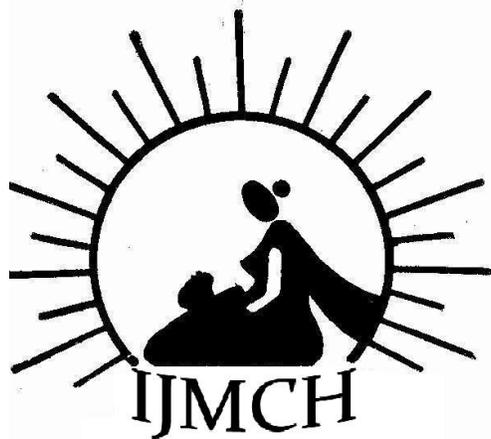


Determination of retinol concentration in cord blood by liquid chromatography and its association with weight and sex of neonates.

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What is the concentration of retinol in cord blood of neonates delivered in a maternity hospital of Guwahati City of Assam, India.

## Determination of retinol concentration in cord blood by liquid chromatography and its association with weight and sex of neonates.

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### Abstract:

**Research Question:** What is the concentration of retinol in cord blood of neonates delivered in a maternity hospital of Guwahati City of Assam, India.

**Objectives:** 1) To estimate retinol concentration in cord blood by High performance Liquid Chromatography

2) To evaluate its association with birth weight and sex of neonates.

**Materials and methods:** Cord blood of neonates after delivery was collected and retinol concentrations were estimated by Reversed Phase High Performance Liquid Chromatography. Serum retinol concentrations were then correlated with birth weight and sex. Student's t test is used to find out the significance between two observed values.

**Participants:** 63 neonates delivered in a maternity Hospital of Guwahati city were considered for this study.

**Results:** Out of 63 neonates 49.2% were female and 50.79% were male. 24% of all the neonates have deficient cord retinol <math>10\mu\text{g}/\text{dl}</math>. Mean cord serum retinol was higher in normal birth weight group (>2500g) than the low birth weight group (<2500g). There was significant positive correlation of cord serum retinol with birth weight ( $r=0.408$ ) in case of low weight neonates.

**Conclusions:** The level of retinol in low birth weight neonates correlated with birth weight. Low cord serum retinol reflects poor vitamin A status of the neonates which in turn may affect foetal growth.

**Key words:** Cord serum retinol, High performance Liquid Chromatography, Birth weight

**INTRODUCTION:**

Vitamin A is one of the essential nutrients for health and development of infants<sup>(1)</sup>, which is shown by the reduction in mortality and morbidity by vitamin A supplementation of infants aged 6 months and older in populations with vitamin A deficiency.<sup>(2)</sup> Vitamin A supplementation has been shown to improve growth of children in populations with vitamin A deficiency or xerophthalmia<sup>(3-5)</sup>, suggesting that vitamin A supplementation may be beneficial for growth in population in which vitamin A is a limiting factor. A large number of studies have reported lower maternal serum vitamin A and cord serum vitamin A of neonates to be associated with low birth weight.<sup>(6-8)</sup> However, others have shown no significant improvement in birth weight following supplementation of vitamin A during third trimester of pregnancy.<sup>(9, 10)</sup> In Assam, there is no available information on the vitamin A status of neonates. The present study was conducted to assess the vitamin A status of neonates as a function of sex and weight.

