Transplacental transfer of 25-hydroxy-cholecalciferol

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Summary

Plasma calcium, inorganic phosphorus, alkaline phosphatase, magnesium and 25-hydroxy-cholecalciferol (25-OH-D3) levels were determined on venous blood in 60 Indian mothers during labour. The same analyses were carried out on the cord blood and the infant's venous blood 96 hr after birth. The mean values of plasma calcium, inorganic phosphorus and magnesium were significantly raised in the cord blood compared to those in the maternal blood (calcium and phosphorous $P < 0.001$ and magnesium $P < 0.01$). The mean plasma levels of 25-OH-D3 in the maternal and cord blood were 11.7 ng/ml and 9.6 ng/ml respectively, the cord blood level being about 81% of the maternal. There was a highly significant correlation between these concentrations ($r = 0.80$, $P < 0.001$). The plasma 25-OH-D3 was subnormal in 12 pregnant mothers at labour and 14 infants.

The study highlights the low vitamin D state of Indian pregnant mothers and their newborn infants. The plasma 25-hydroxy-cholecalciferol in pregnancy may reach subnormal levels which are often associated with clinical osteomalacia. This, in turn, adversely affects the vitamin D state of the infant.

Introduction

The fetus is dependent upon the mother for the supply of vitamin D. Traditionally, infants are not exposed to the sun for a few weeks after birth; hence they are dependent either on the supply of vitamin D in the milk or on the stores of vitamin D obtained during intrauterine life by transplacental transfer. Vaishnav (1975) from North India reported a high incidence of osteomalacia in women during the reproductive period; 20% of his patients were first seen during pregnancy. Deficiency of vitamin D during pregnancy is likely to affect the fetus and

infant adversely. Congenital rickets has been observed under such circumstances (Ford *et al.*, 1973). The status of vitamin D is best assessed by assay of plasma 25-hydroxy-cholecalciferol (25-OH-D3) (Haddad and Chyu, 1971). A study of maternal-fetal relationship of vitamin D has been made, using the measurements of 25-OH-D3 levels in mother and infant.

Subjects and methods

This study was carried out at Armed Forces Medical College, Pune, India. Pune is situated in the Deccan plateau of South India where there is ample sunshine throughout the year and hardly any seasonal variation during the study period between March to October.

Sixty healthy Indian mothers and their infants were studied during labour. The mean gestational age was 39 weeks (range 32-41 weeks) and only 8 were born before 35 weeks of gestation. No vitamin D supplements were given during pregnancy. The mean dietary vitamin D intake was 140 i.u./day (range 70 and 160 i.u./day depending on whether they were vegetarian or non-vegetarian). Thirty two were male infants and 28 were female. The mean birth weight was 2.8 kg (1.8-3.5 kg). The birth weight was more than 2.5 kg in 38 infants, 2.0-2.5 kg in 21 and only 1.8 kg in one infant. All infants were breast fed and closely observed for clinical signs of hypocalcaemia for 96 hr from birth.

Maternal venous blood and the cord blood were collected, in heparinized tubes during labour and kept at 4°C until centrifugation. Blood was also withdrawn from the femoral vein of infants about 96 hr after birth. Plasma was separated within 2 hr and stored at -20°C until analysed. Plasma calcium, inorganic phosphorus, total proteins, specific gravity, magnesium and alkaline phosphatase were per-

TABLE 1. Biochemical findings (mean $\pm$ s.d.) in maternal, cord and infant blood

Parameter	Maternal blood (A) <i>n</i> = 60	Cord blood (B) <i>n</i> = 60	Infant's venous blood at (C) 96 hr <i>n</i> = 60	<i>P</i> value	
				A v. B	B v. C
Calcium (mmol/litre)	2.42 $\pm$ 0.12	2.82 $\pm$ 0.26	2.36 $\pm$ 0.27	<0.001	<0.001
Phosphorus (mmol/litre)	1.13 $\pm$ 0.33	1.47 $\pm$ 1.07	1.43 $\pm$ 0.46	<0.001	>0.05
Alkaline phosphate (u./litre)	134.26 $\pm$ 48.42	124.04 $\pm$ 23.64	137.03 $\pm$ 41.04	>0.05	>0.05
Magnesium (mmol/litre)	0.89 $\pm$ 0.11	0.91 $\pm$ 0.10	1.12 $\pm$ 2.61	<0.01	<0.001
25-OH-D3 (ng/ml)	11.73 $\pm$ 6.62	9.59 $\pm$ 5.33	10.2 $\pm$ 5.12	>0.05	>0.05

formed within 24 hr of collection. All plasma calcium values were corrected for variation in total plasma proteins (Berry *et al.*, 1973). Paired samples of maternal and cord blood were analysed for 25-OH-D3 within 2 months using the protein binding radio-immunoassay method of Haddad and Chyu (1971). Correlation coefficient regression equation and paired *t*-tests were calculated and these were treated for statistical significance.

