Journal of Trace Elements in Medicine and Biology 39 (2017) 1–5

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Epidemiology

Serum copper concentrations in hospitalized newborns

Ricardo González-Tarancón[a], Luisa Calvo-Ruata[a], Maite Aramendía[b], Carmen Ortega[a], Elena García-González[a], Luis Rello[a,*]

[a] Department of Clinical Biochemistry, Hospital Universitario "Miguel Servet", Paseo Isabel La Católica 1-3, 50009, Zaragoza, Spain
[b] Centro Universitario de la Defensa-Academia General Militar de Zaragoza, Carretera de Huesca s/n, 50090, Zaragoza, Spain

ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form 27 June 2016
Accepted 30 June 2016

Keywords:
Atomic absorption spectrometry
Copper
Ceruloplasmin
High sensitivity C-reactive protein
Newborn

ABSTRACT

Background: Low serum Cu and ceruloplasmin (Cp) concentrations in newborns can be the first indication of a severe Cu deficient intake or, alternatively, of genetic diseases affecting Cu metabolism. However, Cu and Cp concentrations can also be influenced by other variables that render their quantitative results difficult to interpret. Therefore, it is necessary to identify these variables and stratify Cu and Cp concentrations according to these altering factors.
Methods: Serum Cu and Cp concentrations for 564 hospitalized newborns (0–12 days of life) are stratified according to their age, prematurity (birth weight or gestational age), type of feeding and inflammatory state (assessed by the serum high sensitivity C-reactive protein (hs-CRP) level) to identify potential correlations.
Results: Serum Cu and Cp concentrations are influenced by all four variables analyzed, although inflammation is the most significant: the greater the hs-CRP concentration, the greater the serum Cu and Cp concentrations. Prematurity is also an important factor and preterm infants often show very low Cu and Cp concentrations. Age of life and type of feeding have in turn a more modest effect on these magnitudes, being slightly greater at 3–5 days of age in breastfed newborns.
Conclusions: Inflammation and prematurity are the main variables affecting serum Cu and Cp concentrations in newborns. Therefore, hs-CRP should always be assayed in parallel to Cu status. When there is an inflammatory state proper interpretation of these concentrations can be challenging.

1. Introduction

Copper is an essential trace element that is required for adequate human development [1]. In plasma, approximately 75–90% of Cu is bound to ceruloplasmin (Cp) [2], so Cu concentrations are parallel to those of Cp.

Cu deficiency in newborns as a consequence of an inadequate dietary intake during pregnancy is rare. However, deficiency of this element can arise in the neonatal period by several means [1,3], the most important being produced by genetically determined conditions, such as Wilson's Disease (WD) or Menkes Disease, or by a very low Cu intake in newborns fed with improperly Cu supplemented artificial formulas [4,5]. For this reason, paediatricians often request evaluation of Cu status in hospitalized newborns.

However, interpretation of serum Cu and Cp concentrations in this population is difficult because they also influenced by several variables, such as, prematurity [6,7], type of feeding [8,9] and inflammatory conditions [10]. This is the reason why the use of Cp to screen for WD in the neonatal period has proven unsuccessful [11] or why extreme Cu deficiencies in newborns fed with artificial formulas are often detected after elapsing several months [4,5].

In this paper the concentrations obtained for Cu and Cp in hospitalized newborns are presented. As shown below, proper interpretation of the quantitative results obtained requires the identification and stratification of the different variables influencing these concentrations.

2. Material and methods

2.1. Patients

596 newborns were initially included in the study after obtaining written consent from their parents. All of them were born between January 2009 and December 2010 and were admitted at

Abbreviations: Cp, ceruloplasmin; EQAS, external quality assurance schemes; GFAAS, graphite furnace atomic absorption spectrometer/spectrometry; hs-CRP, high sensitivity C-reactive protein; LOD, limit of detection.

* Corresponding author.
E-mail address: lrello@salud.aragon.es (L. Rello).

http://dx.doi.org/10.1016/j.jtemb.2016.06.010
0946-672X/

the Neonatal Unit or the Neonatal Intensive Care Unit of the Hospital Universitario Miguel Servet (HUMS) for several reasons. The main causes of admittance were prematurity, neonatal jaundice, risk of infection, apnoea or respiratory distress, feeding problems or convulsions. In all cases, serum Cu and Cp concentrations were determined as part of a complete blood analysis study requested by the paediatricians in charge, which is included in *the neurometabolic protocol* established by the Paediatrics Department of the HUMS. Data from 32 babies who died in the following weeks/months after birth due to their severe pathologies and/or extreme prematurity were finally excluded from the study. According to their clinical histories, none of the 564 individuals finally included in the study developed signs of Cu deficiency after their stay at the Neonatal Units of the HUMS and, until November 2015, none has been diagnosed with WD.

To observe the effect of the type of feeding on serum Cu and Cp concentrations, newborns were categorized in three groups: breast, mixed or artificial feeding. The artificial formulas provided contained approximately 3000 ± 20 μg Cu/L for term newborns and 5000 ± 20 μg Cu/L for preterm newborns. The content of Cu in human breast milk is approximately 1200–1500 μg/L [12], although in this case the range of milk Cu concentrations among mothers is very wide, and values ranging from 30 to 2190 μg/L have been reported [13]. In any case, the exact Cu intake in each newborn was unknown, as the amount of milk/artificial formula ingested was not documented.

