Original Article

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The Levels of Calcium and Magnesium, and of Selected Trace Elements, in Whole Blood and Scalp Hair of Children with Growth Retardation

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Abstract

Objective: Metals such as copper (Cu), zinc (Zn), iron (Fe) are essential for human beings. Chronic metabolic disturbances may result from an excess or deficiency of these metals. Ca and Mg are also nutrient elements and play an important role in biological systems. Thus, it is very important to check regularly trace elements concentration in the body. The purpose of this study was to measure the content of Fe, Cu, Zn, Ca and Mg in whole blood and hair of children with growth retardation compared to that of controls.

Methods: A quantitative elemental analysis of whole blood and scalp hair of children with constitutional growth retardation (n=27) and matched controls (n=21) was used to find out correlation and possible changes, between growth retardation and healthy controls. Atomic absorption spectrophotometric (AAS) analysis of quantitative method was used to determine iron, zinc, copper, calcium and magnesium levels of whole blood and scalp hair.

Findings: The whole blood levels of Fe and Zn were significantly lower in children with growth retardation (P<0.05), but there were no differences in Cu, Ca and Mg concentrations in whole blood between children with growth retardation and healthy controls. The hair levels of Fe, Zn, Ca and Mg were significantly lower in children with growth retardation when compared to that of controls (P<0.05). The Cu concentrations in the hair of children with growth retardation and healthy controls showed no significant differences (P>0.05).

Conclusion: The usefulness and significance of these elements in growth retardation should be discussed more detailed in the light of the most recent data.

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Key Words: Whole Blood; Hair; Trace Elements; Growth Retardation; Children

Introduction

Trace element deficiencies and excesses are known to affect numerous biological functions in humans, including physical growth, psychomotor development and immunity[1,2]. Thus, it is very important to check trace elements concentration regularly in the body.

Growth retardation of humans has been linked to systemic conditions such as iron deficiency anemia, inflammatory bowel disease, celiac disease, occult renal disease, growth hormone deficiency, genetic syndromes, emotional deprivation, constitutional growth delay and other causes. In addition, several studies[3-6,7] have demonstrated that the growth of children is affected by deficiencies or imbalances of elements such as copper (Cu), zinc (Zn), iron (Fe), calcium (Ca) and magnesium (Mg). As a biological tissue, scalp hair is unique in that it remains isolated

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from human metabolic activities and indicates the concentration profiles of elements in individuals at the time period. Hair analysis has been used to evaluate the trace element status in the body[8]. Unlike blood, serum and urine, the hair provides historical information on concentrations of trace elements in the body as well as the nutritional condition over a long time[9,10]. In metal toxicology, especially for trace elements, samples of whole blood and not serum/plasma are most often used for biological monitoring. One reason may be that the concentrations are higher in blood than in serum or plasma and, thus, possible to detect with conventional techniques[11]. The measure of a metal in blood may reflect recent absorption of it. However, whole blood levels may show variability during the day. Thus, hair gives a better estimate of the total body intake of certain elements than those of blood or urine[12-14]. In addition, hair analysis provides information about intracellular accumulations of trace elements.

We also preferred examinining hair of the children, since whole blood does not have stable balance of trace elements. In the light of these facts, we studied Fe, Zn, Cu, Ca and Mg levels in hair and whole blood of children with constitutional growth retardation and compared them with those in controls.

Subjects and Methods

Totally 48 children (27 with growth retardation, 21 controls) aged 4-12 years were included in this study. Children lived in the city Elazig in eastern Turkey. A questionnaire requesting information on age, sex, place and duration of residence, food habits, family occupation, socio-economic status and health status was filled out. Healthy children with normal physical and mental development and no chronic disease were included in the study as the control group. Growth retardation was diagnosed on the basis of clinical and biochemical findings. The mean age of the children with growth retardation and controls was 8.59±2.78 and 8.31±2.88 years, respectively. All children were studied prior to treatment in Pediatrics Department of the Firat Medical Center of Firat University.

Unless stated otherwise, all chemicals used were of high-purity reagent grade. In all analytical work, double-distilled water was used. All glass materials were stored in 1 mol/L^{-1} nitric acid when not in use. In the digestion and extraction procedures, concentrated nitric acid (65%, Merck) and hydrogen peroxide (35%, Merck) were used. Stock solutions of Ca, Mg, Zn, Cu, and Fe (1,000 mg/L^{-1}) were prepared by dissolving $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Zn(NO_3)_2$, $Cu(NO_3)_2$, and $Fe(NO_3)_2$ salts in 1.0 mol/L^{-1} nitric acid.

In order to prevent possible contamination, beakers were cleaned before hair samples were placed in them. The beakers were washed with chromic acid and rinsed with distilled water; and then 1.0 mL of HNO_3/H_2O_2 mixture (2:1) was added to the beakers. After boiling and evaporating, the beakers were cleaned by adding 1.0 mol/L^{-1} HNO_3 to the 0.1-0.2 mL remaining residue.

