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Glucose-6-Phosphate Dehydrogenase (G-6-PD) Status, Aflatoxins and Neonatal Jaundice

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Summary

Ahmed H, Hendrickse RG, Yakubu AM and Maxwell SM. Glucose-6-Phosphate Dehydrogenase (G-6-PD) Status, Aflatoxins and Neotanal Jaundice. *Nigerian Journal of Paediatrics 1995; 22:3.* In the present study of G-6-PD status, aflatoxins and neonatal jaundice, involving, 78 jaundiced and control in-born babies and 124 jaundiced and control out-born infants, G-6-PD was deficient in more male jaundiced babies (in-and out-borns) than in the controls ($P<0.001$). Aflatoxins were detected from cord blood in 30.0 percent of all the in-born babies and from peripheral blood in 24.2 percent of all the out-born infants. Relationship between G-6-PD activity in the 77 jaundiced and control in-born babies, prenatal exposure to aflatoxins and the presence of jaundice, revealed no correlation between cord blood concentration of aflatoxins and G-6-PD activity in either the jaundiced babies ($P>0.05$), or in the controls ($P>0.05$). The frequency of aflatoxin detection in peripheral blood in relation to G-6-PD status and the presence or absence of jaundice, showed that the detection level was independent of the G-6-PD status of the in-and out-born jaundiced babies ($P = 0.1753$) and of the controls ($P = 0.3901$). The present findings suggest that *in-utero* transfer of aflatoxins does not seem to predispose the G-6-PD-deficient baby to any extra risk of developing jaundice, nor does peripheral blood aflatoxin pose any additional risk to the G-6-PD-deficient baby in the development of jaundice.

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Introduction

AFLATOXINS are mycotoxins produced by certain strains of *Aspergillus flavus*. These

fungal toxins are hepatotoxic to a wide variety of animals, though species susceptibility varies greatly.[1] Widespread exposure to aflatoxins in food and breast milk consumed by children in tropical Africa has been reported.[2 3] These toxins also occur in sera, urine and stool obtained from children in tropical Africa.[2-4] *In-utero* transfer of aflatoxins and the deleterious effects on neonatal pigs have been demonstrated.[5] Transplacental transfer of aflatoxins to babies has also been reported from several African countries including Nigeria.[6] The effects of foetal and neonatal aflatoxin exposure in man are, however, not very clear; these need urgent investigation.

The liver is the main target organ for aflatoxin toxicity and the very young animal species are more susceptible to aflatoxicosis than adults.[5 7] Aflatoxins are very potent generators of free radicals believed to be important in the pathogenesis of liver damage by aflatoxins.[8] Although the epoxy derivative of aflatoxin is formed exclusively in the liver, peroxidation of membranes is not limited to this organ alone.[8 9] Aflatoxins have thus , emerged as an unsuspected risk factor in neonatal jaundice. Hypothetically, free radicals produced by aflatoxins may induce oxidative damage on the red blood cells of the G-6-PD deficient infant whose red cells have impaired ability to cope with oxidant stress susceptibility to damage. The purpose of the present study was therefore, to investigate firstly, the relationship between prenatal exposure to aflatoxins, G-6-PD status of new-born babies and subsequent development of neonatal jaundice and secondly, the frequency of aflatoxin detection in peripheral blood of jaundiced and control babies in relation to G-6-PD status.

Subjects and Methods

The study was jointly carried out by us at the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and by workers at the Department of Tropical Paediatrics and International Child Health, Liverpool, United Kingdom (UK). The subjects comprised two sets of babies, namely: in-born neonates at the ABUTH and out-borns with neonatal jaundice, who were admitted to the emergency paediatric unit, ABUTH. In-born babies who did not subsequently develop neonatal jaundice, 28 days after birth, constituted the controls for those in-borns who developed significant jaundice (total serum bilirubin $\geq$ 170 μmol/L, 10mg/dl). The controls for the out-borns consisted of other newborn babies matched for age and sex.

