Original article

Correlation between the levels of anti- A/B IgM/IgG antibodies and cord blood bilirubin levels in haemolytic disease of the new-born

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ABSTRACT

Introduction: Haemolytic Disease of the new-born due to ABO incompatibility has emerged as an important cause of unconjugated hyperbilirubinemia in neonates.The presence of IgG antibody in ABO HDN is one characteristic that differentiates it from the other types of haemolytic diseases of the new-born and titres of maternal IgG anti-A/B have been shown to be associated with the risk of ABO-HDN and cord blood bilirubin concentrations.

Aim: The present study was carried out to evaluate the relationship between the level of maternal IgG anti-A/B titres in group O mothers and cord blood bilirubin levels of Group A or B babies born to them at the time of birth.

Results: Group I (A and B children who developed jaundice) Group II (A and B children who did not develop jaundice) and Group III (Blood Group "O" children) were enrolled. Group I babies had higher serum bilirubin levels which was statistically significant. Maternal anti A IgG antibody levels were found in more number of mothers in group I and II while in the cord blood, both anti A IgG and anti B IgG levels were low in all the groups. Although a positive correlation was found between the total bilirubin and antibody titres, the relationship between the variables was weak.

Conclusion: In this era of early discharge of new-born from the hospital, monitoring of cord blood bilirubin levels is warranted to find out new-borns with ABO incompatibility who are at greater risk for developing hyperbilirubinemia.

Key words: Haemolytic disease of the new born (HDN) – cord blood bilirubin level - Maternal IgM and IgG anti A/B titres

INTRODUCTION

Haemolytic Disease of the new-born due to ABO blood group incompatibility (ABO-HDN) has emerged as an important cause of unconjugated hyperbilirubinemia in neonates [1].Hence it is necessary to have a simple and reliable test to predict development and severity of hyperbilirubinemia due to ABO-HDN. Haemoglobin (Hb) levels, haematocrit (Hct) counts, reticulocyte counts, direct Coombs test results, bilirubin levels, and immunoglobulin G (IgG) titters of cord blood and maternal anti-A/anti-B titters have been suggested as approaches for predicting the severity of hyperbilirubinemia in ABO HDN[2]. Presence of IgG antibody in ABO HDN is one characteristic that differentiates it from the other types of haemolytic diseases of the new-born [3, 4].Titres of maternal IgG anti-A/B have been shown to be associated with the risk of ABO-HDN[5]. Cord blood bilirubin concentrations have been used to measure in utero haemolysis[6], and genetically determined differences in bilirubin metabolism have been documented[7].

AIM:

The present study was carried out to evaluate the relationship between the level of maternal IgG anti-A/B titres in South Indian group O mothers and cord blood bilirubin levels of Group A or B babies born to them at the time of birth.

METHODS:

The present prospective study was carried out in the Obstetrics & Gynaecology Department of Government Kilpauk Medical College Hospital, Kilpauk, Chennai 600010 Tamilnadu, India. The protocol of the study was approved by the Institutional Ethics Committee and written informed consent was obtained from all study participants prior to commencement of the study related procedures.

"O" group term pregnant mothers, who were Rh positive and whose blood Hb levels, were more than 10g/dl were selected for the present study. Pregnant mothers with history of known hypersensitivity or medical disorders complicating pregnancy, as well as those mothers with hepatic, or renal diseases were excluded from the study. At the time of entry into the study, basic information about the patient such as age parity was recorded. No complications during pregnancy were reported in the mother's clinical records.

At the time of delivery of the baby, cord blood (15 ml) was collected in the delivery -room in three test tubes and labelled A, B &C.In the first test tube labelled as A, 3 ml of blood was collected with an anticoagulant (EDTA). This was utilized for identifying blood group of the baby as well as estimation of haemoglobin and reticulocyte count.In the test tube labelled as B, 2 ml of blood was collected without an anticoagulant. The blood was allowed to clot and stand for 1 hour. Serum was separated by centrifuging at 3000 revolution per min for 15 minutes and was utilized for the estimation of bilirubin within 1 hour.In the test tube labelled as C, blood was collected without an anticoagulant. The blood was allowed to stand for 1 hour. Serum was separated by centrifuging at 3000 revolution per min for 15 minutes. The serum was stored at -20°C for antibody estimation. Serum separated from five millilitres of blood collected from the mothers under aseptic precautions and stored at -20°C was used for antibody estimation. ABO haemolytic disease was diagnosed when Hct was <45%; reticulocytes amount, >4.5%; and hyperbilirubinemia was present [8]. Estimation of IgG anti-A or anti-B was done after partial neutralization or inactivation of IgM anti-A or anti-B by the following methods: a) Partial neutralization of IgM anti-A or anti-B by blood group substance A or B; b) Inactivation of IgM antibody by thiol reagents and c) Dithiothereitol and looking for clumps or agglutination.

Continuous variables were expressed as mean ± SD and categorical variables as the number and percentage. Student's t-test and chi square-test were used for comparisons between sex and groups. Analysis of variance (ANOVA) was applied for comparisons. The correlations among total, direct, indirect bilirubin levels, haemoglobin and reticulocyte levels of the cord blood and antibody titres of cord blood were determined using Spearman correlations. Multiple linear regression analysis was done to test the dependence of titre of cord blood on antibody titre and bilirubin levels. A p value of <0.05 was considered as statistically significant.

