

FETO-MATERNAL MINERAL HOMEOSTASIS—A STUDY IN THREE PREGNANT GROUPS

IMTIAZ AHMAD* AND TASNIM MAJID**

Department of Biochemistry, Postgraduate Medical Institute, Lahore, Pakistan

(Received June 25, 1992; revised December 11, 1993)

To assess the relationship between maternal and fetal mineral homeostasis, serum, calcium, magnesium inorganic phosphorus total protein, albumin and alkaline phosphatase concentrations in cord serum from 30 preterm 30 term and 30 term low-birth-weight neonates were compared with the material serum. There was a significant, relationship between the two compartment. A strong positive correlation was observed between the mineral levels of term and preterm fetometernal compartment. No correlation was present between the mineral levels of term low birth weight group except magnesium. Results of paired t-test indicate significant differences among the mineral levels of three pregnant groups.

Key words: Calcium, Magnesium, Phosphorus, Albumin.

Introduction

Pregnancy imposes major changes in the mother's nutritional requirements and mineral metabolism. Several reports in the last few years have dealt with the fetomaternal relationship of mineral particularly in term and preterm neonates [1,2]. Cord serum mineral levels have been shown to be directly related to the maternal serum concentrations [3]. Studies in which calcium, inorganic phosphorus and magnesium concentration were considered, all showed that there was a positive fetomaternal gradient without reaching a consensus on whether a relationship existed between the two pools [4].

Calcium and magnesium metabolism in the pregnant women and fetus represent a complex process with a number of intricate and interrelated components. A primary features seems to be the active transport of minerals from mother to fetus. Thus neonatal tetany, neonatal hypocalcemia, neonatal hypoparathyroidism and a defect of tooth enamel are all associated and are probably due to vitamin-D and mineral deficiency in the mother during pregnancy [5]. The purpose of the present study was to assess simultaneously the fetomaternal relationship of serum calcium, magnesium, inorganic phosphorus, total protein, albumin and alkaline phosphates in three pregnant groups and their neonates.

Experimental

Patients. The present study was undertaken on ninety pregnant women and their neonates with predominantly normal pregnancies. Patients using medication as drugs (antidiabetic, antihypertensive, vitamin and mineral supplementation during the last trimester of gestation were excluded from the study subjects. These subjects were selected from obstetrics and gynaecological units of Services Hospital and Lady Willing-

don Hospital Lahore during 1989-90. The gestational age and birth-weight of the neonates were taken from the case sheets. The study subjects were divided into three groups:

Group I. Pregnant women deliver full term normal birth-weight neonates.

Group II. Pregnant women deliver full term low-birth-weight neonates.

Group III. Pregnant women deliver preterm neonates.

The blood samples were collected at delivery from mothers and infants. The maternal blood was withdrawn from the cubital vein using a tourniquet while recumbent. The neonatal blood was obtained from the placental end of the umbilical cord after it was clamped and cut soon after the pulsation had ceased.

Maternal and cord blood samples were centrifuged and the serum frozen at 20° until used. Serum ionic calcium, total calcium, magnesium, inorganic phosphorus, total protein, albumin and alkaline phosphatase [6-12] were analyzed on a Labsystems FP-901 chemistry analyzer.

Results are expressed as mean (standard deviation). The significance (P values) of the difference in mean concentrations between the maternal and cord sera was evaluated by paired student "t" test and their correlation by linear regression analysis.

Results and Discussion

The biochemical data from the paired maternal and cord sera in full term normal birth weight neonates are shown in Table 2. Meanionic calcium, total calcium and inorganic phosphorus of cord blood (1.21, 2.49, and 1.43 mmol/l) were significantly higher than the maternal blood (1.05, 2.07 and 0.91 mmol/l, P<.001). No statistical difference were found between the mean magnesium levels of maternal and cord

* Present address: Dept. of Chemistry, Govt. F.C. College, Lahore.

** Fatima Jinnah Medical College, Lahore, Pakistan.

blood (0.89 and 0.87 mmol/l). A highly significant difference was present between the cord and maternal blood levels (62.6 and 54.6 g/l) of total protein in the group ($P<.001$), however, no significant difference was observed in the albumin levels, (35.1 and 37.7 g/l). Alkaline phosphatase levels of cord and maternal blood show highly significant difference (489.5 and 350.1 U/l, $P<.001$). Cord serum ionic calcium, total calcium magnesium, protein, albumin and alkaline phosphatase are positively correlated with the maternal sera (Table 2).

