COMPARATIVE STUDY OF SERUM PROTEINS OF MOTHERS AT DELIVERY AND OF CORD BLOOD

by

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In view of the paramount importance of serum proteins, the study of serum proteins of mother and child has attracted the attention of many workers.

Studies on variations in blood total proteins in pregnancy and in the newborn seem to have been started in 1926 by Plass and Mathew. Since the advent of electrophoretic techniques, comparative study of serum protein fractions in pregnancy and in the cord blood was done by a few (Longsworth *et al,* 1945; Moore *et al,* 1949; Beach *et al,* 1952; Urban Furuhjelm, 1955; Oberman *et al,* 1956; and Tulzer, 1959). In India Menon *et al* (1958, 1959) studied the pattern in pregnancy and in the newborn babies. Jasani and Pandya (1964) studied the protein pattern only in normal cord blood.

As not much work has been done on the comparative study of protein fractions of mothers at delivery with that of the cord blood in Indian women the present study was taken up. Incidentally, an attempt was made to find out whether this study would throw light on the origin of foetal proteins.

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Material

The subjects for investigation consisted of 20 healthy females and 94 pregnant women admitted for delivery. Only well nourished women were selected for this study to eliminate the interfering factors arising out of malnourishment. The ages of the subjects varied from 16 to 35 years, the presenting parities varying from 1 to 9. The non-pregnant healthy women selected for this study belonged to the same age group as the pregnant ones. Out of 94 mothers, two gave birth to twins. The protein values of the twins along with those of their mothers are given separately in Table III. Of the remaining 92 mothers, 10 showed signs of pre-eclampsia. Of the 92 babies born, 18 babies were premature, assessed on the basis of the international standard of birth weight of 2500 gm. or below as evidence of prematurity.

Mothers' venous blood samples were collected at the time of delivery from the median cubital vein. The umbilical cord was clamped in two places, one 3 inches from the foetus and the other 2 inches distal to it, and cut in between. The clamp towards the placental end of the cord was released and the blood allowed to flow freely into a clean dry test tube and allowed to clot. Analysis was done

immediately after the serum was separated.

Methods

The method used in this study was that of Wolfson *et al.* (1948). However, certain modifications had been introduced by way of substituting the original Weichselbaum reagent with a modified reagent of Gornall *et al.* (1949). A calibration curve was drawn from the protein of pooled normal sera, the total protein of which was determined by microkjeldahl nitrogen analysis. The optical densities obtained with biuret reagent for total protein, albumin and other fractions of each case were read from the calibration curve.

Results

The mean values of total proteins and the various protein fractions in normal non-pregnant, normal pregnant, pre-eclamptic mothers and mothers of premature babies, as well as those in the respective cord blood of the latter three, have been compiled in Tables I and II along with statistical data.

It is observed that there is a statistically significant fall in total proteins in mothers at delivery as compared to that of normal non-pregnant women. This fall is exaggerated in pre-eclampsia cases. The fall in total protein in pre-eclampsia is statistically significant when compared to that of normal mothers at delivery whereas no such drop is seen in mothers of premature babies.

The cord blood, irrespective of the nature of pregnancy, showed values for total protein between 5.0 to 6 gm% in the majority of cases. When compared to the blood of corresponding mothers, the cord blood total protein of normal and premature babies shows a statistically significant fall.

Albumin

There is a statistically significant fall in serum albumin in mothers at delivery in all three groups when compared to the serum albumin of normal non-pregnant women. The decrease is more marked in mothers with pre-eclampsia and mothers of premature babies. The albumin in the cord blood of all the three groups is found to be elevated by 10-15% over that of respective mothers and are statistically significant. But when compared to normal non-pregnant adult values there is a fall in albumin in cord blood. Even though a slight difference is observed in the albumin value of cord blood of premature babies as compared to that of normal babies, they are not of statistical significance.

Globulins

There is a statistically significant rise in the total globulin fraction at delivery in normal mothers and mothers of premature babies when compared to normal non-pregnant women. But in pre-eclampsia there is a fall in total globulins. There is a statistically significant fall in total globulin in cord blood in all the three series when compared to their corresponding mothers and also normal non-pregnant cases.

Mothers with pre-eclampsia show a statistically significant fall in total globulin as compared to normal mothers. This seems to be due to a fall in the beta-globulin fraction.

