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EVALUATION OF SOME HAEMATOLOGICAL PARAMETERS OF NEONATAL JAUNDICE

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ABSTRACT

The effect of jaundice on haematological parameters were carried on thirty full term neonates (15 males and 15 females) who presented with clinical jaundice and 10 control group (without jaundice). The parameters assayed include bilirubin (total, conjugated and unconjugated), ABO and Rhesus blood grouping, glucose-6- phosphate dehydrogenase (G-6-PD) prothrombin time (PT) platelet and Reticulocyte count. Mothers' samples were also collected for the blood grouping. Pre-term babies and babies with other disease conditions apart from jaundice were excluded. Total, conjugated and unconjugated bilirubin levels of 214.49±163umol/l, 40.88±44umol/l and 173.61 ±111.6umol/l respectively were recorded. Mean levels of 18.9±6.6umol/l, 8.8umol/l and 10.1 ±44umol/l total, conjugated and unconjugated bilirubin levels were recorded also in control population. The bilirubin levels were quite high in all the patients when compared with the mean normal control subjects ($P<0.01$). Eleven patients were G-6-PD deficient with mean bilirubin levels of 307±150umol/l and 249±127umol/l for total and unconjugated bilirubin respectively.G-6-PD patients showed higher mean bilirubin level when compared with non G-6-PD subjects. Total and unconjugated bilirubin of non G-6-PD subjects were as fellows 163+53umol/l and 130±75umol/l respectively ($p<0.05$). The ABO and Rh blood grouping of both mother and babies were done and compared. Thirteen patients were ABO incompatible with their mothers. The mean bilirubin levels in ABO incompatible jaundiced patients were 174.9umol/l and 139.4umol/l for total and unconjugated bilirubin respectively. Only two patients were Rh incompatible. Mean prothrombin time of 21.8±12.6 seconds was recorded also and compared with control 14.4+0.5 seconds. There was significant difference between mean (PT) when compared with control ($P<0.05$). Prothrombin time of G-6-PD and ABO incompatible patients did not differ with other patients Mean platelet level and reticulocytes of 139x109±.9/l, 1.27± 0.56% respectively were recorded. There were significant variation in both platelet and reticulocyte count when compared with control 196x109±28/l and 2±0.3% respectively ($P<0.05$).

KEYWORDS: Haematological parameters, neonatal jaundice, ABO and Rh Blood group.

INTRODUCTION

In all countries many children die or suffer from serious illness about the time of birth. In tropical countries the problem is much greater and many more newborn babies become ill or die because medical care is frequently inadequate, while the health of mother is poor and the surroundings into which the babies is born is often dirty and insanitary (Jellifer 1980).

Some of these illness includes jaundice, Haemorhage, vomiting, mastitis, Tetanus, neonatorum, thrush, septicaemia, skin infections, umbilical infection, respiratory infection, diarrhea.

Among the above mentioned, jaundice is one of the commonest hence some are uncommon. Jaundice is a common clinical problem in newborn. Although the need to diagnose and treat hyperbilirubinemia in the healthy term newborn has been controversial (Augustine 1999).

Jaundice (Iceterus) is a condition characterized by raised bilirubin level in the blood (hyperbilirubinaemia). Minor degrees are only detectable chemically (latent jaundice) major degrees are visible inmay remain in the skin and the patient still appear jaundiced although the plasma total bilirubin level has returned to normal (Baron 1969).

Jaundice can be physiological or pathological and may be due to the following:

Obstruction anywhere in the biliary tract (obstructive jaundice), excessive haemolysis of the blood cells (haemolytic jaundice), toxic or infective damage of liver cell (hepato cellular jaundice).

Some babies develop slight jaundice from the third day or later, but this is only slight, and these infants do not become ill and the condition disappears. This is so-called physiological jaundice and is caused by the destruction of red cell that takes place after birth and to the fact that the liver is not fully developed. However, if the jaundice is present on the first day of life or that it becomes very deep, it may be due to an excessive destruction of the baby's blood and is a serious condition which may require blood transfusion or even exchange transfusion hence pathological jaundice**.**

If the jaundice occurs in a baby with umbilical infection and enlargement of the liver, it may be due to spread of such infection to the liver and should be treated with antibiotics such as a mixture of penicillin and streptomycin or tetracycline. If jaundice is accompanied by the passage of dark urine or very pale stools it may be due to a congenital abnormality of the bile duct of the liver or hepatitis, it is occasionally one of the earliest signs of congenital syphilis.