Results of comparison and correlation between maternal and cord blood in full term low birth weight neonates are shown in Table 3. Serum ionic and total calcium levels show no statistical difference (0.94 and 0.99 mmol/l, 2.16 and 2.15 mmol/l). Phosphorus level of cord blood (1.59 mmol/l) was significantly higher ($P<.001$) than maternal blood (0.85 mmol/l). Levels of magnesium, total protein and albumin in cord blood (0.82 mmol/l, 63.0 and 40.5 g/l) was significantly lower ($P<.001$) than maternal blood (1.17 mmol/l, 70.8 and 47.9 g/l). Alkaline phosphatase level of cord blood (219.4 U/l) were also significantly lower ($P<.01$) than the maternal blood (258.2 U/l). There was a highly significant positive correlation ($P<.001$) between paired maternal and fetal blood for total protein ($r=0.630$) albumin ($r=0.805$) and alkaline phosphatase ($r=0.847$) in the group. No

correlation was observed for serum calcium and phosphorus while serum magnesium show a significant correlation ($r=0.3951$, $P<.01$).

Paired t-test and correlation comparison of maternal and cord blood of premature neonates shows that ionic calcium levels of maternal blood (1.14 mmol/l) were not significantly different from the cord blood (1.16 mmol/l), but total calcium show a significant difference ($P<.01$). Phosphorus and magnesium levels of cord blood (2.13 and 1.05 mmol/l) were significantly higher ($P<.001$) than the maternal blood (1.62 and 0.82 mmol/l). Total protein and albumin levels were lower while alkaline phosphatase levels of cord blood were significantly higher than the maternal blood ($P<.001$). A highly significant positive correlation was observed between cord and maternal blood of calcium, phosphorus and magnesium levels (Table 4). Alkaline phosphatase had a highly significant, positive correlation ($r=0.894$, $P<.001$).

It is generally accepted that fetal and prenatal mineral homeostasis depend on endocrine and nutritional factors. Our data in these pregnant groups show higher concentrations of minerals and alkaline phosphatase and lower concentration of albumin and total protein in the cord blood than in the maternal blood with a consistent correlation between the two compartments (Tables 2-4).

Paired t-test results of present study indicate that term normal weight neonates had significantly higher mineral levels than the maternal blood except magnesium. No significant difference between maternal and cord values for magnesium had also been reported by Verity *et al.* [13]. Results of our study in term low birth weight neonates show that phosphorus levels was significantly higher ($P<.001$) in cord blood than maternal blood whereas reverse was true for magnesium levels (Table 3). Total serum calcium and ionic calcium levels

TABLE 1. DISTRIBUTION OF FETAL AGE AND WEIGHT.

Number of groups	Gestational age (weeks)	Birth weight (grams)
Full term normal birth weight	39.3±1.0	2975.5±377.2
Full term low birth weight	38.5±1.0	2080.0±257.3
Premature	31.4±2.7	1716.5±345.8

TABLE 2. COMPARISON AND CORRELATION OF FETO-MATERNAL SERUM MINERALS, TOTAL PROTEIN, ALBUMIN AND ALKALINE PHOSPHATASE IN FULL TERM NORMAL BIRTH WEIGHT NEONATES BY PAIRED 't' TEST AND LINEAR REGRESSION ANALYSIS.

	Ionic calcium (mmol/l)	Total calcium (mmol/l)	Inorganic phosphorus (mmol/l)	Magnesium (mmol/l)	Total protein (g/l)	Albumin (g/l)	Alkaline phosphatase (U/l)
Maternal (Mean±SD)	1.05±0.17	2.07±0.24	0.91±0.33	0.89±0.27	54.6±9.1	35.1±7.1	350.1±231.1
Cord (Mean±SD)	1.21±0.19	2.49±0.37	1.43±0.55	0.87±0.32	62.6±11.6	39.8±10.3	489.8±276.6
Number of Pairs	30	30	30	30	30	30	30
Difference (P value)	$P<.001$	$P<.001$	$P<.01$	NS	$P<.001$	NS	$P<.001$
Correlation (r)	0.588	0.809	0.274	0.423	0.660	0.860	0.953
Significance (P value)	$P<.001$	$P<.001$	NS	$P<.001$	$P<.001$	$P<.001$	$P<.001$

was not significantly different from each other. Total serum protein, albumin and alkaline phosphates levels were significantly lower ($P < .001$ and $< .01$, Table 3) in cord blood than the maternal blood. These results are in accordance with finding of Cockburn *et al.* [14] and Delvin *et al.* [2] who reported that the levels of total and ionic calcium in cord blood regularly exceed than in maternal blood by an average of 0.5 and 0.25 mmol/l. Magnesium is marginally higher and phosphorus substantially so.