TABLE I

Mean values of protein fractions in the mothers and normal non-pregnant women along with statistical data (gms%)

Group	No. of cases		Total protein	Albumin	Globulin			
					Total	Alpha	Beta	Gamma
1. Normal non-pregnant	20	Mean	7.40	4.18 (56.5)	3.22 (43.5)	1.01 (13.6)	1.16 (15.7)	1.05 (14.2)
		S.D.	0.3364	0.1661	0.2975	0.2704	0.2571	0.2294
2. Normal mothers	64	Mean	6.67	2.87 (43.1)	3.80 (56.9)	1.23 (18.4)	1.38 (20.7)	1.19 (17.8)
		S.D.	0.6206	0.5526	0.6733	0.3771	0.3448	0.2257
Normal mothers versus normal non-pregnant		'p'	<0.001	<0.001	0.01-0.001	Not significant	0.05-0.01	0.05-0.01
3. Mothers of premature babies	18	Mean	6.35	2.65 (41.7)	3.70 (58.3)	1.18 (18.7)	1.26 (19.8)	1.26 (19.8)
		S.D.	0.5020	0.3927	0.4570	0.2923	0.3640	0.2857
Mothers of premature babies versus normal non-pregnant		'p'	<0.001	<0.001	0.01-0.001	Not significant	Not significant	0.05-0.01
4. Mothers with pre-eclampsia	10	Mean	5.70	2.65 (46.5)	3.05 (53.5)	1.08 (18.9)	0.92 (16.2)	1.05 (18.4)
		S.D.	0.5673	0.5103	0.5182	0.2706	0.3571	0.3758
Pre-eclampsia mothers versus normal non-pregnant		'p'	<0.001	<0.001	Not significant	Not significant	Not significant	Not significant
5. Mothers with pre-eclampsia versus normal mothers		'p'	<0.001	Not significant	<0.001	Not significant	<0.001	Not significant
6. Mothers of premature babies versus normal mothers		'p'	Not significant	Not significant	Not significant	Not significant	Not significant	Not significant

NOTE : The figures in brackets denote per cent of total protein.

TABLE II

Mean values of protein fractions in cord blood and the statistical data (gms%)

Group	No. of cases		Total protein	Albumin	Globulin			
					Total	Alpha	Beta	Gamma
1. Cord blood of normal mothers	64	Mean S.D.	6.13 0.6230	3.37 (55) 0.4861	2.76 (45.0) 0.5480	0.83 (13.5) 0.3637	0.90 (14.7) 0.3184	1.03 (16.8) 0.2313
'p' values when compared with corresponding mothers		'p'	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
'p' values when compared with normal non-pregnent		'p'	<0.001	<0.001	0.01-0.001	Not significant	0.05-0.001	Not significant
2. Cord blood of mothers of premature babies	18	Mean S.D.	5.93 0.6553	3.15 (53.1) 0.4063	2.78 (46.9) 0.6753	0.74 (12.5) 0.3466	0.84 (14.2) 0.3521	1.20 (20.2) 0.3076
When compared with corresponding mothers		'p'	0.05-0.001	<0.001	<0.001	<0.001	<0.001	Not significant
3. Cord blood of mothers with pre-eclampsia	10	Mean S.D.	5.70 0.5757	3.75 (59.1) 0.5671	2.33 (40.9) 0.5291	0.76 (13.3) 0.2583	0.62 (10.9) 0.3289	0.95 (16.7) 0.2093
When compared with corresponding mothers		'p'	Not significant	0.01-0.001	0.01-0.001	0.05-0.01	Not significant	Not significant
4. Cord blood of babies of pre-eclampsia mothers versus normal cord		'p'	0.05	Not significant	0.05-0.01	Not significant	<0.001	Not significant
5. Cord of premature babies versus normal cord		'p'	Not significant	Not significant	Not significant	Not significant	Not significant	0.05-0.01

NOTE :—Figures in brackets denote per cent of total protein.

TABLE III

Proetin pattern in two cases of twins (gms%)

	Total protein	Albumin	Alpha globulin	Beta globulin	Gamma globulin
MOTHER A	4.4	1.43	1.49	1.01	0.51
Twin I female 1.41 Kgs.	3.68	1.91	1.01	0.35	0.41
Twin II Male 1.56 Kgs.	4.44	2.38	1.05	0.53	0.48
MOTHER B	5.0	1.74	1.03	1.03	1.20
Twin I male 2.38 Kgs.	6.3	2.11	1.17	1.79	1.23
Twin II female 1.95 Kgs.	5.7	2.77	0.99	0.74	1.20

Alpha Globulin

No statistically significant alteration is found in this fraction in mothers of the three groups as compared to normal non-pregnant values. In the cord blood there is a uniform decrease in alpha globulin which is statistically significant in all the three series when compared to that of their respective mothers.

Beta Globulin

There is a statistically significant rise in beta globulin only in normal mothers when compared to that of the normal non-pregnant. There is a statistically significant fall in beta globulin in mothers with pre-eclampsia as compared to that of normal mothers.

Statistical analysis shows that beta globulin is decreased only in normal and premature babies as compared to that of their mothers. When cord blood of mothers with pre-eclampsia and mothers of premature babies is compared with normal cord blood, only in pre-eclampsia the cord blood shows a significant fall in beta globulin.

