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APPLIED METHODOLOGY

Direct analysis of human blood (mothers and newborns) by energy dispersive X-ray fluorescence

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Abstract

This work is an application of energy dispersive X-ray fluorescence (EDXRF) as analytical technique for trace element determination in human tissues. Potassium (K), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), bromine (Br), rubidium (Rb) and lead (Pb) were determined directly in blood samples from 66 mothers at delivery after full-term pregnancies. The corresponding 66 cord-blood samples of the newborns were also analysed, in order to find element correlations between maternal and newborn blood at birth. The studied samples were obtained from mothers aged between 15 and 39 years old, the gestational age being between 35 and 41 weeks and the newborns' weight between 2.310 and 4.310 kg. Samples were lyophilised and analysed without any chemical treatment.

Very low levels of Pb were found both in maternal and fetal cord blood samples. Cu values ranged from 3 to 13 $\mu g\, g^{-1}$, both for mothers and children. A correlation between Cu and Fe concentrations in maternal and fetal cord blood was found. Zn is considered as one of the key elements in newborn health. Concentrations between 10 and 40 $\mu g\, g^{-1}$ were measured. A positive correlation between Br levels in mothers and children was observed. Positive correlations for mothers were observed between Zn and Rb as well as K and Fe. The corresponding correlations in fetal cord blood samples were not observed, however positive correlations were found between Ca and K; Cu and Fe. The mean concentrations for each element were similar in maternal and in fetal cord blood, except for Cu and Zn, being higher in maternal samples. No correlations between element concentrations and pathologies of the mothers were observed.

Keywords: EDXRF; Biological samples; Human cord blood; Trace elements; Element analysis

Introduction

Energy dispersive X-ray fluorescence spectrometry (EDXRF) was used for quantitative analysis of potassium (K), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), bromine (Br), rubidium (Rb) and lead (Pb) in maternal and newborn cord blood at birth.

Blood is the medium for transport of trace elements in the whole human body. The transport of essential elements from mother to fetus varies during gestation and the transport of proteins may change during pregnancy. Trace elements are present in cord blood and reach the fetus. Therefore, whole blood, plasma or serum are convenient samples for determination of trace element concentrations in order to establish a pattern for fetal development. The role of several elements in fetal growth is well documented in literature [1]. A number of

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studies have been carried out in whole cord blood and serum of newborns and element concentrations have been correlated for mother's and child's health [2–4].

Zn is known to be essential for normal embryogenesis, fetal growth and protein synthesis, and low Zn levels suggest the existence of fetal growth problems [5,6]. Cu is also of particular importance, especially early in life, for the development and maintenance of myelin [5].

The impact of inadequate or excess intake of certain nutrients during pregnancy on embryonal and fetal development has been studied [7]. Deficiency of trace elements during intrauterine existence is closely related to mortality and morbidity in the newborn [1,7,8]. The mechanism of transport of trace elements from the mother to the newborn is still not well known. Since the transport of several essential elements across membranes is competitive, many divalent ions compete with each other and positive or negative correlations between them may exist.

The effect of gestastional age on cord blood plasma Cu, Zn and Mg has been studied by atomic absorption spectrophotometry [9]. An increase of the Zn concentration in maternal plasma with the increase of gestational age was observed, but a decrease with increasing gestational age in cord blood plasma. On the contrary, Cu concentration decreased in maternal plasma with the increase of gestational age, while in cord blood there was an increase with gestational age. Serum of newborns has also been studied by inductively coupled plasma mass spectrometry (ICP-MS) to compare the element concentrations with those of adults and young infants [10]. The X-ray fluorescence technique was also applied to study human tissues and body fluids, including whole blood and serum [11,12]. Blood samples have also been studied by proton-induced X-ray emission (PIXE) [13].

The purpose of this work was to determine the element concentrations of K, Ca, Fe, Cu, Zn, Br, Rb and Pb in maternal and fetal cord blood at birth and to investigate whether the concentrations of these elements in maternal and fetal cord blood depend on gestational age, birth weight and age of the mother. Moreover, we intended to establish correlations between elements in fetal cord blood, in maternal cord blood and between them. This study aims to be a contribution for a better understanding of the mechanism of trace element transport from the mother to the child, and of the effect of excess or deficiency in certain elements in fetus development.

Material and methods

Samples

Blood samples were provided by the obstetric department of Garcia de Orta Hospital in Almada, Portugal. The 66 cord blood samples were collected post-partum, the mothers being aged between 15 and 39 years. Regarding the delivery labours, 50 were normal, 9 were with forceps and 7 were caesareans. Nine mothers were smokers and 57 were not. Thirty-five babies were male and 31 female, and their weight ranged from 2310 to 4310 g. All except 2 newborns were healthy. The gestation period ranged from 35 to 41 weeks.

Blood samples were collected from the umbilical cord, which is the structure that connects the placenta to the fetus. The umbilical cord consists in a long tube, with three blood vessels: a vein and two arteries. In the vein, the oxygenated blood with nutrients flows from mother to fetus, and in the arteries, the venous blood and the excretion products flow from the fetus to the mother. In the placenta, the fetal blood and the maternal blood flow very close to each other, separated by the placenta membrane, which allows a nutrient exchange between mother and fetus.

The post-partum tissues were removed using normal surgery equipment, always the same for all the samples.

Sample preparation

The samples were frozen in sealed plastic bags for transportation from the hospital to the laboratory for further analysis. Prior to analysis, blood samples were lyophilised for 72 h at -60° and low pressure (ca. 0.1 atm). The time was optimised for total removal of water. Following this procedure, a powder was obtained of each sample in a polyester mill, and the samples were stored under controlled humidity conditions. For analysis the samples were pressed into pellets of 2.0 cm in diameter and 1 mm thickness. A minimum of three pellets of each sample were produced to reduce the error of analysis. Each pellet, without any substrate, was glued on a Mylar film and put directly on a sample holder in the X-ray beam for element determination. An EDXRF system was used. Mylar film and glue were previously checked for contamination control.

During the grinding of the sample preparation, special care has been paid to avoid contamination, as well as during the whole procedure. All the used materials were of polyester, to avoid any contact with metals.

Experimental setup

The spectrometer used in this work for EDXRF analysis consisted of a commercial X-ray tube (PW 1140; 100 kV, 80 mA) equipped with a changeable secondary target of molybdenum (Mo) [14]. With this setup it was possible to obtain virtually a monochromatic source, with energies of the K_α and K_β lines of Mo of 17.44 and 19.60 keV, respectively.