In the premature neonates since the cord calcium levels is related to gestational age a normal fall in calcium, is likely to extend into the hypo-calcemic range [15,16]. Serum magnesium concentration is higher in preterm neonates and may be associated with decreased muscles tone [17]. Our data regard-

ing premature neonates shows that total calcium levels was significantly ($P < .01$) lower than the maternal serum calcium but no differences were observed in the ionic calcium levels. Phosphorus magnesium and alkaline phosphates were significantly higher than the maternal compartment ($P < .001$, Table 4). Plasma alkaline phosphatase activity a screening test for rickets in preterm neonates was studied by Kovar *et al.* [18].

In conclusion the most interesting finding is the absence of a maternal to fetal gradient of ionized calcium in low birth weight and preterm neonates. This impaired active transport of calcium across the placenta in these neonates indicates possible reasons for the absence of a calcium gradient. This might includes inadequacies of Vitamin D, parathyroid hormone or parathyroid hormone related peptide. The high concentrations

TABLE 3. COMPARISON AND CORRELATION OF FETO-MATERNAL SERUM MINERALS, TOTAL PROTEIN, ALBUMIN AND ALKALINE PHOSPHATASE IN FULL TERM LOW BIRTH WEIGHT NEONATES BY PAIRED 't' TEST AND LINEAR REGRESSION ANALYSIS.

	Ionic calcium (mmol/l)	Total calcium (mmol/l)	Inorganic phosphorus (mmol/l)	Magnesium (mmol/l)	Total protein (g/l)	Albumin (g/l)	Alkaline phosphatase (U/l)
Maternal (Mean±SD)	0.94±0.17	2.16±0.16	0.85±0.19	1.17±0.21	70.8±4.7	47.9±4.7	258.5±139.5
Cord (Mean±SD)	0.99±0.14	2.15±0.30	1.59±0.28	0.82±0.14	63.0±10.2	40.5±7.0	219.4±126.1
Number of pairs	30	30	30	30	30	30	30
Difference (P value)	NS	NS	$P < .001$	$P < .001$	$P < .001$	$P < .001$	$P < .01$
Correlation (r)	0.068	0.066	0.158	0.395	0.630	0.805	0.847
Significance (P value)	NS	NS	NS	$P < .01$	$P < .001$	$P < .001$	$P < .001$

TABLE 4. COMPARISON AND CORRELATION OF FETO-MATERNAL SERUM MINERALS, TOTAL PROTEIN, ALBUMIN AND ALKALINE PHOSPHATASE IN PREMATURE NEONATES BY PAIRED 't' TEST AND LINEAR REGRESSION ANALYSIS.

	Ionic calcium (mmol/l)	Total calcium (mmol/l)	Inorganic phosphorus (mmol/l)	Magnesium (mmol/l)	Total protein (g/l)	Albumin (g/l)	Alkaline phosphatase (U/l)
Maternal (Mean±SD)	1.14±0.14	2.15±0.21	1.62±0.28	0.82±0.15	49.4±8.4	43.7±7.2	647.9±271.2
Cord (Mean±SD)	1.16±0.3	2.00±0.3	2.13±0.48	1.05±0.25	43.3±6.4	31.4±6.3	716.3±326.6
Number of Pairs	30	30	30	30	30	30	30
Difference (P value)	NS	$P < .01$	$P < .001$	$P < .001$	$P < .001$	$P < .001$	$P < .001$
Correlation (r)	0.285	0.409	0.645	0.522	0.678	0.409	0.894
Significance (P value)	NS	$P < .02$	$P < .001$	$P < .001$	$P < .001$	$P < .02$	$P < .001$

of phosphorus in preterm and low birth weight neonates compared to term neonates also supports the contention of impaired parathyroid hormone metabolism.