The HUMS Ethics Committee approved the study.

2.2. Instrumentation

Cu was determined in a graphite furnace atomic absorption spectrometer (GFAAS) ZEEnit 600 with Zeeman correction (Analytik Jena, Jena, Germany); Cp concentrations were determined by nephelometry on an Immage 800 apparatus (Beckman Coulter, Brea, CA, USA) and hs-CRP concentrations were measured by turbidimetry on the AU analysers (Beckman Coulter). Serum was the specimen of choice in all cases.

The analyses were made at the Department of Clinical Biochemistry of the HUMS. This laboratory participates in several national and international external quality assurance schemes (EQAS) and performs successfully in all three magnitudes analyzed.

It is worth mentioning that the GFAAS method for Cu determination used in this work, adapted from the one published by Almeida and Lima [14], only requires a minimum quantity of serum (10 μL). When using Flame Atomic Absorption Spectrometry (FAAS), the analytical technique of choice in most laboratories for serum Cu measurement, hundreds of microliters of sample are required, a volume not always available for this population especially, as in this case, when multiple tests are requested. Serum samples were 100-fold diluted with a 0.028 M HNO_3 solution and 10 μL of the diluted sample was directly introduced in the GFAAS spectrometer by means of an autosampler. Two replicate measurements were carried out for each determination. Optimized conditions for best detection power were achieved by deploying the Cu line at 324.8 nm, a pyrolysis T of 1200 °C and an atomization T of 2000 °C, which yielded a limit of detection (LOD) of 4.2 μg/L for the undiluted sample. This LOD is slightly poorer than those reported in other works measuring serum Cu by GFAAS: 4.0 μg/L in the work by Correia et al. [15] (1/80 sample dilution) or 0.98 μg/L in the work by Almeida and Lima [14] (1/25 sample dilution). This fact might be a direct consequence of the greater sample dilution factor deployed in this case (1/100). In any case, the LOD achieved is clearly fit for purpose, as all samples analyzed had Cu concentrations above the limit of quantification. Precision of the technique, evaluated from analysis of quality control materials, was 3.7% for 801 μg Cu/L and 6.2% for 1340 μg Cu/L. Bias, estimated by EQAS performance (OELM Serum, Trace Elements External Quality Assessment Scheme organized by the Société Française de Biologie Clinique), ranged between −5.1% and +3.2%, with a total Z score mean of −0.4.

2.3. Sampling conditions and contamination issues for Cu determination

Following standard phlebotomies, venous blood samples were drawn into siliconized trace metal tubes (Vacutainer trace element serum, reference 368380. Becton Dickinson, Franklin Lakes, NJ, USA). After centrifugation, 100 μL serum aliquots were transferred to metal free polypropylene tubes (1.8 mL Nunc cryotube vials, catalog number 363401. Roskilde, Denmark) and immediately frozen at −20 °C until Cu analysis. All Cu assays were performed less than 1 month after collection.

All laboratory material used for analysis (glassware, pipette tips, autosampler cups) was previously immersed for 24 h in a 0.28 M HNO_3 solution, followed by rinsing with Milli-Q water. As serum Cu concentrations in newborns are expected to be in the order of hundreds of μg/L, any additional precaution to avoid contamination was considered unnecessary. In fact, blank absorbance values were always in the range of 0.0020–0.0030 s^{-1}, while 1 μg/L Cu aqueous standard solutions provided absorbance values in the 0.0120–0.0140 s^{-1} range, a signal that can be easily differentiated from blank levels.

2.4. Statistical analyses

The sociodemographic variables were categorized using established or study-specific cut-offs. Thus, prematurity was conventionally defined as birth weight or gestational age below 2500 g or 36 weeks, respectively. Days of life at sample collection were arbitrarily separated into 3 groups, 0–2, 3–5 and 6–12 days. To study the effect of the inflammatory state on serum Cu and Cp concentrations, serum hs-CRP concentration was also stratified defining 3 intervals: normal (<5 mg/L), slightly elevated (5–20 mg/L) and overtly high (>20 mg/L).

All categorical variables (birth weight, gestational age and days of life at sample collection) are presented as medians (25th–75th percentiles). Normality of Cu and Cp concentrations in each subgroup was assessed using the Kolmogorov–Smirnov test. As these concentrations were not normally distributed in any of the cases, they are also reported as medians (2.5th–97.5th percentiles).

Differences in Cu and Cp concentrations were assessed by U-Mann-Whitney or Kruskall-Wallis tests when comparing two or more than two subgroups, respectively. Differences with p values below 0.05 were considered to be statistically significant. Finally, correlation between serum Cu and hs-CRP concentrations was assessed using the Spearman's correlation coefficient (in that case, serum hs-CRP concentration was considered to be a continuous variable).

Statistical analyses were conducted with SPSS software (IBM Company, SPSS Statistics version 18.0, United States).

3. Results

The main altering factor influencing Cu (and Cp) serum concentrations in newborns is inflammation, even more than prematurity.

The characteristics of the group of patients under study according to the main variables considered are shown in Table 1. Tables 2 and 3 show the serum Cu and Cp concentrations obtained, respectively, for each of the newborn subgroups considered. In each of these subgroups, the greater the hs-CRP, the greater the Cu and Cp concentrations (p^b value, last column in Tables 2 and 3). As expected, term babies (>2500 g or >36 weeks) have serum Cu and Cp concentrations greater than preterm newborns, but these