QUANTITATIVE ASSAY OF UMBILICAL CORD BLOOD PORPHYRINS - A SPECTROPHOTOMETRIC STUDY

S. C. SHARMA,* F. HANDA AND K. S. SIDHU

Summary

Quantitative assay of free erythrocyte porphyrins was done in umbilical cord blood of 100 newborn babies by spectrophotometric method. The mean level of free erythrocyte protoporphyrin in umbilical cord blood was found to be 74.56 μg/100 ml packed erythrocytes with a standard deviation of $\pm$ 28.06 & that of free erythrocyte coproporphyrin to be 0.34 μg/100 ml packed erythrocytes with a standard deviation of $\pm$ 0.93 respectively. Relationship between free erythrocyte coproporphyrin & their correlations with period of gestation, birth weight & sex of the newborns were found to be statistically significant,

Very few workers like Schwartz and Wikoff[1], Hsia and Page[2], Prato et al[3], Ventura and Meduri[4] and Wranne[5] have done quantitative estimation of free erythrocyte porphyrins in umbilical cord blood and that too in a small number of cases, using different techniques. The present study was undertaken to establish normal levels of free erythrocyte protoporphyrin (EPP) and free erythrocyte coproporphyrin (ECP) in umbilical cord blood of 100 newborn babies and to establish significant correlations if any.

Material and Methods

For the present study Remington's[6] sensitive and reproducible technique based on the Broadsheet 70 (Revised Broadsheet 36), 1971 was used. Umbilical cord blood samples were obtained from 100 newborns at birth, delivered in the Obstetric Department of this hospital. No asphyxia, congenital anomaly, disease or birth injury had been observed in these newborns. Birth weights ranged from 2000 - 3400 g with a mean of 2630 g.

These babies were born after normal pregnancy and uncomplicated labour occuring between 15 days before to 14 days after the expected date of delivery. Age of the mothers was between 18 - 40 years with a mean age of 25.9 years. Haemoglobin in mothers ranged between 8.0 - 11.5 g/100 ml with a mean haemoglobin of 9.4 g/100 ml. Parity of mothers ranged from 1st - 9th para with 3rd para as the mean parity. Besides, there was no Rh incompatibility between the mother and newborn, no complication or medication (except supportive iron and vitamin therapy) during pregnancy.

Results

Normal levels of cord blood porphyrins and their correlation

The mean EPP level was 74.56μg/100 ml packed erythrocytes with a

From the Department of Dermato-Venereology, Government Medical College & Rajindra Hospital, Patiala & Department of Chemistry, Punjabi University, Patiala (Punjab), India

* Presently at Gertrude Riding House, Newshan General Hospital, Belmont Road, Liverpool L6 4AF UK

Received for publication on 8—4—78

range of 28.60 - 148.10 μg/100 ml and mean ECP level was 0.34 μg/100 ml with a range of 0.00 - 4.53 μg/100 ml. The highest EPP value of 148.10 μg/100 ml corresponded to the highest ECP value of 4.53 μg/100 ml and the third highest EPP value of 139.30μg/100 ml corresponded to second highest ECP value of 3.72 μg/ 00 ml. This correlation between EPP and ECP was found to be positive and statistically significant (t value was 7.87; much higher than 1.98, table value t for 98 D.F. at P = 0.05).

Thus, increase in the levels of EPP corresponded to an increase in the levels of ECP.

Correlation with sex of newborns
Protoporphyrin:

Mean level of EPP of 80.82 μg/100 ml packed erythrocytes with a range of 31.26 - 148.10 μg/100 ml in male newborns was higher than the mean level of EPP of 67.20 μg/100 ml with a range of 26.60 - 135.15 μg/100ml in female newborns and this difference in the levels was found to be statistically significant (t value was 4.09) Table 1.

Coproporphyrin:

Mean level of ECP of 0.51 μg/100 ml packed erythrocytes with a range of 0.00 - 4.53 μg/100 ml in male newborns was higher than the mean level of ECP of 0.15 μg/100 ml with a range of 0.00 - 2.53 μg/100 ml in female newborns and this difference in the levels was found to be statistically insignificant (t value was 0.28) Table 2.

Correlation with birth weight
Protoporphyrin:

Correlation between EPP and birth weight was positive and statistically significant (t value was 7.00) Fig 1.

Fig. 1. RELATIONSHIP BETWEEN BIRTH WEIGHT AND CORD BLOOD PROTOPORPHYRIN.