Hair samples were collected from 48 children aged 4 to 12 years. Hair samples were cut from an area close to the occipital region of the scalp and only the first 0.5-1.0 cm of hair was used for analysis. The hair was in new, sealed, clear polypropylene tubes and stored at 4 ºC during its transportation to the laboratory. Polypropylene tubes were rinsed at least five times with a double-distilled water and 1:1 HNO_3:H_2O mixture. The hair samples were washed with 25mL 1% (w/v) sodium laurel sulphate solution over 3 h with stirring. Then they were filtered and washed five times using acetone:ethanol (1:1) mixture and distilled water. After drying 0.1-0.2 g hair samples were placed into the beakers and then wet-digested in duplicate 2 mL of 65% nitric acid (suprapure, Merck) at 85°C for 4 h and at 110°C for 6 h. Each beaker was then carefully shaken and boiled until 0.1-0.2 mL of residue remained. After cooling, the solution was diluted to 10 mL with deionized water and kept until analysis.

Whole blood was collected in Vacutainer heparinized 7 mL tubes. A 3-5 mL aliquot of each blood sample was placed into the beakers and then wet-digested in duplicate with 65% nitric acid (suprapure, E. Merck, Darmstadt, Germany) and analytically grade perchloric acid (ultrapure, E. Merck, Darmstadt, Germany) (3:1) at 110°C for 6 h. Each beaker was then carefully shaken and boiled until 0.1-0.2 mL of residue remained. After cooling, the solution was diluted to 10 mL with

deionized water and kept until analysis.

The internal standard stock solution consisted of a solution of the selected internal standards, Fe, Zn and Cu, at a concentration 5 µg/mL. Internal standardization was used for correction of non-spectral interferences. Therefore, the concentration of each analyte was related to the concentration of internal standard. In the whole blood and hair samples, Ca, Mg, Zn, Fe and Cu contents were measured with Perkin Elmer A. Analyst 400 Model AAS. Each hair and blood sample was analyzed in duplicate.

All results were expressed as mean±SD. Differences between groups were analyzed for significance using the Student's *t*-test. All calculations are carried out on a Microsoft Office EXCEL2007 program for Windows Vista. Statistical significance was defined as $P<0.05$.

Findings

Fe, Cu, Zn, Ca and Mg levels in whole blood and scalp hair of children with constitutional growth retardation and controls, performed in this work, are shown in Tables 1-2. The age of children ($P=0.73$) showed no significant difference between controls and children with growth retardation ($P<0.05$). The mean±SD concentrations in the whole blood and scalp hair of children with growth retardation were, respectively, Fe 315±98.54 µg/mL and 14.01±4.14 µg/g, Zn 8.63±2.96 µg/mL and 157±25.10µg/g, Cu 0.94± 0.38 µg/mL and 9.97±3.99 µg/g, Ca 111.57±39.10 µg/mL and 1168±231 µg/g, Mg 34.90±8.42 µg/mL and 168±49.87 µg/g. As is seen in Tables, the whole blood levels of Fe and Zn were significantly lower in children with growth retardation when compared to those of controls ($P<0.05$). The scalp hair levels of Fe, Zn, Ca and Mg also were significantly lower in children with growth retardation when compared to those of controls ($P<0.05$).

A significant difference was found in the whole blood levels of Fe ($P=0.0041$) and Zn ($P=0.00073$), while Cu ($P=0.49$), Ca ($P=0.25$) and Mg ($P=0.23$) showed no significant difference between children with growth retardation and controls ($P<0.05$). Fe ($P=0.0023$), Zn ($P=2.1\times10^{-6}$), Ca ($P=0.0009$) and Mg ($P=0.0054$) levels in hair showed significant difference, while Cu ($P=0.057$) indicate no significant difference between children with growth retardation and controls ($P>0.05$).

Discussion

There are few studies of trace elements, Ca and Mg in scalp hair and serum/plasma of children with growth retardation. This study was performed in our hospital, at Firat Medical center, with the aim of comparing the Fe, Zn, Cu, Ca and Mg levels in scalp hair and whole blood of the children with constitutional growth retardation, which was diagnosed on the basis of constitutional growth delay and control groups. Constitutional growth delay is one of the variants of normal growth commonly encountered by the pediatrician. Growth then decelerates to near or below the 3rd percentile for height and weight. Studies of growth hormone secretion and other studies are within normal limits. Bone age is closer to height age than to chronologic age[23].