**MATERIALS AND METHODS:**

Cord blood was collected from the placental end of the umbilical cord immediately after delivery. Blood samples were centrifuged to separate the serum. Serum was analysed immediately whenever possible otherwise stored at -20°C. All the analysis were carried out in dim light in order to avoid photo degradation of retinol compounds. Birth weight and sex of the neonates were recorded immediately after birth. The study protocol has been approved by the ethical committee of Gauhati University constituted for this purpose. Retinol was extracted from serum samples by mixing with water and then retinyl acetate as internal standard was added followed by protein extraction with ethanol. It was then extracted thrice with hexane. The supernatants were pooled and evaporated under reduced pressure and reconstituted in 100 ml Methanol and injected (20µl) into the HPLC system for analysis. The extracted retinol was then analysed by using a Shimadzu system composed of two pumps and a photodiode array detector (PDA SPD-M10AVP). The separation was achieved on asupelcosil-LC-8 (25cm x 4.6mm, 5µm) column. The chromatography was carried out using a step gradient elution mode in which eluent A was MeOH:H<sub>2</sub>O (85:15) and eluent B was MeOH:DCM (80:20). Retinyl acetate (internal standard) and retinol was detected and characterised by comparing their retention time with their standards and also from their characteristic UV spectrum as measured by PDA detector. Retinol and Retinyl acetate were estimated from the peak area of the corresponding HPLC chromatogram with the help of standard curves. The samples were analysed in triplicate and data were expressed as means ± SD. Analysis of collected data was done.

**RESULTS:**

Data in Table I show birth weight and cord serum retinol in relation to sex. The 63 neonates included in the present study were 31 females (49.20%) and 32 males (50.79%). The weight of both female and male neonates was found to be almost same. Comparison of mean cord serum retinol between male and female neonates shows that mean cord serum retinol between the two groups are almost same ( $p=0.3651$ ; t test).

Table I: Birth weight and cord serum retinol of neonates as a function of sex

Sex	N(%)	Weight(Kg)	Cord retinol( $\mu\text{g}/\text{dl}$ )
Female	31 (49.20)	2.54 $\pm$ 0.46	14.13 $\pm$ 5.60
Male	32 (50.79)	2.59 $\pm$ 0.47	14.74 $\pm$ 7.11

Values are mean  $\pm$  SD

Data in table II reveal that 15 neonates representing 24% of all the neonates have deficient cord retinol ( $<10\mu\text{g}/\text{dl}$ ) while the majority (59%) of them have cord retinol ( $10\text{-}19\mu\text{g}/\text{dl}$ ). 11 neonates (17 %) have cord serum retinol within the acceptable level ( $\geq 20\mu\text{g}/\text{dl}$ ). The results in the above table indicate that 87% of female neonates have cord serum retinol  $<20\mu\text{g}/\text{dl}$  as compared to male neonates (78%).

Table II: Distribution by sex of cord serum retinol level

Sex	Mean cord serum retinol level ( $\mu\text{g}/\text{dl}$ )			Total N (%)
	$<10$ N (%)	10-19 N (%)	$\geq 20$ N (%)	
Female	8 (25.80)	19 (61.29)	4 (12.90)	31 (100)
Male	7 (21.87)	18 (56.25)	7 (21.87)	32 (100)
Total	15 (23.80)	37 (58.73)	11 (17.46)	63 (100)

On statistical analysis using the t test, the mean cord serum retinol was significantly higher in normal birth weight group ( $18.37\mu\text{g}/\text{dl}$ ) than the low birth weight group ( $9.83$ ).

Table III: Birth weight in relation to the level of cord serum retinol

Retinol level ( $\mu\text{g}/\text{dl}$ )	Birth Weight(Kg)		P value
	$< 2.500$ (n=29)	$\geq 2.500$ (n=34)	
Cord serum	9.83 $\pm$ 3.08	18.37 $\pm$ 5.80	6.996

The figures 1(a) and 1(b) depict the scatter diagram showing the correlation of cord serum retinol with the birth weight in case of neonates having low birth weight and normal birth weight respectively.