Relevant history was obtained in respect of each of the subjects and controls; a clinical examination was also undertaken, including assessment of gestational age, using the scoring system that has been found suitable and reliable for the assessment of maturity in African neonates.[10]
Total and conjugated serum bilirubin, estimated by the photoelectric method,[11] was performed daily in all the jaundiced babies until the total bilirubin fell below 170μmol /L (10mg/dl) on two consecutive days and only once in the controls. Other laboratory investigations carried out on all jaundiced babies and controls included ABO blood group, Rh type, direct Coomb's test, as and when indicated, and G-6-PD estimation, performed firstly, by the photometric dye uptake screening method[12] and secondly, by

the spectrophotometric quantitative method.[13] Other investigations, where relevant, included total and differential white cell counts, reticulocyte counts, red blood cell morphlogy, red cell indices and counts, platelet count, blood culture and swabs for culture. Maternal ABO blood group and Rh type, antibodies (haemolytic and anti-A, anti-B) were carried out, as and when indicated, according to the method described by Worlledge *et al.*[14]

Cord and peripheral blood was obtained for the assay of the six major aflatoxins (B_1, B_2, G_1, G_2, M_1, M_2) and aflatoxicol, from all the in-born infants, except that, for the in-born controls, the peripheral blood was obtained on day 28 of life. Peripheral blood alone was obtained from the out-born babies for the aflatoxin assay. Ten milliliters (ml) of the cord blood were collected into stoppered plastic test tubes from the maternal end of the cord, after it had been clamped and severed. The blood was allowed to clot at room temperature, centrifuged at 3000 rpm for five minutes and serum separated and stored at -20^0C. Two ml of the peripheral venous blood obtained from the subjects and controls, were treated similarly. All the sera were transported frozen in insulated containers containing dry ice to Liverpool for the aflatoxin assay. Prior to assay by high performance liquid chromatography, aflatoxins were extracted by the method described by Nelson *et al,*[15] which is more sensitive than other methods.[16]

Informed parental consent was obtained orally before an infant was entered into the study which was approved by the Ethical Committee of ABUTH. Statistical analysis was carried out, using the Chi-square test and Student's 't' test. Where figures were small, Fisher's exact probability test was used. Correlation coefficients were determined where appropriate. For the calculation of correlation coefficient, actual figures for aflatoxins were used, while for construction of the scattergram, $\log_e$ was used for convenience.

Results

There were 78 in-born babies, 38 (48.7-percent) of whom developed jaundice and 40 (51.3 percent) who did not; these non-jaundiced 40 babies constituted the controls for the 38 jaundiced infants. Total serum bilirubin in the 38 babies ranged from 171 to 342 µmol/L (10.1 to 20.1mg/dl) with a mean of 251µmol/L (14.8mg/dl). Table I lists possible aetiological factors in the 38 in-born jaundiced babies. The commonest aetiologic factors were sepsis plus G-6-PD deficiency in eight (21.1 percent) and sepsis plus LBW in four (10.5 percent) of the cases. The G-6-PD status of the 78 in-born babies (jaundiced and controls) are summarized in Table II. G-6-PD deficiency was more frequent in the jaundiced males than females ($X^2 = 9.700$, df = 2, $P<0.001$) and marked in males than females ($X^2 = 9.444$, df = 2, $P<0.0001$) in the control group. G-6-6PD deficiency was more frequent in the jaundiced males than in control males ($X^2 = 10.041$, df = 1, $P<0.0015$). Among the females, there was no difference in G-6-PD activity between the controls and jaundiced infants ($X^2 = 1.891$, df = 2, $P>0.05$).

Cord blood aflatoxin was assayed in 37 of the 38 in-born infants with neonatal jaundice and aflatoxin was detected in 14 (37.8

TABLE I

Aetiological Factors in 38 jaundiced In-born Babies.