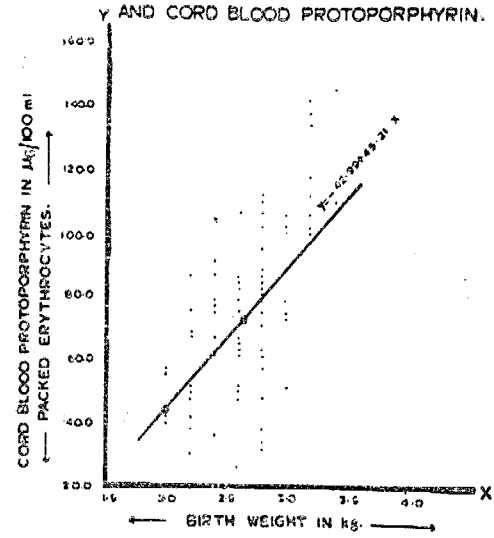


TABLE 1

Correlation of sex of newborns with EPP in cord blood.

Sex	No. of cases	EPP (μg/100 ml)		± SD	± SE	t Value
		Mean	Range			
Male	54	80.82	31.26–148.10	29.79	3.95	4.09
Female	46	67.20	26.60–135.15	25.56	3.81	

TABLE 2

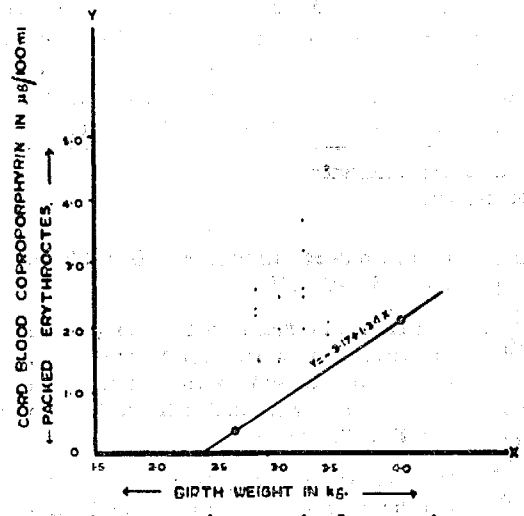
Correlation of sex of newborns with EPP in cord blood.

Sex	No. of cases	EPP (μg/100 ml)		± SD	± SE	t Value
		Mean	Range			
Male	54	0 51	0.00–4.53	1.14	0.16	0.28
Female	46	0.15	0.00–2.53	0.56	0.08	

Coproporphyrin:

Correlation between ECP and birth weight was positive and statistically significant (t value was 6.06) Fig 2.

Fig. II. RELATIONSHIP BETWEEN BIRTH WEIGHT AND CORD BLOOD COPROPORPHYRIN

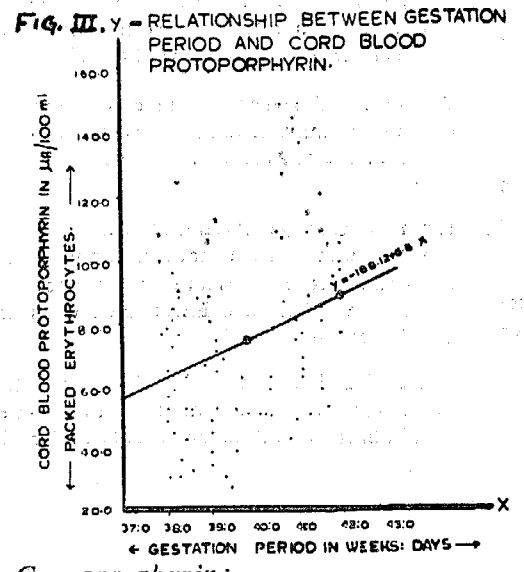


Correlation with period of gestation

Protoporphyrin:

Correlation between EPP and period of gestation was positive and statistically significant (t value was 3.59) Fig 3.

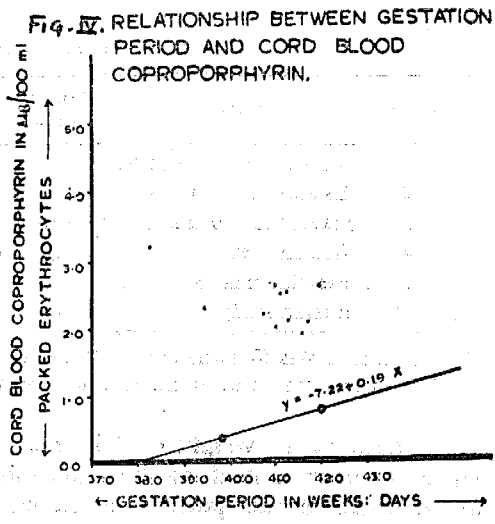
Fig. III. RELATIONSHIP BETWEEN GESTATION PERIOD AND CORD BLOOD PROTOPORPHYRIN.