Results

The mean plasma calcium corrected for total proteins ($P<0.001$) inorganic phosphorus ($P<0.001$), magnesium ($P<0.01$) and alkaline phosphatase were higher in the cord blood than in the maternal blood (Table 1). Figure 1 shows the relationship between the concentrations of 25-OH-D3 in 60 paired samples of the maternal and cord plasma. There was a highly significant correlation between the sets of measurements ($r=0.80$, $P<0.001$). Determination of the linear regression in the maternal plasma 25-OH-D3 on the cord plasma 25-OH-D3 gave the equation $y=0.38x+4.99$ where y is the cord plasma 25-OH-D3 and x is the maternal plasma 25-OH-D3. There was no correlation between the cord plasma 25-OH-D3 and the infant's birth weight.

None of the infants developed convulsions, apnoea, cyanosis or tetany during the period of observation from birth to 96 hr. The significant biochemical changes during the period were a reduction of plasma calcium and a rise in plasma magnesium ($P<0.001$). Plasma calcium was significantly low in infants weighing under 2.5 kg at 96 hr ($P<0.05$) but was poorly correlated with plasma 25-OH-D3 (Table 2). The latter was subnormal (less than 10.0 ng/ml) in 12 mothers and 14 infants.

FIG. 1. Relationship between the concentration of 25-OH-D3 in 60 paired samples of maternal and cord plasma.

Discussion

During fetal life, calcium ions are actively transferred from the mother to the fetus via the placenta where there is an active transport mechanism involving calcium and magnesium stimulated adenosine triphosphatase system. The placental 'calcium pump' results in a relatively hypercalcaemic fetus during pregnancy (Ramberg, Delivoria and Crandall, 1973). Fetal plasma parathyroid hormone level remains low and undetectable for this reason (David and Anast, 1974). Inorganic phosphorus and magnesium also

TABLE 2. Biochemical findings (mean $\pm$ s.d.) in 60 infants aged 96 hours according to their birth weight

No.	Birth weight <2.5 kg 22	Birth weight $\geq$ 2.5 kg 38	P Value
Plasma calcium (mmol/litre)	2.17 $\pm$ 0.30	2.41 $\pm$ 0.21	<0.05
Plasma alkaline phosphatase (u./litre)	140.01 $\pm$ 38.11	135.89 $\pm$ 4.22	NS
25-OH-D3 (ng/ml)	9.47 $\pm$ 4.958	12.07 $\pm$ 4.663	NS NS = >0.05

actively cross the placenta (Stone and Pritchard, 1970).

Although 25-OH-D3 levels in the cord blood have been measured in some countries during recent years no such studies have yet been made in India. The cord 25-OH-D3 levels in the Caucasian population have been 23 μ g/litre in St Louis, U.S.A. (Hillman and Haddad, 1974), 16.2 ng/ml in U.K. (Dent and Gupta, 1975) and 8.0 ng/ml in Belgium (Bouillon, Bealan and Moore, 1977). In U.K. where foods are not supplemented with vitamin D, the cord 25-OH-D3 in Asian immigrants is 6.5 ng/ml (Dent and Gupta, 1975). In a vitamin D-supplemented dark skinned population in New York, U.S.A., it is 23 μ g/litre (Rosen *et al.*, 1974). In the present study the cord 25-OH-D3 was 9.6 ng/ml. In India, vegetable cooking oils are fortified with vitamin D (175 i.u. of vitamin D per 100 g of vegetable oil) and there is ample sunshine in many parts of the country. The positive correlation between maternal and cord free 25-OH-D3 has established a 'facilitated' transfer of this steroid through the placenta (Bouillon, Bealan and Moore, 1977). Hence, infants born with subnormal plasma 25-OH-D3 are likely to have depleted stores of vitamin D.

Reeves (1979) has argued that a dietary supplement of vitamin D is not necessary in the sunnier parts of the world. Although there is sufficient sunshine available the pregnant mothers do not habitually expose themselves to the sun.

Subnormal plasma 25-OH-D3 levels in 20% of pregnant women and 23% of their new born infants indicate a relatively low vitamin D state. This study shows that the supplements of vitamin D are indicated for Indian mothers during pregnancy.

Acknowledgment

The authors are grateful to Medical Research Council, U.K. and Armed Forces Medical Research Council, India, for generous grants to conduct the study and to the Director General, Armed Forces Medical Services, for his kind permission to publish the results.

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