

Maternal Determinants of Cord Blood Immunoglobulin Levels: a Preliminary Report

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Abstract

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Background and Objective: Immunoglobulin G (Ig G) is essential for the promotion of growth and protection particularly during neonatal life. Although various aspects of Ig G have been studied, we are unaware of any study relating maternal social factors to neonatal Ig G levels. This study aims at determining the relationship between maternal factors and the cord serum Ig G.

Methods: This was a prospective longitudinal cohort study involving administration of questionnaires on women with normal pregnancy confirmed by the obstetrician. Specimens of umbilical blood were obtained at delivery from mother/neonate pair and the serum Ig G levels determined on the samples using enzyme linked immunoassay. These levels were correlated with certain maternal factors such as age, vaccination with tetanus during the current pregnancy, illnesses such as malaria fever during pregnancy among others. Exclusion criteria included sepsis, chorioamnionitis and chronic illnesses.

Result: Cord blood was obtained from 86 mother/ neonate pairs (47 females and 39 males). The mean \pm SD serum Ig G was 1297.8mg/ml \pm 995.6. The mean age of the mother was 27 \pm 3 years, while the gestational age of the neonates was 37.6 weeks \pm 2.8. The mean cord serum Ig G levels in neonates of mothers <25 years of age and mothers >25 years of age were 1611.2 mg/ml and 1183.5mg/ml respectively (p = 0.038).

The social classes of the mothers included low 51(59.3percent), middle 21(24.4 percent) and high 14(16.3 percent) with mean cord serum Ig G of 941 mg/ml, 1157.4 mg/ml and 1476.7 mg/ml respectively.

Malaria fever in pregnancy apparently occurred in 31(36 percent), 29 of whom had one to two episodes while the remaining had ≥ 3 episodes. The mean cord serum Ig G levels in neonates of mothers with and without malaria fever were 1526.3mg/ml and 1089.6mg/ml respectively (p = 0.016).

The duration was < 12 hours among 32(37 percent) mothers in whom the cord serum IgG levels was 2156mg/ml, while the duration was >12hours in 54(63 percent) with mean cord serum Ig G of 921mg/ml respectively (p = 0.005). Conversely, there was no correlation with the other variables.

Conclusion: There was significant correlation between cord serum Ig G levels and malaria fever in pregnancy, maternal age and duration of labour.

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Introduction

THE lack of opsonic antibodies is an important risk factor for the immunity of newborns to infections caused by many bacteria with polysaccharide capsules that cause serious infections in newborns.^{1,2} Maternal transport of immunoglobulins to the fetus occurs transplacentally beginning around the 8th to 10th week of gestation and accelerating during the last trimester. This constitutes the fetal humoral immune activity leading among other things to nonspecific stimulation of different cellular receptors and induction of immunoregulation that are essential for fetal development, growth and survival.^{3, 4, 5, 6}

Furthermore, a deficiency of Ig G has been reported to predispose to infections, stunted growth and death.^{5,6,7} Various factors have been associated with low neonatal serum Ig G. Placental malaria and maternal HIV infection have been reported to be associated with a decrease in neonatal IgG antibody transfer. In addition, raised maternal total serum IgG titres were correlated with reduction in neonatal serum IgG antibody.^{8,9} However, there had not been a previous report of the relating maternal social factors and antenatal care to the neonatal Ig G level. The goal of this initial report is to document fetal immunoglobulin levels and identify maternal factors and antenatal events which are significant in the determination of these levels.

Hypothesis

Our hypothesis is that maternal factors and antenatal events are significant correlational variables in the determination of fetal immunoglobulin status.

Patients and Method

This was a prospective longitudinal cohort study of the sociodemographic data of mothers with normal pregnancy and the serum Ig G of their neonates at delivery.

The study took place at the University College Hospital Ibadan and Bilal Mission Hospital, Agodi, Ibadan. The University College Hospital is a tertiary center with specialized obstetrics facilities and an estimated average deliveries of 100 per month, while Bilal Mission is a primary care centre managed by a retired trained nurse and supervised by a general practitioner. The service is mainly maternity with an estimated average of 60 deliveries per month. The University College Hospital caters for all social classes while most of those who patronize Bilal Mission belong to the lower social class.

The sample size of 118 mother/neonate pairs was calculated using an online module.¹⁰ Our patients were consecutive mothers with normal pregnancy as judged by the obstetrician. Pregnant women with history of sepsis, chorioamnionitis and other chronic medical illnesses such as asthma, allergy, diabetes, and hypertension were excluded.

