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Original article

Copper concentrations in Egyptian infants with cholestasis: A single center study

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ABSTRACT

Background and study aims: Hepatobiliary cholestatic disorders produce excess copper (Cu) retention in the liver, which is toxic and may cause hepatitis, fulminant hepatic failure, cirrhosis and death. In this study, we measured hepatic Cu and tested its correlation with serum Cu (S. Cu) and serum ceruloplasmin (S. ceruloplasmin) in cholestatic infants.

Patients and methods: 41 cholestatic infants were enrolled as cases and 11 healthy infants as control subjects. S. Cu and S. ceruloplasmin were done for all infants and hepatic Cu was measured in the liver specimen in cases.

Results: Cases were 63.5% males with their age ranging between 1 and 7 months, while control subjects were 45.5% males with an age range between 3 and 18 months. Among cases, 41.5% had biliary atresia and 58.5% had intrahepatic cholestasis. Cholestatic infants had significantly higher levels of S. Cu and S. ceruloplasmin than control subjects and their hepatic Cu concentration was significantly higher than literature control. Infants with biliary atresia showed higher levels of Cu indices, with no statistical significance. Serum and hepatic Cu levels positively correlated with each other and with S. ceruloplasmin. Results of ROC curve showed that S. Cu was highly sensitive and specific for predicting hepatic Cu concentration at cut-off 181 μg/dl.

Conclusion: Serum and hepatic Cu concentrations were markedly elevated in patients with cholestasis and positively correlated with each other and with S. ceruloplasmin. S. Cu level can predict hepatic Cu concentration.

Introduction

Copper (Cu) is a trace element essential for the growth and differentiation of cells. It is an integral part of numerous enzyme systems. Excess hepatic Cu has been shown to cause liver injury in humans, it may lead to hepatitis, fulminant hepatic failure, cirrhosis and death [1].

Biliary excretion is the major elimination route of Cu, as more than 80% of absorbed Cu is excreted in bile. Exocytosis of lysosomal Cu across the canalicular membrane is the major source of biliary Cu, which is then bound to large proteins (e.g., metallothionein) that prevent reabsorption by the small intestine [2,3]. Pathologic processes that interfere with biliary excretion of Cu, such as intrahepatic and extrahepatic hepatobiliary cholestatic disorders, produce Cu retention in the liver, generally in the lysosomal fraction of hepatocytes. Cu accumulated is released into the circulation with increased fraction of free Cu relative to Cu-bond to ceruloplasmin leading to elevated plasma Cu and ceruloplasmin levels [3,4].

Liver Cu concentration is the best indicator of Cu status and is the standard to compare the performance of any test used to detect Cu overload. However, this invasive procedure is only justified when there is evidence of liver damage as a result of Cu overload [5]. The newborn human can tolerate 5–100 folds the hepatic Cu content of normal adults, which is physiologic and necessary to overcome the negative Cu balance in the early postnatal period. After birth, hepatic Cu content falls rapidly, reaching adult levels by 3 months of age [3,6–8].

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Serum Cu (S. Cu) and serum ceruloplasmin (S. ceruloplasmin) concentrations are the most widely used laboratory indicators to assess Cu status [5]. Both S. Cu and S. ceruloplasmin experience changes related to age and sex and are increased by other conditions not related to Cu status (inflammatory or infectious processes and neoplasms) [9]. Their use is limited by the nonexistence of standardized assays [5].

After birth, S. Cu increase from birth level of 31 μg/100 ml to the adulthood level of 98 μg/100 ml by 7–12 months [3]. Ceruloplasmin is a blue-colored Cu-containing α2- globulin synthesized in the liver and secreted into the plasma after incorporation of six Cu ions in the secretary pathway. It is the key protein in Cu metabolism**.** In the human newborn, ceruloplasmin levels measured by the oxidase enzymatic assay are 1.8–13.1 mg/dL, in older children it is between 20 and 45 mg/dL [3].

The aim of this work is to evaluate S. Cu and S. ceruloplasmin as indicators of hepatic Cu levels in infants with cholestasis.

Patients and Methods

This is a crosssectional study that involved 41 cholestatic infants who are attending the Paediatric Hepatology Unit, Cairo University Children's Hospital. Recruitment was done over 2 years. The study protocol was approved by the ethical committee and patients' enrollment was done after obtaining an informed consent from one of the parents. Eleven healthy infants whose parents agreed to share in the study were enrolled as a control group.

