EVALUATION OF CERULOPLASMIN IN NEONATAL SEPTICEMIA

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ABSTRACT

Serial serum ceruloplasmin (Cp) levels were estimated in healthy and septicemic neonates, using single radial immunodiffusion. In 25 healthy neonates mean Cp levels were 19.82 mg/dl at birth, 18.20 mg/dl at 12-24 hours, 17.26 mg/dl at 14±4 days and 17.68 mg/dl at 28±4 days of life. For the entire neonatal period the mean Cp levels were computed to be 18.24 mg/dl.

In 20 culture positive, septicemic neonates, mean Cp levels were 27 mg/dl at onset of disease, 28.65 mg/dl 12-24 hours later and 36.2 mg/dl after 7±3 days of start of illness ($p < 0.001$ for all sampling intervals as compared to healthy group values in first month of life). The mean Cp levels were unaffected by gestational age in both groups. In the septicemic neonates, the mean Cp levels in dying neonates did not differ significantly from recovering neonates for all sampling intervals. It is concluded that estimation of serum Cp levels may help in diagnosis of neonatal septicemia, but it is not useful as an early diagnostic aid or for prognostication.

Key words: *Ceruloplasmin, Neonate, Septicemia.*

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Received for publication February 8, 1990; Accepted April 19, 1990

Septicemia is an important cause of neonatal morbidity and mortality. Early diagnosis and prompt institution of appropriate therapy are the key to successful management of this problem. However, clinical features of neonatal sepsis are non-specific and this may delay the appropriate diagnosis. To overcome this problem investigators have evaluated several acute phase proteins such as C-reactive protein(1-6), haptoglobin(2), alpha-1-antitrypsin(2,7), orosomucoid(6), prealbumin(6) and alpha-2-macroglobulin(7), as early diagnostic aids. Of these, only C-reactive protein (CRP) has been extensively studied as a result of which it has an established role as an early diagnostic aid in neonatal sepsis(3,5).

Ceruloplasmin (Cp) is also an acute phase protein(4,8,9). However, its utility as an early diagnostic aid in neonatal septicemia has not been adequately studied. The present study was, therefore, designed to evaluate the role of ceruloplasmin in the early diagnosis of neonatal sepsis.

Material and Methods

This prospective study was done on babies delivered in the Obstetric Units of Lok Nayak Jai Prakash Narain Hospital between September, 1985 and February, 1986.

The babies were divided into two groups based on certain selection criteria. The study group comprised 20 neonates with culture positive septicemia. Of these 8 were full-term (≥37 weeks gestation) and 12 pre-term (<37 weeks gestation); 11 were males and 9 females. A preliminary selection into the study group was based on clinical findings(10), or a strong suspicion of septicemia. However, those neonates who had a negative blood culture were

subsequently excluded from the study. Also excluded were neonates with birth asphyxia (5 minute Apgar ≤5). The control group comprised 25 healthy neonates born after an uneventful prenatal and natal course, who subsequently remained well during the first month of life. Of these 18 were full term and 7 preterm; 11 were males and 14 females.

Serial samples from the study group were taken at initial suspicion of septicemia (sample labelled S1); 12-24 hours after the first sample (S2); and 7±3 days after the first sample (S3). Of these 20 neonates, 15 died as a result of disease, while 5 recovered. Of the 15 septicemic neonates who succumbed, the 7±3 day sample (S3) could only be collected from 5.

From the control group, serial samples were taken at birth from the umbilical cord (sample labelled CB); at 12-24 hours of age (D1); at 14±4 days age (D14); and at 28±4 days age (D28).

In all samples, ceruloplasmin levels were estimated by single radial immunodiffusion (SRID) in agar gel(11). Monospecific antiserum was obtained commercially (Moloy Laboratory Inc. Diagnostic Division, U.S.A.) as was the necessary reference standard (Kallestad Laboratories Inc, Austin, U.S.A.).

Student's 't' test and Wilcoxon rank sum test were used to assess the significance of the differences between the groups.

Results

Of the 20 septicemic neonates, 18 had Gram negative infection (*Klebsiella:* 8 out of 18) and only 2 had Gram positive infection (both *Staph..aureus*)

In the group of healthy neonates, mean serum Cp levels were 19.82 mg/dl at delivery (cord-sera), decreasing to 18.2 mg/dl at 12-24 h of life. At 2 weeks of age the levels were 17.26 mg/dl and at 4 weeks of age 17.68 mg/dl. For the entire neonatal period the mean Cp levels were computed to be 18.24 mg/dl (*Table I*). In comparison the mean serum Cp levels in the septicemic group of neonates were significantly higher at start of illness and remained so till 1 week later (*Table I*).

