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Reference Values of Umbilical Cord and Third-day Cystatin C Levels for Determining Glomerular Filtration Rates in Newborns

A Bahar, Y Yilmaz, S Unver, I Gocmen and F Karademir

Department of Paediatrics, Gülhane Military Medical Academy Haydarpaşa Training Hospital, Istanbul, Turkey

The aim of this study was to determine reference values for serum cystatin C at, and 3 days after, birth, and to determine if the concentration was influenced by gender, gestational age or bilirubin level. Umbilical cord and peripheral venous blood was taken, and serum cystatin C, creatinine, and total and direct bilirubin levels were measured. The mean concentration of cystatin C was not significantly different between cord blood and blood taken on day 3 (1.36 ± 0.35 mg/l and 1.35 ± 0.33mg/l, respectively). Comparison of subgroups, divided by gender, duration of gestation and bilirubin levels, using the Mann–Whitney *U*-test and Wilcoxon analysis, showed no effect of these parameters on cystatin C levels.

KEY WORDS: Cystatin C; Newborn; Neonates; Cord blood; Hyperbilirubinaemia

Introduction

Cysteine proteases are proteolytic enzymes involved in many pathological processes and found in the lysosomes of cells. Their role is crucial in normal cellular metabolism, being fundamental to intracellular protein turnover, degradation of collagen, and cleavage of precursor proteins. Cysteine protease inhibitors, of which the cystatin super-family is one example, constitute the final regulatory step in the control of cysteine proteases.[1] Cystatin C, the most studied cystatin, is present at high concentrations in many body fluids including seminal, cerebrospinal and synovial fluids.[2] It is excreted only by the kidney, and readily passes through glomeruli due to its low molecular weight and positive charge at physiological pH.[3]

Measurement of creatinine clearance is widely used for determining glomerular filtration rate (GFR) in clinical practice.[4,5] This method, however, takes a long time to perform as it needs a 24-h urine collection.[6,7]

Cystatin C has been demonstrated to reflect GFR better than other low molecular weight proteins, including creatinine, with sensitivity and specificity values of 70% and 100%, respectively.[8,9] There are, however, only a few studies reporting reference values of this protein in umbilical cord or peripheral blood samples obtained from infants and children.[5,10]

This study aimed to determine reference values of cystatin C in umbilical cord and peripheral venous blood taken from 3-day-old neonates. These values were analysed in different subgroups of neonates, comprising gender (male or female), gestational age (pre-term or term), and presence or absence of significant hyperbilirubinaemia.

Patients and methods

PATIENTS

The study was carried out on consecutive babies, born to women followed antenatally at the Department of Gynaecology and Obstetrics of Gülhane Military Medical Academy Haydarpaşa Training Hospital, between June 2001 and July 2002. Written, informed consent was given by the parents, and the local ethics committee approved the study. Newborns with any renal pathology, systemic illness, congenital anomaly, conjugated hyperbilirubinaemia, or whose mothers had chronic or pregnancy-induced hypertension, pre-eclampsia/eclampsia, or any other systemic illness were excluded from the study.

METHODS

Blood samples were taken from the umbilical cord at birth, and peripheral veins of the same babies on the third day of life (day-3 samples). Samples were separated by centrifugation at 1509.3 ***g*** for 5 min, within 6 h of collection, and serum was stored at –20 °C prior to the measurement of cystatin C, and total and direct bilirubin levels.

Bilirubin was measured by the Jendrassik–Grof method using a Beckman Coulter Syncron LX-20 auto-analyser (Fullerton, CA, USA).[11] Cystatin C was measured using a C latex-enhanced immunonephelometric assay (N Latex Cystatin C kit; DADE Behring, Lederbach, Germany), and a BN ProSpec® nephelometer (DADE Behring, Lederbach, Germany).

Birth weight, gestational age, gender, and whether the subject had significant hyperbilirubinaemia were recorded in each case. Neonates with gestational ages < 37 weeks and ≥ 37 weeks were deemed to be pre-term and term, respectively.[12] Hyperbilirubinaemia was defined as a serum total bilirubin level of ≥ 2 mg/dl at birth, ≥ 8 mg/dl on day 1, ≥ 12 mg/dl on day 2, ≥ 15 mg/dl on day 3 and ≥ 17 mg/dl thereafter.[13]

STATISTICAL ANALYSIS

Serum cystatin C levels were analysed in the total study group, and in subgroups with respect to gender, gestational age and significant hyperbilirubinaemia. The Mann–Whitney *U*-test and Wilcoxon test were performed to compare results between the subgroups. Statistical significance was set at $P < 0.05$.

Results

Blood samples were taken from the umbilical cord of 112 newborns (60 female and 52 male) and from 98 (58 female and 40 male) 3-day-old neonates. Fourteen patients did not attend for follow-up visits. The demographic characteristics of the study group are shown in Table 1.

The mean (± SD) level of cystatin C was found to be 1.36 ± 0.35 mg/l in cord blood samples, and 1.35 ± 0.33 mg/l in day-3 blood samples, which was not significantly different. The mean concentrations of cystatin C in samples, subdivided according to gender, gestational age and bilirubin levels, are shown in Tables 2 – 4.