References

1. L. S. Hilman, S. Rojanasentahet, E. Slatopolsky and J. G. Haddad, *Pediatric Res.*, **11**, 736 (1977).
2. E. E. Delvin, F. H. Gloneux, B. L. Salle, Li. David and J. P. Varenne, *Arch. Dis. Child.*, **57**, 754 (1982).
3. J. J. Steichen, R. C. Tsang, T. L. Gratton, A. Hamstra and H. F. Deluca, *N. Engl. J. Med.*, **302**, 640 (1980).
4. R. M. Pitkin, D. I., Cruikshank, C. W. Schauburger, W. A. Reynolds, G. A. Williams and G. H. Hargis, *Pediatrics*, **66**, 77 (1980).
5. R. M. Pitkin, *Am. J. Obstet. Gynaecol.*, **151**, 99 (1985).
6. S. C. Conceicao. D. Weightman, P. A. Smith, M. K. Ward and DNS. Kerr, *Br. Med. J.*, 1103 (1978).
7. M. Gindler and J. D. King, *J. Clin. Path.*, **58**, 376 (1972).
8. C. Bohuon, *Clin. Chim. Acta*, **7**, 811 (1962).
9. G. Gomorri, *J. Lab. Clin. Med.*, **27**, 955 (1942).
10. G. A. Sunderman, *Amer. J. Clin. Path.*, **30**, 112 (1958).
11. J. E. Gustafsson, *Clin. Chem.*, **22**, 616 (1976).
12. O. A. Bessey. O. H. Lowry and M. J. Brock, *J. Biol. Chem.*, **164**, 321 (1946).
13. C. M. Verity, D. Burman, P. C. Beadle, J. B. Holton and A. Morris, *Arch. Dis. Child*, **56**, 943 (1981).
14. F. Cockburn, N. R. Belton, J. K. Brown and T. L. Turner, *Br. Med. J.*, 11 (1980).
15. J. D. Maxwell, O. G. Brooke, I. R. F. Brown, *Br. J. Obstet. Gynaecol.*, **88**, 978 (1981).
16. R. B. Payne, A. J. Little and R. T. Evans, *Clin. Chem.*, **36** (1), 142 (1990).
17. E. F. Donovan, C. R. Tsang, J. J. Steichen, R. J. Strucks and C. May, *J. Pediatr.* **96** (2), 305 (1980).
18. I. Kovar, P. Mayne and D. Burllrops, *Lancet*, **6**, 308 (1982).

The study was carried out by using 0.25 mm thick chromatoplates and 100 ml developing mixture of hexane:ethyl acetate:acid (40:10:1 v/v) for neutral lipids in both cases. The polar lipids of cord blood and of primary foetal foetus were separated by using solvent mixture chloroform:methanol:30% aqueous hydrochloric acid (2:1:1 v/v) for the specific foetal lipids. [7] were used for the identification of polar and neutral lipids.

Identification of fatty acids. The fatty acid composition of polyunsaturated lipids of cord blood and of primary foetus were found out by methylating each lipid fraction with boron trifluoride:methanol (18). The methyl ester fatty acids were identified by thin-layer chromatography (TLC) (Lancaster 304 plates) using gas-liquid chromatography (GLC) (Lancaster 304 plates). The column (1.5 m x 4 mm) prepared by using diethyl-ether:ethyl acetate:hexane (10:10:80 v/v) was used for the separation of fatty acids. The retention times of cord blood and of primary foetus were compared with those of standard fatty acids. The retention times were used as a carrier gas at the rate of 40 ml/min. The initial gas inlet was as an internal standard (9) and the percentage of each fatty acid was determined on the basis of the peak area of myristic acid.

Results and Discussion

The composition of foetal lipids was investigated under the controlled conditions (1) i.e. at $33 \pm 0.2^\circ$ in an incubator to study the effect of temperature on the composition of foetal lipids. The cord lipids and foetal lipids were extracted with chloroform and methanol mixture and non-lipids were removed by Folch washing technique (2). The

lipid fraction was separated on the basis of polarity using the solvent mixture (2) and the results were compared with those of standard lipids. The results are given in Table I.

An effect is made in the present paper to study the changes in the composition of lipids during gestation. The results are given in Table II. The results are compared with those of standard lipids. The results are given in Table III. The results are compared with those of standard lipids. The results are given in Table IV.

Experimental

Gestational. The foetus (20 g) of C. p. was obtained after mating to a female rat for 2 hrs were placed overnight in a place of saturated sodium sulphate solution (12 x 12 x 2 cm). The foetus after covering with wet sack and placed in an incubator (13) at $35 \pm 0.2^\circ$. The washing process was carried out by spraying 50 ml of water on each piece after every 12 hrs within 24 hrs. The washings were picked up and classified into cord blood and primary foetal foetus. The results are given from the washing period having a total length 48, 30 and 30 min respectively. These were done in an oven at 102° for further studies.

Extraction of lipids. The foetal crushed foetus (0.5 g) of a total length of 18, 30 and 30 min were stirred for 1/2 hr with the solvent mixture (4) (30 ml) of chloroform and methanol (2:1 v/v). The supernatant was separated by centrifugation and the experiment was repeated three times with solvent mixture (20 ml) to recover the maximum lipids. The combined supernatants were used for the removal of non-lipids (2). However,