Ceruloplasmin levels were related to maturity of healthy and infected neonates (*Table II*), and also to eventual outcome in neonatal septicemia (*Table III*). It was

TABLE I — *Ceruloplasmin Levels (mg/dl) in Serially Collected Sera from Healthy and Septicemic Neonates (Mean ± SD)*

Day*	Healthy neonates (n=25)	Sample*	Septicemic neonates (n=20)	P value
CB	19.82 ± 13.20	S_1	27.00 ± 10.26	<0.001
D_1	18.20 ± 10.53	S_2	28.65 ± 12.86	<0.001
D_{14}	17.26 ± 12.49	S_3**	36.20 ± 17.33	<0.001
D_{28}	17.68 ± 9.55			

n = number of cases; *See text for explanation; **n = 10.
For testing significance of study group levels, the mean ±SD for ceruloplasmin levels in the first month of life were calculated from healthy group levels (18.24 ± 11.40).

TABLE II—*Ceruloplasmin Levels (mg/dl) Related to Gestational Age in Healthy Septicemic Neonates (Mean ± SD)*

Day*	Healthy Neonates		Sample*	Septicemic neonates	
	Full term (n=18)	Preterm (n=7)		Full term (n=8)	Preterm (n=12)
CB	20.56 ± 14.73	16.50 ± 9.51	S_1	29.00 ± 13.22	25.67 ± 7.40
D_1	17.33 ± 10.55	20.43 ± 10.95	S_2	27.88 ± 10.54	29.17 ± 14.18
D_{14}	18.36 ± 14.02	14.43 ± 7.36	S_3**	36.17 ± 13.27	36.25 ± 22.05
D_{28}	18.61 ± 9.91	15.29 ± 8.81			

*See text for explanations; ** Full term (n=6), Pre-term (n=4).

None of the differences between term and preterm babies were significant (P>0.05) in either healthy or septicemic neonates.

observed that mean Cp levels, for all the various periods of sampling, were unaffected by gestational age of the neonates in both healthy and septicemic groups (*Table II*). Similarly, Cp levels did not vary significantly in either the group which recovered from the infection, or the group which succumbed to infection, either at the start or later during the course of the illness (*Table III*).

Discussion

Ceruloplasmin is an alpha-2-globulin, which accounts for the major portion of copper in plasma(4). A pronounced inborn deficiency of this protein is characteristic of Wilson's disease(12). In 1955, Markowitz *et al.*(13) demonstrated that oxidase activity of Cp is elevated in sera of pregnant women, patients with infectious diseases and the nephrotic syndrome. Rice(8) found a good correlation between serum Cp levels and other acute phase reactants, especially CRP in various disease states.

In a study from India(14), Cp was estimated in normal term, preterm and post-mature neonates and also in infected neonates. Ceruloplasmin was estimated by a colorimetric method(15). The authors concluded that Cp does not cross the placenta and the Cp in cord-blood is the product of

TABLE III—*Ceruloplasmin Levels (mg/dl) in Serially Collected Sera from Septicemic Neonates Related to Outcome (Mean ± SD)*

Sample*	Ceruloplasmin levels		p value
	Recovered (n=5)	Died (n=15)	
S_1	27.40 ± 8.45	26.87 ± 10.80	>0.05
S_2	30.00 ± 12.34	28.20 ± 13.00	>0.05
S_3	32.00 ± 19.68	40.40 ± 13.34	>0.05

*see text for explanation; **recovered (n=5); died (n=5).

10. Glasgow LA, Overall Jr JC. Infections of the newborn. *In*: Nelson Textbook of Pediatrics, 12th edn. Eds Behrman RE, Vaughan III VC, Nelson WE. Philadelphia, WB. Saunders Company, 1983, pp 399-416.

11. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem 1965, 2: 235-254.

12. Schienberg IH, Gitlin D. Deficiency of ceruloplasmin in patients with hepaticolenticular degeneration (Wilson's disease). Science 1952, 116: 484-485.

13. Markowitz H, Gubler CJ, Mahoney JP, Cartwright GE, Wintrobe MM. Studies on copper metabolism. XIV. Copper, ceruloplasmin and oxidase activity in sera of normal human subjects, pregnant women, and patients with infection, hepatolenticular degeneration and nephrotic syndrome. J Clin Invest 1955, 34: 1498-1508.

14. Husain Z, Hameed F, Jamil AA. Serum ceruloplasmin in neonates. Indian Pediatr 1982, 19: 829-832.

15. Ravin HA. An improved colorimetric enzymatic assay of ceruloplasmin. J Lab Clin Med 1961, 58: 161-168.