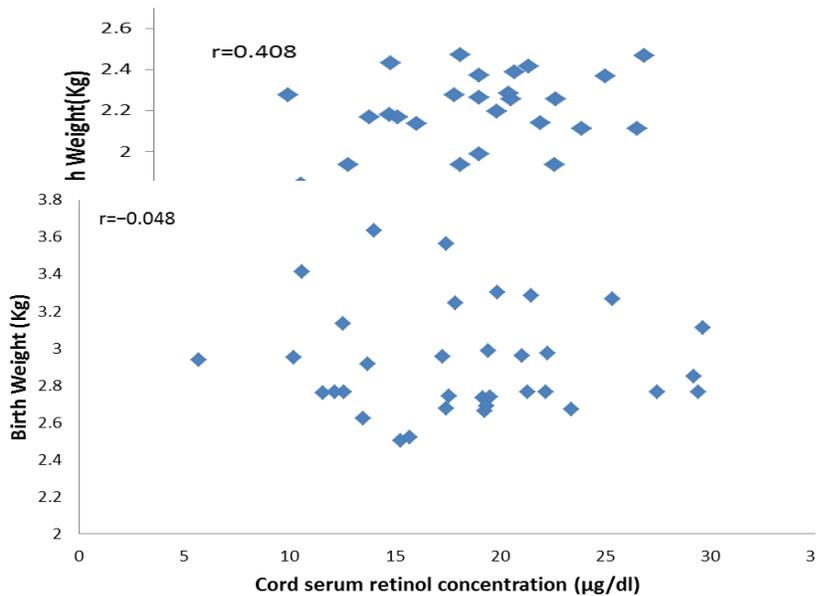


Fig. 1 (a)

Fig. 1(b)

From above figures 1(a) and 1(b) it is

the figures 1(b) it is

seen that there was significant positive correlation of cord serum retinol with birth weight ( $r=0.408$ ,  $p=2.32>0.05$ ) in case of low weight neonates but it is seen that the negative correlation ( $r=-0.048$ ) of cord serum retinol with birth weight in case of normal weight neonates is not significant ( $p=0.27$ ).

#### **DISCUSSION:**

In this study the mean cord serum retinol between male and female neonates was found to be almost same which is in accordance with that reported by Shenai *et.al.*<sup>(11)</sup> On the other hand previous studies have shown that male cord serum retinol level was significantly lower than that of female.<sup>(12, 13)</sup> In our study 46% neonates were of low birth weight which indicates poor nutrition in mothers. Cord serum retinol level was found to be higher in normal birth weight neonates compared to low birth weight neonates. Low cord serum retinol may reflect poor vitamin A status of the new-born and the mother, which in turn may affect foetal growth. For instance, the studies of Navarro *et. al*<sup>(7)</sup>, Gazala *et.al*<sup>(14)</sup> and Agarwal *et.al*<sup>(15)</sup> have shown lower cord serum vitamin A levels in neonates of low birth weight than in normal weight neonates. We found a negative correlation between birth weight and cord serum retinol in normal birth weight neonates. This may be because cord serum retinol is not the only factor to determine birth weight. The other factors such as mother's nutritional status, placental weight etc. may also have considerable influence on birth weight in case of normal birth weight. However, a significant correlation between birth weight and cord serum retinol was observed in low weight neonates. A number of studies have reported cord serum vitamin A level of neonates with low birth weight or prematurity.<sup>(7, 16)</sup> This emphasizes the vital role of vitamin A in the foetal growth which has been reported.<sup>(17, 18, 19)</sup> In our study we adopted the measurement of cord serum levels as the criteria for assessing the retinol concentration in the neonates. However measuring serum retinol concentration has some disadvantages. Serum retinol concentration reflects the body vitamin A status only when the liver retinol stores are seriously depleted and inflammation affects serum retinol concentration. Indirect methods such as relative dose response (RDR), modified relative dose response test (MRDR), tracer dilution technique are more sensitive. However these tests cannot be widely used as they are expensive. Thus, serum retinol concentration continues to be widely used to assess vitamin A status. We analyzed retinol levels in relation to birth weight but not with other parameters of neonatal anthropometry, which might have yield important information. However, it is well known that in neonates with poor foetal growth, birth weight remains the most important determinant of outcome.

#### **CONCLUSION:**

Low cord serum retinol reflects poor vitamin A status of the neonates which in turn may affect foetal growth. Since the level of retinol in low birth weight neonates correlated with birth weight, supplementary vitamin A may be beneficial. Similar studies with larger group of neonates and with more parameters may be useful.

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