Factor	*No of Cases*	*Percent of Total*
Sepsis + G-6-PD deficiency	8	21.1
Sepsis + LBW	4	10.5
Sepsis alone	3	7.9
Sepsis + ABO inc + cephalhaematoma	2	5.3
Sepsis + ABO inc + LBW	1	2.6
Sepsis + G-6-PD defc + ABO inc	1	2.6
G-6-PD defc alone	3	7.9
G-6-PD defc + LBW	1	2.6
G-6-PD defc + ABO inc	1	2.6
G-6-PD defc + ABO inc + LBW	2	5.3
LBW alone	2	5.3
LBW + ABO inc	1	2.6
ABO inc alone	2	5.3
Unknown	7	18.4
Total	38	100.0

LBW = Low birthweight
ABO inc = ABO incompatibility
G-6-PD defc = G-6-PD deficiency

TABLE II

G-6-PD Status of 38 jaundiced In-born Babies and their 40 Controls

Status	*Jaundiced*		*Control*	
	Male	*Female*	*Male*	*Female*
Deficient	15(53.6)	1(10)	2(8.3)	-
Intermediate	-	2(20)	-	5(31.25)
Normal	13(46.4)	7(70)	22(91.7)	11(68.75)
Total	28(100)	10(100)	24(100)	16(100)

Figures in parentheses represent percent of total

percent), while in the 40 controls, aflatoxin was detected in nine (22.5 percent). Total aflatoxin detection in both jaundiced and control in-born babies was thus, 23(30.0 percent) of the 77 babies. There was no difference (X^2 = 1.488, df = 1, P = 0.2225) between aflatoxin detection in the 37 jaundiced in-borns and the 40 controls. The mean aflatoxin concentration of 3575pg/ml (range, 13 to 238177pg/ml) in the 14 jaundiced in-born babies, was higher than in the nine aflatoxin-positive in-born controls ($P<0.05$). The concentration, ranging from 214 to 238,177pg/ml and mean, 82,481pg/ml, of aflatoxin B_1 (most toxic of the aflatoxin fractions) was higher in the jaundiced babies than in the controls (range, 474 to 2,216pg/ml mean, 1342pg/ml).

Aflatoxins were detected in peripheral blood of nine (24.3 percent) of the 37 jaundiced in-born babies and of 12 (30.0 percent) of the 40 in-born controls. This difference in peripheral blood detection of aflatoxin between the jaundiced and control in-born babies was not significant (X^2 = 0.092,df = 1, P = 0.76). The concentration of aflatoxins detected in peripheral blood of the jaundiced in-born babies ranged from 24 to 23,749pg/ml (mean, 5,086pg/ml), while the concentration ranged from 5 to 2,315pg/ml (mean, 1018pg/ml) in the in-born controls. Peripheral blood concentration of aflatoxins in the jaundiced in-borns was higher (t = 1,845, df =19,P<0.05) than in the in-born controls. The relationship between G-6-PD activity of all the 77 in-born babies (jaundiced and control), prenatal exposure to aflatoxins and the presence or absence of jaundice is shown in Fig. There was no correlation between cord blood aflatoxin concentration and G-6-PD activity

in either the jaundiced babies, (r =0.00933, P>0.05), or in the controls (r = 0.1326, P>0.05).