Participants were recruited into the study following ethical approval by the UI/UCH ethical committee (UI/IRC/07/023). The pregnant mothers who were in the last trimester of pregnancy were counseled, after which questionnaires were administered to them during their last antenatal clinic which were usually 2 - 4 weeks before the estimated date of delivery. Information sought included immunization with the three doses of tetanus vaccines, episodes of malaria fever, antenatal care during pregnancy, duration of

labour, foetal weight and gestational age. The questionnaire was administered to the mothers in the last antenatal clinic between 2 - 4 weeks before estimated date of delivery. The questionnaire was pretested initially by both the authors and assistant together to serve as training for the assistants and later by the assistants alone.

At delivery foetal cord blood was taken from the umbilical vein using a 21G needle and 5 ml syringe. Serum was extracted from the specimen within 24 hours by normal centrifuging and the use of a Pasteur pipette. The samples were stored at -80°C, pooled together and analysed for Ig G levels.

Immunoglobulin Measurement

Serum levels of Immunoglobulin G were quantified by the single radial immunodiffusion method. A 3% noble agar was prepared in phosphate buffered saline (PBS, pH7.2) containing 0.2% sodium azide. One milliliter of each antiserum (anti-human immunoglobulin class) was mixed with 7ml of PBS. Eight milliliters of the 3% noble agar was thoroughly mixed with the diluted antiserum. The mixture was carefully poured on a glass plate placed on a leveler avoiding the formation of air bubbles. The agar-antiserum mixture was allowed to set and wells of 3 mm in diameter were made in the agar with a circular metal punch. The punched agar was carefully removed from the plate with the smooth edge of pipette attached to a vacuum pump. Several dilutions (25%, 50%, 100% and 200%) of the standard serum were prepared in PBS. Using a 5ml micro-dispenser, the sera and standards were applied to the punched wells. The plate for Ig G estimation was put into a humid chamber and incubated for four hours. The diameter of the precipitation ring was measured along two perpendicular diagonals to the nearest 0.1mm using eye precision viewer. The standard curves for the Ig G were plotted on a semi-log graph paper and the concentrations of the test and control samples were read off the standard curve.

Statistical analysis

The main outcome variables were the serum levels of Ig G in the neonates. Tetanus vaccination was judged adequate if there were 2 doses within 4 weeks of delivery, and inadequate if less or none. We depended on self report of malaria fever by the mother, this was assessed as malaria fever if confirmed by the doctor or self - treatment with antimalaria was followed by resolution. Antenatal care was judged adequate if there was booking within 8 - 12 weeks of pregnancy and antenatal clinic appointment was not missed more than once by the pregnant mother. The socioeconomic class was defined as high (I and II), middle (III) and low (IV and V) based on occupation, income earning and

education of the parents.^{11,12}

The data was initially explored using the stata software, variables were analysed by unpaired t-test both for equal and unequal variance using the variance ratio function of the Stata software to determine the appropriate use of the Satterthwaite's correction for the degrees of freedom. Level of statistical significance was set at $p < 0.05$ for all the analyses.

Result

There were 86 neonates/mother pairs. The neonates were made up of 47 females and 39 males. The range of the serum Ig G was 234 - 6538mg/ml, 1297.8mg/ml \pm 995.6 (mean \pm SD) and median value of 934mg/ml.

The mean serum Ig G in the females was 1374.3 mg/ml while in males it was 1272.1mg/ml.

The distribution of the social class of the mothers was as follows: Fifty one (59.3 percent) belonged to the low social class, 21(24.4percent) to the middle class and 14(16.3percent) to the high class. The mean fetal serum Ig G were 1476.7mg/ml in high social class (1 & 2), 1157.4 mg/ml in the middle class (3) and 941mg/ml in the low social class (4 & 5), Figure 1.

The mean serum Ig G in the fetuses of mothers with age <25years was 1611.2 mg/ml, while the mean in the fetuses of mothers between 25 - 29 years was 1383.5 mg/ml and the fetuses of mothers between 30 - 34 years had 919.4 mg/ml.

The duration of labour ranged from 1 - 72 hours, with a mean \pm SD of 12 \pm 3.8). The mean cord serum Ig G was 2156mg/ml in 32(37percent) neonates whose mothers were in labour for <12 hours compared to a mean level of 921mg/ml in 54(63%) neonates whose mothers were in labour for >12hours.