Inclusion criteria

- Infants below 24 months with cholestasis
- Both sexes
- Normal synthetic liver functions (prothrombin time and serum albumin)
- Haemoglobin and platelet counts within acceptable levels.
- No associated infections or comorbidities (e.g. cholangitis)
- Parental agreement to sign the informed consent

All cholestatic infants were subjected to

Demographic and anthropometric data included age, sex, weight and length. All infants underwent a thorough history taking and full clinical assessment. Liver profile including total serum bilirubin (normal range: 0.2–1 mg/dl), direct serum bilirubin (normal range 0.1–0.3 mg/dl), aspartate aminotransferase (AST) (normal range 10–38 IU/L), alanine aminotransferase (ALT) (normal range 10–41 IU/L), alkaline phosphatase (ALP) (normal range 45–122 U/L) and albumin (normal range 3.5–5 g/dl). These tests were measured by auto-analyzer (Abbott AXSYM system-UK). Prothrombin time and concentration (normal range: 75–100%) were determined.

Serum Copper was measured in μg/dl (GBS scientific equipment, Australia). Serum ceruloplasmin was measured by using radial immune-diffusion plates for accurate quantitative determination by Biocientifica SA (Argentina) [10].

Needle liver biopsy was performed as a part of the workup for cholestatic infants at our site where sophisticated laboratory tests and genetic study are not available. It was done after obtaining special biopsy-related informed consent from one of the parents. It was mandatory to rule out surgical causes (e.g. biliary atresia) and to detect pathological pattern of inflammation. In infants fit to undergo biopsy, it was performed using Sure Cut needle. Liver tissues were divided into two sections; one for histopathological examination and the other for assessment of tissue copper contents.

For histopathological examination, the biopsy specimens were fixed in 10% formalin solution and processed by the paraffin technique. The paraffin blocks were serially sectioned by a microtome. The paraffin sections were stained by hematoxylin and eosin.

For assessment of tissue copper contents, biopsy specimens (about 0.5 cm in length) were dried in a vacuum oven, weighed and digested in concentrated nitric acid in a 100 °C oven for 1 h. Cu level was measured by atomic absorption spectroscopy (AAS) using the graphite furnace (Part No. 01-009-00; GBS Scientific Equipment Pty. Ltd., Dandenong, Victoria, Australia). The working range of the standard used was 2.5–10 μg/ml at wave length 327.4 nm. This method is sensitive up to 0.050 μg/ml level in tissue samples.

Control group were recruited from infants aged below 2 years attending the general outpatient clinic, complaining of mild upper respiratory tract infection; for example, rhinopharyngitis. The eleven enrolled infants underwent blood sampling for assessment of S. Cu and S. ceruloplasmin.

Statistical Methods

Data were collected and tabulated. Statistical Package for Social Science (SPSS, Chicago, IL 60606-6412, USA) program version 17.0 was used for data analysis. Mean and standard deviation (SD) and median and interquartile range (IQR) were estimates of quantitative data while frequency and percentage were estimates of qualitative data. Differences in serum level of Cu indices and hepatic Cu between cases and controls and between groups were done using Student's *t* test for S. ceruloplasmin and Mann Whitney *U* test for S. Cu and hepatic Cu. Spearman's correlations were done to test for linear association between indirect Cu indices and hepatic Cu levels.

Receiver operator characteristic (ROC) curve was drawn to test the association of Cu indices and hepatic Cu content in cholestatic infants and to calculate their best cutoff levels. The cut-off for hepatic Cu used was 92 μg/g dry weight [11]. Data for S. Cu have been normalized by log transformation. The best cut-off for indirect Cu indices were obtained from the ROC curve and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated. Level of significance was two-sided p value ≤ 0.05 for all statistical analyses.

Results

The present study included 41 cholestatic infants, their ages ranged between 1 and 7 months; 26 (63.5%) of them were males. All cases were clinically jaundiced. They had direct hyperbilirubinaemia, with elevated level of ALT, AST and ALP. Histopathology showed a picture of intrahepatic cholestasis in 24 (58.5%) infants and biliary atresia **(BA)** in 17 (41.5%) infants. Diagnosis of BA was confirmed during surgery. Clinical and laboratory characteristics of infants with cholestasis are shown in Table 1.

The control subjects were 11 infants; 6 (54.5%) of them were females, and their median and IQR for age was 12 [12–15] months, ranged between 3 and 18 months. They were apparently healthy without any symptoms or signs of liver disease (e.g. no jaundice or organomegaly).

In cases with biliary atresia, the main pathological findings were distorted architecture, hydropic/ballooning degeneration of hepatocyte. Portal areas showed marked fibrotic expansion with mixed inflammatory cellular infiltrate +/− oedema and proliferated distorted bile ducts. Occasional fibrous septa and portal-portal bridging were observed. In cases with intrahepatic cholestasis, there was diffuse ballooning degeneration with giant cell transformation and steatosis. Portal area showed mild expansion with cellular infiltrate. PAS diastase resistance globules were negative in all cases. Although all cases showed hepatocellular degeneration

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