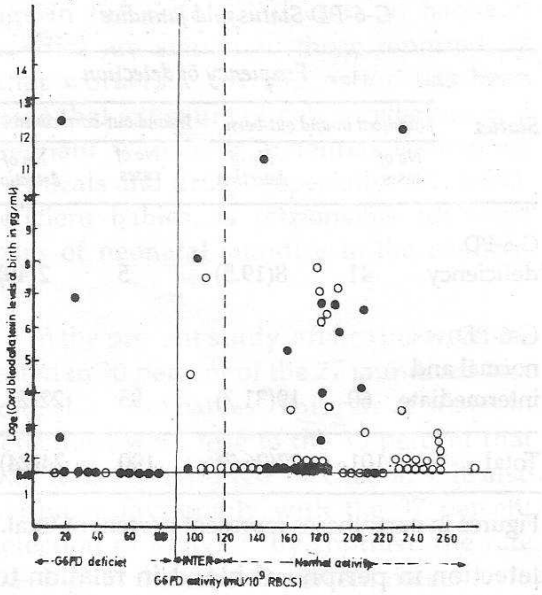

Fig. Relationship between cord blood aflatoxin levels at birth, G-6-PD activity and development of neonatal jaundice in 77 in-born babies. ● = Developed jaundice o=Not jaundiced. Each point represents an infant. INTER = Intermediate G-6-PD activity. *Horizontal line across $\log_e$ (1.6) means no aflatoxins detected.

Total peak serum bilirubin among the 64 out-born babies with jaundice, ranged from 181 to 1054μmol/L (10.6 to 62mg/dl) with a mean of 347μmol/L, while that of the 60 controls ranged from 12 to 109μmol/L (0.7 to 6.4mg/dl) and a mean of 50μmol/L (2.9mg/dl). In Table III are summarized the aetiologic factors in the 64 out-born babies with jaundice. As in the jaundiced in-born babies, sepsis plus G-6-PD deficiency was the commonest factor, being 13 (20.3 percent) of the 64 patients. Table IV contains the G-6-PD status of the 64 jaundiced out-born babies and their 60 controls. There was a significant difference (X^2 = 5.393, df = 2, P<0.05) between males and females of these jaundiced out-born babies and also between male and female out-born controls (X^2 = 10.541, df = 2, P<0.001). Between the jaundiced and the controls (males and females combined), there was a significant difference (X^2 = 12.474,df = 1, P<0.001).

Aflatoxins were detected in the peripheral blood of 18 (28.1 percent) of the 64

TABLE III

Aetiological Factors in 64 jaundiced Out-born Babies

Factor	*No of Cases*	*Percent of Total*
Sepsis + G-6-PD deficiency	13	20.3
Sepsis alone	9	14.0
Sepsis + LBW	7	10.9
Sepsis +ABO inc	4	6.2
Sepsis + G-6-PD defc + ABO incl	1	1.6
Sepsis + G-6-PD defc + LBW	1	1.6
Sepsis + cephalhaematoma	1	1.6
Sepsis +ABO inc + LBW	1	1.6
Sepsis + cephalhaematoma + LBW	1	1.6
Sepsis + ABO inc + LBW	1	1.6
G-6-PD defc alone	4	6.2
G-6-PD defc + LBW	3	4.7
G-6-PD defc +ABO inc	1	1.6
G-6-PD defc + ABO inc + cephalhaematoma	1	1.6
LBW alone	5	7.8
ABO-inc alone	6	9.3
Unknown	5	7.8
Total	64	100.0

LBW = Low birthweight, ABO-inc = ABO-incompatibility, G-6-PD defc = G-6-PD deficiency.

TABLE IV

G-6-PD Status of 64 jaundiced Out-born Babies and their 60 Controls

Status	Out-Born			
	Jaundiced		Control	
	Male	Female	Male	Female
Deficient	20(54.1)	5(18.5)	2(7.7)	1(2.9)
Intermediate	-	1(3.7)	-	11(32.3)
Normal	17(45.9)	21(77.8)	24(92.3)	22(64.8)
Total	37(100)	27(100)	26(100)	34(100)

Figures in parentheses represent percent of total

jaundiced out-born babies and of 12 (20.0 percent) of the 60 controls; total aflatoxin detection was therefore 30 (24.2 percent) of the 124 out-born babies. There was no difference (X^2 = 0.7156, df = 1, P = 0.3975) at this level of detection between the patients and controls. Total concentration of aflatoxins in the jaundiced out-borns ranged from 20 to 10,239pg/ml (mean, 1561pg/ml) and from 89 to 6561pg/ml (mean, 1736pg/ml) in the controls. The difference in this mean concentration between the patients and the controls was not significant (P>0.05). The disparity between the jaundiced in-borns in relation to peripheral blood aflatoxin concentration, may be related to differences in the ages at presentation. The mean age of jaundiced in-borns at presentation was 3.6 days, while it was 28 days in in-born controls (P<0.05). By contrast, the mean age of the jaundiced out-born babies was 5.5 days at presentation, while that of the controls was 6.0 days (P>0.05).