Different conditions are associated with a prolonged cholestatic jaundice in the neonatal period, viral hepatitis; biliary atresia and choledocal cyst are the most frequent causes. Laboratory findings are necessary, although they do not permit an etiologic diagnosis in all cases. Serial ultrasonographic study could be proposed for the evaluation of biliary excretion before and after feeding, in order to differentiate between these three conditions (Haddan *et al*., 1998). Prolonged neonatal jaundice, beyond day 14 of life is very common and of concern to the clinician. Although risk factors such as hemolysis, hypoxia, hypoglycemia, pre-maturity infection must always be considered, the main criteria of a potential severity of neonatal hyperbilirubinemia is the serum concentration of free bilirubin or if it cannot be determined, the bilirubin/albumin ratio. However, in common practice the therapeutic decision is usually based upon the level of total bilirubin serum concentration. Phototherapy and albumin infusion control mist of severe hyperbilirubinaemia, and the use of exchange transfusion is rare nowadays (Labbrune, 1998).

Jaundice is a common fatal manifestation in the neonates, as such all babies with jaundice on the first day of life or with severe jaundice should be seen by a doctor (Jellife 1980).

Objectives of the study

a. To ascertain the effect of jaundice on coagulation. Whether there is a bleeding risk.
b. To find out whether there is a sex differences in the bilirubin level,
c. The severity of jaundice on G-6-PD deficient patients.
d. To compare jaundice level in different blood groups.

MATERIALS AND METHODS

Subjects

Blood sample were collected from a total of forty neonates in new born special care unit (NBSCU) in University of Nigeria Teaching Hospital, Enugu. Their age ranges from 1 day to 1 week. 30 subjects were neonates with high level of bilirubin of above 2 µmol/l while 10 subjects were from neonates with normal bilirbin levels of below 2µmol/l. Premature babies were excluded from this study.

SAMPLE COLLECTION

Precautions were taken on proper collection of the sample; the samples were collected directly into the bottles using only the needles and not the syringes. This is because the veins of the neonates are too tiny that they cannot withstand the negative pressure in the syringe. Their veins collapse easily.

About five ml of blood were collected from each of the neonates (both the jaundiced and the normal subjects). The samples were collected in EDTA bottles for the following test, platelet count, reticulocyte count. blood grouping, G-6-PD in heparinized bottle. Samples for prothrombin time test were collected in trisocinum citrate bottle (that is an anticoagulant of choice in conjugations studies.

However, sample for bilirubin test were collected in a plain bottle.

BILIRUBIN ESTIMATION (the methods adopted was that of Baker (1985)

Serum is diluted with water and diazotized with sulphanilic acid in the presence of diphylline as an accelerating agent. Ascorbic acid is added to stop the reaction and to prevent haemoglobin from interfering with the azo -coupling. The azo bilirubin so formed is then measured at an alkaline pH.

PROCEDURE

Into five test-tubes labeled S (standard) SB (Standard blank), B(Blank),TB (Total bilirubin) and CB (Conjugated bilirubin).

TB: To 0.2ml serum 0.8ml water was added, then add 0.5ml diazo reagent. Followed by 2 ml dyphyline. Stand for 10min, 0.1ml ascorbic acid was added.

CB: 0.2ml serum was added to 0.8ml water, followed by 0.5ml diazo reagent. Stand for 10mins, then add 0.1ml ascrobic acid added and read spectrophotometrially followed immediately by 2ml dyphyilline. S. To 0.2ml standard (200umol) add 0.8ml water, then add 0.5ml diazo reagent, followed by 2ml dyphylline. Stand for 10min, and then add 0.1 ml ascrobic acid.

SB: 0.8ml water was added to 0.1ml ascrobic acid and 0.5ml diazo reagent, followed immediately by 0.2ml standard and 2ml dyphylline.

B. To 0.8ml water add 0.1ml ascrobic acid 0.5ml diazo reagent, followed immediately by 0.2ml serum and 2ml dyphylline.

To all tubes add 1.5ml alkaline tartrate and read the asbsorbance at 6000nm, zeroing the instrument with water. This should be done soon after adding the tartrate.

CALCULATION

$$\text{Serum bilirubin in umol/l} = \frac{\text{Absobance of TB or CB - Absorbance of B}}{\text{Absorbance of S - Absorbance of SB}} \times 200$$

RETICULOCYTE ESTIMATION

The method adoption was cited from of Dacie and lewis Practical Haematology (1994).

SUPRAVITAL STAINING

Retculocyte contain reminants of ribosomal ribonucleic acid which is present in larger amounts in the cytoplasm of the nuecleated cells. Ribosomes has the ability of reacting with basic dye such as brillant creslyl blue to form a blue precipitate of graunules or filaments.