There were 67(78 percent) neonates with normal weight (>2.5kg) and 19(22 percent) of low birth weight (<2.5kg) with mean serum Ig G of 1229.6 and 2068.1mg/ml respectively.

Maternal tetanus vaccination was complete in 78(91percent) while it was incomplete in 8(9percent), the corresponding mean cord serum Ig G levels were 1293.2 mg/ml and 1681.2 mg/ml respectively.

Malaria fever in pregnancy was diagnosed in 31(36 percent); 29(93.5 percent) of whom one to two episodes during pregnancy while two(6.5 percent) had three or more episodes. The mean cord serum Ig G in the neonates of mothers with malaria fever

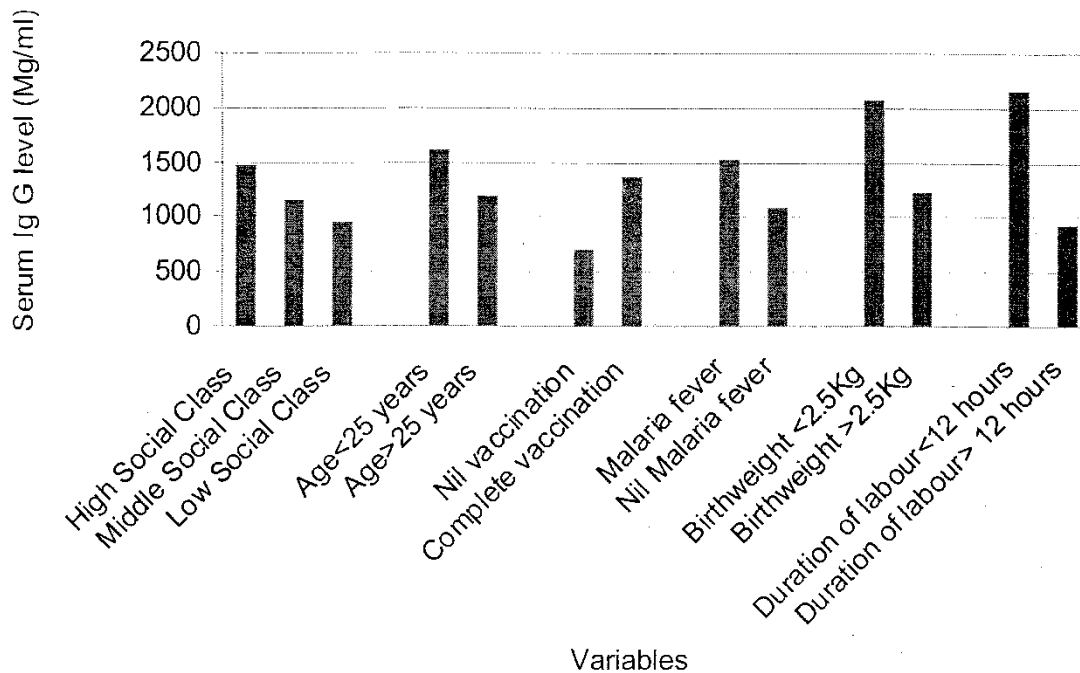


Fig 1: Neonatal serum Ig G levels and the variables

The ages of the mother at pregnancy ranged from 19 years to 42 years (mean \pm SD=27 \pm 3); while the gestational age of the neonates ranged between 32 weeks and 42 weeks with a mean \pm SD of 37.6 \pm 2.8 weeks.

during pregnancy was 1526.3 mg/ml and in those whose mothers did not suffer malaria, the level was 1089.6 mg/ml respectively.(Table 1).

Multivariate analysis revealed significant correlation with malaria fever in pregnancy ($p = 0.016$), age of