Table V shows the frequency of aflatoxin

TABLE V

Frequency of Aflatoxin Detection in Peripheral Blood, G-6-PD Status and Jaundice

Status	Frequency of detection			
	Jaundiced in-and out-born		In-and out-born control	
	No of cases	No of detection	No of cases	No of detection
G-6-PD deficiency	41	8(19.5)	5	2(40)
G-6-PD normal and intermediate	60	19(31.7)	95	22(23.2)
Total	101	27(26.7)	100	24(24)

Figures in parentheses represent percent of total.

detection in peripheral blood in relation to G-6-PD status and the presence or absence of jaundice. The frequency of detection was independent of G-6-PD status of the in-and out-born jaundiced babies combined (X^2 = 1,1837, df = 1, P = 0.1753). The frequency of detection was also independent of G-6-PD status of the controls combined (X^2 = 0.7391, df = 1, P = 0.3901).

Discussion

The G-6-PD status of the 78 jaundiced and control in-born babies in the present study, has revealed deficiency of the enzyme in more jaundiced males than females. The deficiency was also more frequent in jaundiced males than in control males. Similarly, there was a significant difference between males and females of the 64 jaundiced out-born babies, as well as between male and female out-born controls. In both in-and out-born babies, the commonest

aetiologic factors were sepsis plus G-6-PD deficiency and sepsis alone in 21.0 percent and 10.5 percent, respectively. These findings in the aetiologic factors in neonatal jaundice are similar to those reported by other workers.[17] A suggestion has been made that exposure of newborn babies and pregnant women to potential icterogenic chemicals and drugs, especially to G-6-PD-deficient babies, is responsible for most cases of neonatal jaundice in the country.[18]

In the present study, aflatoxins were detected in 30 percent of the 77 jaundiced and control in-born babies. This rate of aflatoxin detections was close to the 35 percent that was recently reported in Ghana;[6] it also compares favourably with the 37 percent detection in Kenya.[4] By contrast, the rate was higher than the 12 percent detection in Jos.[6] The difference in the overall aflatoxins detection seems to be related to the extent of maternal aflatoxin exposure in different places.[6] The finding of significant concentration of aflatoxin in the jaundiced in-born babies than in the controls, indicates either *in-utero* exposure to high aflatoxin concentration that predisposed the babies to the development of jaundice, or an inability of the jaundiced babies to metabolize aflatoxins as efficiently as the controls. Regarding aflatoxin detection in the 124 out-born jaundiced babies and the controls, it was difficult to reach a firm conclusion on the influence of the aflatoxins on the development of the neonatal jaundice, as these substances were detected from the peripheral blood.

Possible mechanisms in the development of neonatal jaundice in G-6-PD deficient babies exposed to aflatoxins, is based on scientific speculations. However, the present findings would suggest that *in-utero* transfer of aflatoxins does not seem to predispose the G-6-PD-deficient baby to extra risk of developing jaundice. Additionally, an analysis of aflatoxin concentration in peripheral, as against the concentration in cord blood, in relation to G-6-PD status and development or otherwise of jaundice, did not reveal that peripheral blood aflatoxin posed any additional risk to the G-6-PD-deficient baby in the development of jaundice. As has been shown above, in the relationship between G-6-PD activity in all the 77 jaundiced and control in-born babies, prenatal exposure to aflatoxins and the presence or absence of jaundice, there was no correlation between cord blood aflatoxin concentration and G-6-PD activity in either the jaundiced babies or the controls.

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