429).

3.15The Hp and ABO blood group

3.15.1O group

The distribution of HP1-1, Hp2-2 and Hp 2-1 phenotypes in O positive newborns were (67.7%), (29%) and (3.3%) respectively. While, the distribution of Hp1-1, Hp2-1 and Hp2-2 phenotypes in O negative newborns were (100%) Hp1-1.

3.15.2Group A

The distribution of Hp1-1, Hp2-1 and Hp2-2 phenotypes in A positive newborns were (64%), (36%) and (0) respectively, while the distribution of Hp1-1, Hp2-1 and Hp2-2 phenotypes in A negative newborns were (40%), (40%) and (20%) respectively.

3.15.3Group B

The distribution of Hp1-1, Hp2-1 and Hp2-2 phenotypes in B positive newborns were (58.3%), (33.3%) and (8.3%) respectively. The distribution of Hp1-1 phenotype was (100%) in B negative newborns .

3.15.4Group AB

The distribution of Hp1-1 phenotype was (100%) in AB positive newborns .No correlation was found between Hp and ABO Group (P.value= 0.894).

4.Discussion and Conclusions

The biologic functions of Hp can be related to its ability to bind haemoglobin or to modulate immune response [(21)]. Incidence of Hp 1-1 among the HDN newborns was found to be much higher (66.25%) than Hp2-1 and 2-2, and this might due to greater binding capacity of Hp 1-1 to hemoglobin. This study confirmed that, Hp 1-1 was still the most distributed phenotype in African country .Based on the global geographical distribution of haptoglobin phenotypes, Hp1-1 was took 53% from other phenotypes. Moreover, the study was agreed with Kirk *et al.* (1970),

who found that, the higher levels of Hp 1-1 in children probably enhanced their chances of surviving postnatal hemolytic disease caused by ABO incompatibility since this protein could reduce iron loss and kidney damage during hemolysis[22]. On the other hand, we did not find any different in distribution of Hp phenotypes according to sex. Both males and females newborns were showed high percent of Hp1-1 in distribution. On the other hand we found the highest hemoglobin concentration was related with Hp1-1 and the lowest concentration was related to Hp2-2, and these findings were interpreted the strong property of binding free hemoglobin for Hp1-1 rather than Hp2-2. In accordance the highest total white blood cells count was related to Hp2-1, and this might described that Hp2 subjects were characterized by a higher immune reactivity. Also we observed the lowest count of RBCs, PCV and indices was related to Hp2-2. The study also closely agreed with Elagib*et al.* (1998), who found that Hp1-1 phenotype was significant increase (60.8%) in Sudanese patients with uncomplicated and complicated (cerebral) falciparum malaria [24]. Although there was ABO and Rh blood group heterogeneity in all newborns with HDN, but still Hp1-1 was taken the highest incidence. The most frequent ABO group in patients was O Rh positive. However, it remains unclear the association between the lowest level of direct and indirect bilirubin and Hp2-2 in our study, perhaps to the small data of Hp2-2 phenotype in the study.

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