Table 1: The mean and standard deviation values of the whole blood trace elements in children with growth retardation and controls

Trace Elements	Children with growth retardation Mean (SD) µg/mL	Controls Mean (SD) µg/mL	*P* value*
Age	8.59 (2.78)	8.31 (2.88)	0.4
Calcium	111.57 (39.10)	124.26 (7.29)	0.2
Magnesium	34.90 (1.76)	39.39 (3.21)	0.2
Fe	315.03 (20.54)	391.78 (14.57)	0.004
Zinc	8.63 (0.62)	13.18 (1.06)	0.001
Cu	0.94 (0.08)	1.02 (0.07)	0.5

*Student's *t*-test ($P=0.05$); SD: Standard Deviation

Table 2: The mean and standard deviation values of the hair trace elements in children with growth retardation and controls

Trace Elements	Children with growth retardation Mean (SD)µg/g	Control Mean (SD) µg/g	*P* value*
Age	8.59 (2.78)	8.31 (2.88)	0.4
Calcium	1168 (231)	1417 (245)	0.001
Magnesium	168 (50)	206 (40.00)	0.005
Iron (Fe)	14 (4.14)	18.03 (4.33)	0.004
Zinc	157 (25.10)	218 (42.29)	0.001
Copper	9.97 (3.99)	11.83 (2.53)	0.5

*Student's *t*-test (*P*=0.05); SD: standard Division

Family income of the children with growth retardation is lower than that of control groups; they have low socio-economic status, are uneducated, and have been fed mainly carbohydrate diet.

Fe levels in whole blood were significantly higher, while it was significantly lower in hair of both groups. Whereas Zn, Cu, Ca and Mg concentrations in blood were significantly lower when compared with hair of both groups. On average, the following order of elements concentration was observed: Ca > Mg > Zn > Fe > Cu in scalp hair and Fe > Ca > Mg > Zn > Cu order in whole blood for both groups. Previous studies, dependent on growth retardation types, reported some conflicting results with Fe, Zn, Cu, Ca and Mg levels in scalp hair and in whole blood. It has been previously shown that hair Zn levels in malnourished children had been found to be significantly lower while Fe and Cu levels were significantly higher[15]. In a different study, no significant associations were found between the serum concentrations of Ca, Cu, Mg, Zn and growth retardation[17]. In our study, hair Zn and Fe levels in children with growth retardation were significantly lower and Cu level in hair showed no significant difference when compared with controls (*P*>0.05). Widely prevalent growth retardation, susceptibility to a variety of infections, and cognitive impairment has been related to zinc deficiency in the developing world[19,32,33]. Zinc is essential element for the normal growth of human beings. Several zinc-dependent enzymes are involved in the synthesis of nucleic acids and proteins and, hence, in the fundamental processes of cell replication and differentiation and, ultimately, growth[34,35,37]. Furthermore, zinc deficiency has been shown to reduce insulin-like growth factor I production and growth hormone levels[36]

Previous studies showed that Zn levels in hair, blood or serum of growth retardation children were significantly lower[3-6]. In other studies, hair Zn values were detected to be a good indicator of nutrition status and the essentiality of Zn clearly plays an important role in growth and sexual function[15,20,21]. Low serum level of Zn is associated with the retardation in both somatic growth and pubertal maturation[22]. In our results both hair and whole blood Zn levels of children with growth retardations were significantly lower when compared with controls.

Iron deficiency in children is associated with the retardation in growth. It has been previously shown that the iron deficiency may affect psychomotor development, but does not appear to affect growth[23]. Some authors suggested that the effect of iron on growth was supported by the findings that zinc has a positive stimulatory effect on growth only when iron levels are adequate[5,39,40]. According to our study results, both hair and whole blood Fe levels of children with growth retardation were significantly lower when compared with controls.

Cu is contained in some key enzymes involved in the synthesis of erythrocytes and participates in the oxidizing chain in mitochondria. Tiredness, hepatic and renal problems and anemia can be consequences of a deficiency of this metal. Generally, previous studies show that hair, blood or serum Cu levels were found significantly higher in different types of growth retardation[3,6]. Present results showed that Cu content in hair and in whole blood of the children with growth retardation showed no significant difference when compared with controls.

Some reports have described the inhibitory effect of magnesium and pyrophosphate on the dissolution and growth of calcium oxalate[24]. Ca is the fundamental constituent of bones. This metal is involved in muscular contraction, functional integrity of the nervous system, cardiac activity and blood coagulation. On the other hand, magnesium can be effective in fetal growth[25]. Our data shows that although no significant difference was found in whole blood Ca and Mg contents of the children with growth retardation, Ca and Mg levels in scalp hair were significantly lower when compared with controls.

Accumulation of elements in scalp hair and in whole blood of children has been found different as depending on age, sex, living regions, ethnic and geographic origin, and nutritional condition[6,16,26-28]. This study showed that the measured levels of Fe, Zn, Cu, Ca and Mg in whole blood or in hair were lower when compared with referent values from the literature[29-31].

Conclusion

The elements such as Fe, Zn, Cu, Mg and Ca are not only essential for development and growth but they are also necessary for functioning of immune system and metabolism of anti-oxidants. Thus, it is very important checking these elements concentration regularly in the body. These results together with previous studies[5,15,16,18] demonstrate that the elements such as Fe, Zn, Cu, Ca and Mg may play an important role in children with growth retardation. In addition, socio-economic status, dietary habits, uneducated parents appear to affect the growth of children with constitutional retardation.

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Conflict of Interest: None

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