Gamma Globulins

When compared to normal non-pregnant women there is a statistically significant increase in gamma globulin in normal mothers and mothers of premature babies and not in mothers with pre-eclampsia. There is a significant drop in gamma globulin of the normal cord blood when compared to that of maternal blood. Curiously enough there is no significant alteration in the gamma globulin of the cord of the other two series as compared to that of the corresponding mother's blood. Compared to normal cord blood there is a significant increase in gamma globulin in the cord blood of premature babies.

An attempt was made to find out whether there is any correlation between the birth weight and total protein content in mature and premature infants. From Table IV it is

TABLE IV

Birth weight and mean total protein in cord blood

Birth weight in gms.	No. of cases	Mean total protein in gm. %
1500-2000	5	5.99
2000-2500	13	5.80
2500-3000	29	6.13
3000-4000	35	5.91
Total premature infants	18	Mean 5.85+0.610
Total mature infants	64	Mean 6.01+0.556
Premature versus mature.	'p' > 0.1	

seen that there is absolutely no correlation between the birth weight and the protein values in either the mature or premature group, though in general the total protein in the premature group is slightly lower than that of the mature group ('p' > 0.1 and not significant).

The nature of variation of total protein with sex of the child was also examined and it was found that 39 male infants had an average of 5.77 gm% total protein, while 25 female infants had 5.99 gm%.

From Table V which gives the parity and the total proteins, it is obvious that parity has no influence over either the maternal or cord blood proteins.

TABLE V

Parity and mean total protein of normal mothers

Parity	Total No. of cases	Mean total protein in gms. %	
		Maternal	Cord
1st	14	6.56	5.83
2nd	14	6.62	6.10
3rd	12	6.40	5.90
4th	8	6.38	6.06
5th	6	6.35	6.48
6th	2	6.92	6.22
7th	4	6.73	5.93
8th	3	6.22	5.93
9th	1	6.48	5.59

Comments

A fall in total proteins in pregnancy is observed by both Indian and Western workers, and this is mainly due to a fall in albumin. That the total protein of cord blood is about 1 gm. less than that of the maternal blood total protein had been reported as early as 1926 by Plass & Mathew (loc. cit) and later confirmed by other workers.

The hypoalbuminaemia of pregnancy can be explained perhaps as due to inability of the liver, in its synthesis of albumin from amino-acids, to keep pace with the internal loss of nitrogen to the foetus. The loss of nitrogen to the foetus must be by way of placental transfer either of pre-formed albumin or of the amino-acids from which albumin is synthesised. Though there is some experimental evidence brought forth by Brambell and Hemings to indicate transplacental passage of protein in rabbits it requires further work to take it as applicable to human subjects.

Dancis and Shafran, who studied synthesis of plasma proteins in the guinea-pig foetus by isotope studies, have found some evidence to show the transfer of intact maternal plasma proteins to the foetal circulation across the placenta. But the question arises how far this can be applied to the human subjects. They also had evidence to show that the guinea-pig foetus near term can synthesise all plasma proteins except gamma globulin. Gitlin who studied the selectivity of human placenta for transfer of intact proteins to the foetus from the mother had shown that apart from IgG (Immunoglobulin G) only small amounts of other proteins are transferred across the placenta to the foetus. The data in our study had been analysed to see whether the individual protein fractions of the maternal blood are correlated with the respective protein fractions of the cord blood. There was good correlation between maternal albumin and cord blood albumin and also between maternal gamma

globulin and cord blood gamma globulin (P=0.01—0.001 in both premature and mature groups). No such correlation was found in the case of alpha and beta fractions. The positive correlation in the case of albumin and gamma globulin between maternal and cord blood may indicate that there may be transfer of these two proteins from the mother to the foetus. If this is true, we expect equal or slightly lower values of these two fractions in cord blood as compared to those in the maternal blood. But the individual values in our study show that in only four out of sixty-four cases studied, the foetal albumin was lower than that of its mother, whereas in the case of globulin 60 out of 64 cord bloods had lower values than that of its mother. This indicates that probably not much of albumin is transferred across the placenta to the foetus, whereas most of gamma globulin is transferred. It may also be argued, though very unlikely, that high levels of albumin in the cord blood may indicate active transport across the placenta against a concentration gradient or it may be due to slower catabolism of albumin in the foetus than in the mother.

It is highly probable that the foetus synthesises its plasma proteins along with tissue proteins. For the foetal synthesis of proteins, the amino-acid concentration in the tissues must be raised above the equilibrium state. As early as 1917, Morse found that the plasma amino-nitrogen of human cord blood had an average value of 8.7 mg%, while that of the mother had only 6.4 mg%. These findings were confirmed by recent workers. In the maintenance of such a relatively higher concentration of amino acids in foetal circulation, the placenta has to be implicated as playing a vital role. Whether the placenta also synthesises albumin is a disputed question.