Jaundice was noted in 25 neonates on day 3, and all had a direct bilirubin level of < 0.5 mg/dl. The total bilirubin level, however, was > 15 mg/dl in 12 of them.

Discussion

Cystatin C is reported to give more significant results than creatinine for the

TABLE 1:
Demographic characteristics of the neonates entered into the study

	Cord blood	3 days old
Total number of subjects	112	98
Female	60	58
Male	52	40
Body weight (g ± SD)	3240 ± 530	3110 ± 490
Pre-term (gestation age < 37 weeks)	14	14
Term (gestational age ≥ 37 weeks)	98	84
Serum total bilirubin (mg/dl)	1.1 ± 0.6	7.6 ± 4.0[a]
Subjects with hyperbilirubinaemia	5	12
Subjects without hyperbilirubinaemia	107	86

[a]Includes 12 patients with hyperbilirubinaemia and total bilirubin > 15 mg/dl.

TABLE 2:
Comparison of cystatin C levels (mg/l) with respect to gender

	Male mean ± SD (range)	Female mean ± SD (range)	*P*-value[a]
Cord blood	1.38 ± 0.19 (0.77 – 2.43)	1.34 ± 0.39 (0.78 – 2.30)	0.48
3 days old	1.34 ± 0.34 (0.69 – 2.2)	1.35 ± 0.32 (0.86 – 2.40)	0.37

[a]$P < 0.05$ was taken to be significant.

TABLE 3:
Comparison of cystatin C levels (mg/l) with respect to gestational age

	Pre-term[a] mean ± SD (range)	Term[a] mean ± SD (range)	*P*-value[b]
Cord blood	1.40 ± 0.18 (1.30 – 1.66)	1.35 ± 0.37 (0.69 – 2.43)	0.32
3 days old	1.49 ± 0.35 (0.98 – 2.30)	1.32 ± 0.32 (0.78 – 2.40)	0.20

[a]Pre-term was defined as gestational age < 37 weeks while term babies had a gestational age ≥ 37 weeks.
[b]$P < 0.05$ was taken to be significant.

TABLE 4:
Comparison of cystatin C levels (mg/l) with respect to hyperbilirubinaemia

	With hyperbilirubinaemia	Without hyperbilirubinaemia	*P*-value[a]
Cord blood	1.39 ± 0.23 (0.97 – 1.78)	1.36 ± 0.37 (0.69 – 2.43)	0.16
3 days old	1.37 ± 0.30 (0.83 – 2.92)	1.33 ± 0.33 (0.78 – 2.40)	0.12

[a]$P < 0.05$ was taken to be significant.

assessment of renal function in patients with renal disease.[3,14 – 16] Herget-Rosenthal *et al.*[17] reported that, in determining decreased GFR, cystatin C provided a sensitivity of 97% and a specificity of 96%, whereas creatinine provided rates of 83% and 87%, respectively. Beta-2 and alpha-1 microglobulin are inferior to creatinine in assessing GFR.[16]

Increased cystatin C levels have been reported in neonates, but it has been suggested that the increase is not derived from the mother, and there is no correlation between the levels of cystatin C in mother and baby.[18] We found no significant difference between levels of cystatin C in cord blood and day-3 venous blood, supporting the idea that the production and metabolism of cystatin C are independent in the fetus and mother. The elevated levels seen in the first few days rapidly decrease, and by 4 months are within a reference range of 0.7 – 1.38 mg/l.[5,19] We found mean cystatin C levels in the cord blood samples, and day-3 venous blood samples, to be near the upper limit of this previously reported reference range. Our results are therefore consistent with previous studies, and suggest no significant difference between levels of cystatin C in cord blood and day-3 venous blood samples. Premature infants are reported to have the highest levels of cystatin C, and it is suggested that the decrease in levels during the early years of life is an indicator for the maturation of GFR.[5] Our finding that the cystatin C reference range for neonates is higher than for adults supports the view that cystatin C levels reflect the maturation of GFR.[9,19] There was no marked difference between the birth weights of our pre-term and term newborns, suggesting, along with other studies, that birth weight has no effect on cystatin C levels.[5,19]

Bökenkamp *et al.*[19] reported that cystatin C levels showed a mild increase in patients with hyperbilirubinaemia, but other studies have demonstrated that cystatin C is not influenced by levels of bilirubin.[5,18] We found no significant difference between levels of cystatin C in newborns or neonates, with and without hyperbilirubinaemia, a finding consistent with previous studies.[5,18,19] Our results are also consistent with previous studies with regard to cystatin C levels and gender.[2,5,9,10,19]

In conclusion, the levels of serum cystatin C in cord or day-3 blood samples, did not differ significantly when compared as a total group, or divided into subgroups according to gender, gestational age or presence or absence of hyperbilirubinaemia. Cystatin C is regarded as an alternative to creatinine for assessing GFR, but it is not yet accepted as being superior to creatinine. A limited number of studies on the reference values of cystatin C in children are available, so further studies with large numbers of cases are required to assess whether cystatin C should replace creatinine as a test for GFR.

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Address for correspondence
Dr A Bahar
GATA Haydarpaşa Eğitim Hst, Çocuk Servisi 81327, Üsküdar-Istanbul, Turkey.
E-mail: arbahar@hotmail.com