RESULTS

A total of 60 pairs of mothers and babies were included in the present study. Their characteristics are shown in **Table 1**. There were no significant differences between the mother of different groups with respect to age, parity, weight of the mother and their haemoglobin levels as well as the weights of babies in different groups were similar indicating that the three groups were comparable.

The total, direct, indirect bilirubin levels of the cord blood of babies are shown in **Table 2**. Babies who developed jaundice (Group I) had higher total serum bilirubin levels (3.4 vs 1.82) which was statistically significant (p <0.0025) as compared to

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the control group. There was no significant increase in bilirubin levels in group II babies who did not develop jaundice compared to the control group. Ten out of the sixteen children who developed jaundice showed bilirubin concentration equal to or higher than 3 mg/dL whereas only one child in control group showed such values.

Maternal anti A IgG antibody levels were found to be more than 1:32 in significant number of mothers in Group I and II as compared to the control group. Maternal anti B IgG antibody levels were increased more than 1:32 in all the three groups. In the cord blood, both anti A IgG and anti B IgG levels were less than 1:32 in most of the babies in all the three groups (**Table 3**).

Average cord blood haemoglobin levels were comparable and were within the normal range. Those infants who developed jaundice and got admitted in the new-born ward, the level of haemoglobin was found to be decreased to 12 ± 1.3 g. Reticulocyte counts were comparable in all the three groups and the differences were not significant; however the number of reticulocytes were higher in group I.

Although a positive correlation was found between the total bilirubin and antibody titres, the relationship between the variables was weak (r= 0.1051 and 0.1274). The value of R2 the co-efficient determination was 0.011 and the results were not significant. Stepwise multiple regression showed no significant correlation between direct, indirect bilirubin levels, haemoglobin and reticulocyte levels of the cord blood and antibody titres of cord blood.

DISCUSSION

This study carried out to evaluate the relationship between the level of maternal IgG anti-A/B titres in group O mothers and cord blood bilirubin levels of Group A or B babies born to them at the time of birth, indicated that cord blood bilirubin levels were found to be higher in babies who developed jaundice. In this era of early discharge of new-born from the hospital, monitoring of cord blood bilirubin levels is warranted to find out new-borns with ABO incompatibility who are at greater risk for developing hyperbilirubinemia.

In the present study, the severity of haemolysis was mild and higher levels of antibody titre were found in the control group also. Similar higher levels have also been reported due to mosquito bites and intestinal parasitic infections as well as the consequence of vaccination or other antigen exposures [9].

To our knowledge, the current study is the first study to report the relationship between the level of maternal IgG anti-A/B titres in South Indian group O mothers and cord blood bilirubin levels of Group A or B babies born to them at the time of birth in South Indian predominantly Tamil speaking patients. This study emphasised the absence of clinical significance in antibody screening in sera of mothers of hyperbilirubinemia neonates and that cord blood total bilirubin identified infants at risk from severe ABO HDN.

CONCLUSION

Cord blood total bilirubin can be used a single screening test for the early detection of this disorder. Since the number studied is small, it may not be possible to draw generalised conclusions and hence studies carried out at multiple centres across India, are warranted which may throw light on diverse Indian population.

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Table 1 Patient characteristics

Group	Age of mother (years)	Maternal Hb (g/dl)	Weight of mother (kg)	Mode of delivery		Parity of mother			Weight of Baby (kg)	Sex of baby	
				Natural	LSCS	1	2	3		Male	Female
I (n= 16)	23.9 ± 2.0	11.8 ± 1.2	58.63 ± 4.0	16	0	9	7	0	3.0 ± 0.4	9	7
II (n=24)	26.3 ± 3.8	11.6 ± 0.8	60.5 ± 10.8	12	12	8	10	6	2.9 ± 0.3	14	10
Control (n=20)	24.1 ± 2.5	11.4 ± 0.9	58.6 ± 7.17	18	2	10	6	4	2.8 ± 0.2	11	9

Table 2 Cord Bilirubin Haemoglobin levels and Reticulocyte counts

Group	Cord blood bilirubin levels			Hb levels (g/dL)	Reticulocyte Counts (%)
	Total (mg/dL)	Direct (mg/dL)	Indirect (mg/dL)		
Group I (n= 16)	3.4 ± 1.0*	0.9 ± 0.4	2.5 ± 0.7*	17.1 ± 0.6	2.9 ± 0.4
Group II (n=24)	2.5 ± 0.4	0.5 ± 0.2	1.9 ± 0.4	18.2 ± 0.9	2.5 ± 0.2
Control (n=20)	1.8 ± 0.4	0.4± 0.1	1.5± 0.3	18.9 ± 0.7	2.6 ± 0.7

$P < 0.05$

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Table 3 Maternal and Cord Blood IgG Antibody titre

Group	Anti A IgG		Anti B IgG	
	> 1:32	< 1:32	> 1:32	< 1:32
Maternal Blood				
Group I	10	6	10	6
Group II	12	12	6	18
Control	2	18	8	12
Cord Blood				
Group I	0	16	0	16
Group II	0	24	0	24
Control	2	18	6	14

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