Coproporphyrin:

Correlation between ECP and period of gestation was positive and statistically significant (t value was 3.13) Fig 4.

Fig. IV. RELATIONSHIP BETWEEN GESTATION PERIOD AND CORD BLOOD COPROPORPHYRIN.



Discussion

Levels of EPP and ECP obtained in the present study are compared with the findings of earlier workers and our own study of 1976[7] (Table 3). As is evident there is a wide variation in the levels of EPP and ECP in umbilical cord blood porphyrins obtained by different workers. This can possibly be explained on the fact that different analytical procedures have been used in all studies and that the estimation was confined to a small number of cases. Racial differences could also add to this difference.

In this study the highest EPP value corresponded to the highest ECP value. This finding is in confirmation of our own findings in 1976[7] and similar to the findings of Wranne[5]. In addition we could establish a positive and statistically significant correlation between EPP and ECP as in 1976 study[7] which is in contrast to the findings of Wranne[6], who could not establish any such correlation. Thus, increase in EPP levels is accompanied by a corresponding increase in ECP levels.

In the present study we could establish a positive and statistically significant correlation between EPP and ECP

TABLE 3

Levels of cord blood Porphyrins in various studies.

S. No.	Authors	No. of cases	E`P (μg/100 ml)		EPP (μg/100 ml)	
			Mean	Range	Mean	Range
1.	Schwartz & Wikoff (1952)*	2	—	8.1, 5.3	—	178, 69
2.	Hsia & Page* (1954)	6	1.5	0.6–2.8	125	63–203
3.	Prato, Mazza & Fiorina (1959)*	10	7.9	4.4–12.6	72	46–116
4.	Wranne (1960)**	20	2 8	1.1–7.2	54	32–135
5.	Handa & Sharma (1976)	30	0.77	0.00–4.49	74.58	33.10–141.22
6.	Present study (1977)	100	0.34	0.00–4.53	74.56	26.60–148.10

* Schwartz & Wikoff method of 1962 used and plasma included.

** Coproporphyrin included traces of protoporphyrin.

levels and birth weight. This may partially be explained on the basis of an increased erythropoiesis caused by hypoxia in heavier newborns. In addition, EPP and ECP levels were found to be significantly higher in male newborns than female newborns. This too could partly be explained on the heavier birth weights in male newborns, than in female newborns in our study.

Another significant fact established in the present study was a positive and statistically significant correlation between EPP and ECP and period of gestation. This can be explained on the fact that hypoxia of the foetus occurs as the period of gestation advances to term or beyond term which causes increased synthesis of haemoglobin which in turn gives rise to increased EPP & ECP levels in the umbilical cord blood.

Acknowledgements

We are thankful to Mr. V.S. Joshi, Lecturer In Statistics for his help in analysis of the present study.

References

1. Schwartz S and Wikoff HM : The relation of erythrocyte coproporphyrin and protoporphyrin to erythropoiesis, J Biol Chem, 194 : 563, 1952.
2. Hsia DYY and Page M : Coproporphyrin studies in children II. Erythrocyte copro. porphyrin and protoporphyrin levels in normal infants and children, Proc Soc Exp Biol, 86 : 89, 1954.
3. Prato K, Mazza U & Fiorina L : Il metabolismo delle porfirine in condizioni fisiologiche, Arch Sc Med, 108 : 558, 1959.
4. Ventura S and Meduri D : II significato ceinico della protoporifirina IX libera eritrocitaria Noto I, II comportamento della protoporifirina IX in diverse condizione fisiologiche (Con note ditecnica metodologica), Haematologica, 38 : 347, 1954a.
5. Wranne L : Free erythrocyte copro and protoporphyrin, A methodological and clinical study, Acta Paediatr Supp, 122: 49 : 5, 1960.
6. Rimington C : Quantitative determination of porphobilinogen and porphyrins in urine and porphyrins in faeces and erythrocytes, Assoc Clin Path, Broadsheet 70 (Revised Broadsheet 36). 1971.
7. Handa F and Sharma S C : Cord blood porphyrins, A quantitative assay in newborn infants, Acta Paediatr Scand 66 : 285 1977.