METHOD

1. Equal volume of brillant cresyl blue and test sample was added.
2. Mixed well and incubated for 20 minutes
3. The mixture was resuspended and a film was made on a clean grease free slide.
4. The film was allowed to dry and examined unfixed slide under the microscope using oil immersion objectives.
5. 100 red cells were at least counted and percentage of the retics present was calculated.

$$\text{Parentage Retics} = \frac{\text{Relics}}{\text{Retics + RBC}} \times \frac{100}{1}$$

PLATELET COUNT (BRECKER AND CRONIKITE, METHOD CITED IN LYNCH (1983).

Blood was diluted in a diluting fluid (3% ammonium oxalate) that causes lysis of erythrocytes but has no effects on platelet, which was counted in improved Neubauer chamber. A 1 in 20 dilution of EDTA blood was made by adding 0.38ml of diluting fluid and 0.20ml of EDTA blood sample. The suspension was mixed by inversion for 10min. the improved neubauer chamber was charged and filled with the suspension using a glass capillary. The neubauer counting chamber was placed in moist petridish for 20 min to give time for the platelet to settle. The number of platelet which appeared as small refractile bodies in five squares in the central ruled area of haemocytometer was counted microscopically using X10 Objective.

PROTHROMBIN TIME

METHOD (Quick one stage method)

Glass test tubes were used in collection of whole blood and carrying out the test. Whole blood collected in citrate bottles were centrifuged immediately on getting to the laboratory. The entire tests were performed within four hours of collection of blood.

THE TEST

A potent preparation of rabbit brain emulsion was added to citrated plasma. The mixture is then recalcified and the clothing time estimated.

0.1ml of plasma and brain suspension were delivered into the bottom of the test tube placed in a water bath at 37°C, the content of the test tubes were mixed very well and left at 37° for 2 min. After 2 min 0.1ml of 2.55mmol/l calcium chloride was blown into the tube and a stop watch started immediately. The test tube was continuously titled until the first sign of clot was seen and the stopwatch stopped. The clot development marked the end point of the test. The time was recorded immediately the clot was seen (Dacie1994).

Screening Test for Demonstrating Glucose -6-Phosphate Dehydrogenase Deficiency

Method adopted was cited from Dacie and lewis Practical Haematology (1994)

When red cells are treated with sodium nitrite, haemoglobin is oxidized to methaemoglobin and red cell color changes to brown (tube B) in the presence of the red cell enzyme glucose -6-phosphate dehydrogenase, methylene-blue and glucose, methaemoglobin is gradually reduced back to oxyhaemoglobin and during the incubation at 37°C the brown color changes back to red as in tube C. If the enzyme is decreased or absent, methaemoglobin persists and hence the brown color persist

Blood Grouping

Abo and Rhesus Blood Group Determination

This is used on antigen - antibody - reaction. If an antigen (red blood cell) is placed in an environment of its corresponding antibody (antiserum), agglutination reaction will be seen.

The results obtained were statistically analyzed using the student "T" test.

Mean Total, conjugated and unconjugated bilirubin levels of 214.49±163 µmol/l, 40.88±44 µmol/l and 173.61±111.6 µmol/l were obtained respectively. The normal control group presented a mean total, conjugated and unconjugated bilirubin levels of 18.9±6.6, 10.1±5.5 µmol/l and 8.8±4.1umol/l respectively, (see table 1 and 2). There was significant increase in the bilirubin levels between the patients and the control groups.

Eleven patients (36.63%) were found to be G-6-PD deficient. The patients that were G-6-PD deficient

presented with higher mean bilirubin levels **of** 307±150 µmol/l, 249.5±127µmol/l,16.8±70Umol/l for total conjugated and unconjugated bilirubin respectively when compared with non - G-6-PD subjects (Table 4).

Thirteen patients (4.3.3%) were ABO incompatible with their mothers (Table 5) with mean bilirubin levels of 174.9µmol/l, 35.5Umol/l and 139.4 µmol/l (total, conjugated and unconjugated respectively).

All the thirty neonates with clinical jaundice (100%) had prolonged prothrombin time (21.8+12.6 seconds). When compared with the normal controls (14.4± 0.5seconds). This is shown in (table 1).

There was a significant difference in the platelet and Retics count of the jaundice babies 139 x 10^9/l± 0.9 and 1.27± 0.56% respectively when compared with controls 196 x $10^{9/l}$ ±28 and 2±0.3% respectively as shown in (table 1).

Tables 1: Mean Total Bilirubin. u^conjugated bilirubin, retics count, platelet count and prothrombin time.

	Jaundice	Normal	Level Of Sig Significant
Total bilirubin	214.49+ 163 µmol/l	18.9±6.6 µmol/l	P<.01
Unconjugated bilirubin	1 73.61 ±1116 µmol/l	10.1±5.5 µmol/l	P<0.01
Retics count	1.27+0.56(%)	2+0.3(%)	P<0..01
Platelet	139x1 09+ 0.9 (/I)	196x109+28(/l)	P< 0.01
Prothrombin	21 .8±1 2.6 (second)	14.4±0.5sec.	P < 0.01

Table 2: Mean Total Conjugated, Unconjugated Bilirubin Level And Normal Control Bilirubin Level.