While Everbeck and Levans found that the albumin content was higher in the umbilical vein than in the umbilical artery and regarded this difference as indicating participation of placenta in the synthesis of foetal albumin, Dancis who incubated placental tissues with isotopic glycine, found that the placenta can synthesise only gamma globulin but not albumin. Tulzer (loc. cit) found arterio-venous differences in the concentrations of all fractions in cord blood only in foetuses whose intrauterine development was not complete and whose weights did not exceed 3000 gms. He assumes that a temporary permeability of placental membrane to small molecular albumin exists only in the premature babies. As far as the albumin fraction is concerned, it would appear most unlikely that the foetus depends much either on transplacental passage of preformed albumin or on placental synthesis.

In our studies two cases of mothers bearing binovular twins were included. The protein pattern in the sera of mothers and the cords of the twins are given in Table III. The significant difference in the albumin contents of the twins has to be taken as strong evidence against transplacental passage of albumin from the mother. Clemetson found that there are differences in the plasma amino-nitrogen values in the twins. From this, it is obvious that the foe-

tus synthesises its own albumin. Our findings in the two cases of the twins and of almost similar protein pattern in cord blood of all three groups irrespective of the abnormal protein pattern of their mothers indicate more towards foetal synthesis of proteins. This is in agreement with the conclusion of Dancis *et al* that the placenta does not contribute significantly to the plasma proteins of the foetus and that the foetal liver is capable of synthesising all the proteins.

The rise in beta globulin in our study in normal mothers as compared to that of non-pregnant women is similar to findings reported by foreign and Indian workers. The rise may be due to increase in lipids, estrogens and siderophilin.

A statistically significant drop in beta globulin observed in our pre-eclampsia mothers at delivery when compared to normal mothers is in agreement with the finding of Menon *et al*, Mack *et al*, and Kishore and Gupta. Mack in 1955 indicated that there may be a correlation between the observed drop in beta globulin and the premature fall in estrogen levels in blood in toxaemias. The beta globulin in the cord blood of normal and premature babies is uniformly decreased which is statistically significant when compared to the respective mothers. Compared to normal cord blood, the pre-eclampsia cord blood shows a statistically significant drop in beta globulin fraction. This finding is in agreement with Menon *et al*.

One can explain perhaps the rise in albumin and the fall in alpha and beta globulin in foetal blood compared to maternal blood as due to different rates of turnover of the respective proteins in the foetal body.

While the Western workers found a drop in gamma globulin in pregnancy, no such drop could be seen in our studies. On the other hand, there is a statistically significant increase in normal mothers and mothers of premature babies at delivery compared to normal non-pregnant, while in pre-eclampsia there is no signfficant alteration. When compared to normal mothers there is no significant alteration either in pre-eclampsia or in mothers of premature babies. This finding is in agreement with Kulkarni *et al* and De Alvarez *et al*. The increase in gamma globulin in normal pregnancy in Indian subjects may be explained as protection against possible infection as people in tropical countries are exposed to greater risks of infection.

It is seen that gamma globulin of cord blood of premature infants is significantly elevated from that of normal infants, for which we have no proper explanation to offer. Gamma globulin of normal cord blood is significantly decreased when compared to that of its mother, whereas no such alteration was seen in the other two groups compared to their mothers. Our findings of nearly same values of gamma globulins in the case of twins and their mother (Table III) indicate the possibility of transplacental passage of mother's gamma globulin. Dancis and Shafran and Dupan *et al* found by isotopic studies that gamma globulin could pass through the placenta and enter the foetal circulation. This was confirmed by Gitlin. It is also interesting to note that in general, the cord

blood of all foetuses have more or less a similar pattern which is quite different from that of their respective mothers.

Summary

The comparative study of the protein pattern of maternal and cord blood had been taken up in 94 sets of maternal and cord blood and 20 cases of normal non-pregnant women.

In mothers there is a fall in total protein and albumin values. There is a significant elevation of beta and gamm globulin in normal mothers as compared to the values in normal non-pregnant women. The finding of an increase in gamma globulin in normal pregnancy is at variance with those of Western workers but agree with those reported by a few Indian authors.

There is a fall in total proteins in cord blood. There is a significant fall in beta globulin in the cord blood of normal and premature babies as compared to that of their respective mothers. Gamma globulin of cord blood of normal babies is less than that of the mother. Compared to normal babies the gamma globulin of the cord of premature babies showed a statistically significant increase.

Analysis of blood of two cases of binovular twins gave two different protein patterns, suggesting foetal synthesis of proteins, particularly albumin and the possibility of transfer of the gamma globulin fraction across the placenta from the mother to the foetus.

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