Table I
Means of Fetal IgG by covariates n = 86

Covariates	Fetal IgG (mg/ml)	95% Confidence Interval
Age (Years)		
< 25	1611.2	1227.0-1995.3
25 – 29	1383.5	1000.5-1766.5
30 – 34	919.4	704.5-1134.3
35+	934.0	697.6-1170.3
Sex of fetus		
Female	1374.3	981.4-1767.3
Male	1272.2	929.5-1614.8
Gestational age at delivery		
Preterm	2140.4	1080.8-3200.0
Term	1161.2	100.7-1320.7
Antenatal attendance		
None	700.5	242.8-1158.2
Incomplete	1478.8	814.0-2143.6
Complete	1298.9	1066.4-1527.4
Vaccination		
None	700.5	242.8-1158.2
Once	1681.2	613.8-2748.6
Twice	1293.2	1071.8-1514.6
Otitis Media in siblings		
Yes	1084.1	836.2-1332.0
No	1345.1	1106.0-1584.2
Otitis Media in Parent		
Yes	1257.3	801.0-1713.6
No	1310.2	1071.9-1548.5
Malaria fever		
Yes	1526.3	1157.6-1895.0
No	1089.6	882.4-1296.8
Birthweight		
Low	2068.1	905.8-3230.4
Normal	1229.6	1028.3-1430.9
Social Class		
High	1476.7	817 – 2469.5
Middle	1157.4	911 – 2876.3
Low	941	936.2 – 951.6
Duration of labour		
< 12 hours	2156	1105.4-3120.6
> 12 hours	921	868.4-1429.6

mother during pregnancy ($p = 0.038$) and duration of labour ($p = 0.005$). While there was no correlation with other variables: sex of the baby ($p = 0.413$), gestational age ($p = 0.714$), vaccination ($p = 0.454$),

raises issues with the potency of the vaccines given to the mothers. In our environment, loss of cold chain in the storage and transportation of the vaccines may lead to loss of potency, hence impairing the

Table II
Multivariate Analysis using Multiple linear Regression model with Fetal IgG as the Dependent variable

$n = 86$

Covariates	Fetal IgG (mg/ml)	95% Confidence Interval		P value
Variable	Coefficient	95% Confidence Interval		P value
Age	-53.8	-104.4	-3.1	0.038*
Fetal sex	187.2	-269.1	643.4	0.413
Gestational Age	-18.8	-121.2	83.7	0.714
Vaccination	-244.3	-895.5	06.9	0.454
Malaria fever	286.5	56.7	16.2	0.016*
Antenatal Care	319.9	-371.7	1011.4	0.357
Duration of labour	127	54.8	924.7	0.005*
Social class	-58	-81.3	471	0.316

* = Significant

otitis media in parent ($p = 0.367$), antenatal care ($p = 0.93$) and social status ($p = 0.116$; Table 2).

Discussion

The notable findings in this study were significant positive correlation between cord serum Ig G; and malaria fever in pregnancy, maternal age and duration of labour. The high serum Ig G in fetuses of mothers who had malaria fever in pregnancy might be explained by development of immune response to the infection and subsequent passage of the Ig G to fetuses. The elevated cord serum Ig G in neonates following increased duration of labour found in this study may be explained by increased gastrointestinal uptake of Ig G. Suffin *et al*¹³ reported that the Gastrointestinal tract of infant ferrets selectively takes up IgG has been reported to occur over a fivefold concentration gradient in animal studies.^{13, 14, 15} Our impression is that the prolonged duration of labour might prolong the contact of the fetus with maternal blood, hence increased gastrointestinal uptake of Ig G. The reduced fetal serum Ig G seen in mothers with advanced age may be explained by poor metabolic response which occurs with advanced age.

It is also notable that vaccination of the mother in pregnancy did not confer an increase in serum immunoglobulin level in the fetuses, as the mean serum Ig G in the unvaccinated group (1681.2) is higher than the vaccinated group (1293.2). This probably

immunogenicity of the vaccines.^{16, 17, 18, 19} Notably too, this study did not find correlation of the fetal Ig G with social class. Many studies have found increased predisposition of the low socioeconomic class to early onset infections.^{16, 18, 19} However, the lack of correlation of the fetal Ig G with low socioeconomic class may suggest that the predisposition to early onset infection in low socioeconomic class is probably due to other factors which need to be further investigated.

The role of circulating immunoglobulin in fetal immunity includes nonspecific stimulation of cell surface receptor involved in the regulation of neonatal growth, activation of growth hormone and insulin-like growth factor receptors during neonatal life.^{20, 21} In addition the presence of variable Ig domains favors the establishment of a balanced T-cell receptor repertoire, thereby preventing generalized autoimmunity. Various studies have reported growth benefit and protection against infections with the use of adjunct therapy of intravenous immunoglobulins.²² The findings from this study have documented the range of serum Ig G in fetuses of normal uneventful pregnancy. The significance of the fetal serum Ig G in the development of childhood infection particularly otitis media in the first year of life will be examined further in our study. However, this can be applied to other childhood infections, thus establishing the association or causative role of the levels of immunoglobulin.

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