	Jaundice µmol/l	Normal µmol/l	Level Significant
Total Bilirubin	241.49±163	18.916.6	P<0.01
Conjugated Bil.	40.88+ 44	10.1+5.5	P<0.01
Unconjugated Bil.	173.61+111.6	8.8+4.1	P<0.01

Table 3: Mean Total bilirubin, unconjugated bilirubin retics count, platelet count and prothrombin test in mmle and female.

	Male	Female	Level Of Sign.
Total bilirubin	208.0±132.8 µmol/l	221 ±120.6 µmol/l	P>0.7
Unconjugated Bil.	182.2±1112 µmol/l	165±115.27 µmol/l	P>0.6
Retics count	1.22+ 0.5 (%)	1 .3+0.62 %	P>0.6
Platelet count	130x10^9 + 22(K)	147x10^9±34/l	p>0.1
Prothrombin Time	24.1±17.7 sec.	1 9+1 .3 seconds	p>0.2

Table 4: Mean Total And Unconjugated Bilirubin In G-6-Pd And Non G-6-Pd Subjects

	G-6-PD	NON G-6-PD	LEVELS OF SIGN.
Total biliru>irt	307+1 50 µmol/l	161+53 µmol/l	PO.01
Unconjugated	249.5±124 µmol/l	130±75 µmol/l	P<0.01

Table 5: Abo And Rh Incompatibility Compared With Bilirubin Levels Bilirubin Level.

	G-6-PD	NON G-6-PD	LEVELS OF SIGN.
Total biliru>irt	307+1 50 µmol/l	161+53 µmol/l	PO.01
Unconjugated	249.5±124 µmol/l	130±75 µmol/l	P<0.01

Table 6: Incidence of Jaundice Among Abo/Rhesus Incompatibility And G-6-Pd In Degrees And Percentage Number

	Number	Degrees	Percentage
ABO incompatibility	13	156	43.3
Rhesus incompatibility	2	24	6.66
G-6-PD	11	132	36.63
Non ABO/Rh incompatibility. and non g-6-pd	4	48	13.32

DISCUSSION

Forty samples were tested in this study out of this number; ten were from normal control subjects. The blood group (ABO and Rhesus grouping) both of the matter and babies were carried out and the result are shown figure 1. Other tests such as Bilirubin level, glucose -6-phosphate deydrogenase, platelet count.

Reticulocyte count, prothrombin time was also carried out.

Mean, total, conjugated and unconjugated bilirubin levels of 241.49± 163Umol/l, 40.88± 44Umol/l and 173.61 ±111.6Umol/l respectively were recorded. These results affirm hyperbilirubinemia as the cause of neotatal jaundice and this is in parity with works of Loiascon *et al*. (1999), Agrawal *et al*. (1998) and Akaba *et al*. (1999).

Forty three percent of the neonatal jaundice cases were due to ABO incompatibility. This agrees with the findings of Embry *et al.* (1994) and Dawodu *et al.* (1998). Only two cases (6.6%) were due to Rhesus incompatibility. The sample size however is not enough for any meaningful statistical comparison. Azubike (1979) reported that about (5%) of neonatal jaundice in this part of the country was due to RH incompatibility. Neonatal jaundice due to G-6PD was recorded on 11(36.6%) of the patients studied and with high mean bilirubin levels of 307±150Umol/l and 249.5±Umol/l total and unjugated bilirubin respectively. Kaplan (1998) observed that G-6-PD patients present with higher bilirubin levels than ABO and Rh incompatibility. Four G-6-PD patients present with bilirubin levels above 350umol/l, above which calls for exchange blood transfusion and phototherapy.

The entire patient had raised prothrombin time with mean PT of 21.8±12.6 seconds. This agrees with work of Suitor *et al.* (1999).

Mean platelet count of 139 $x10^9$±.9/l was recorded from this work which differs significantly with control platelet count of 196 x 10^9±28/l (PO.05).

In this work we that observed that 1 % Ammonium oxadate could not lyse the patients' red blood cell, even when kept overnight. This could be attributed to high percentage of fetal Haemoglobin (HbF) which resists alkaline denaturation in neonatal samples. Hence a modified method of 3% ammonium oxalate was used to lyse the red cells. This has not been documented in any known write up.

CONCLUSION

In conclusion therefore, there is hyperbilirubinemia in clinical jaundice. This is associated with G-6PD, Rh and ABO incompatibility; there is also increased prothrombin time. There is a reduction in platelet and Retics count when compared with the normal control samples.

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