

Zinc Status in Patients with Telogen Defluvium

Sir,

Zinc deficiency with hair loss may occur in acrodermatitis enteropathica or be acquired due to total parenteral nutrition, alcoholism, malabsorption, wound healing, hemodialysis or sickle cell anemia (1, 2). Telogen defluvium refers to the loss of hair from a disproportionately large number of follicles that have entered the telogen stage of the hair cycle in a relatively synchronous fashion (3, 4). During the earlier stages of the process, there is an increase in the percentage of telogen hair apparent on the trichogram. The increased hair fall usually follows a stressful event by about 2 to 4 months. No specific treatment is currently available.

We have here evaluated the possible role of zinc deficiency in patients with idiopathic telogen defluvium and a control group. In addition to zinc determination in blood plasma, blood cells and hair, we measured two functional indices: albumin and alkaline phosphatase (EC 3.1.3.1.) activity in serum.

MATERIAL AND METHODS

Patients

Sixteen patients (3 males and 13 females) affected by idiopathic telogen defluvium and 16 apparently healthy controls matched for age, sex, hair colour and body mass index (BMI) were recruited. The study was explained in detail by one of the study staff and all subjects gave informed consent. The diagnostic was based on trichogram, which was performed in all subjects. The average age was 30 years, with a range of 14–48 years. The average BMI was 21 kg/m², with a range of 15–28 kg/m². Subjects using dyes, bleaches or other hair treatments were excluded. Subjects with the more common presentations of telogen defluvium, i.e. pregnant or lactating women, women taking oral contraceptives, post-surgical patients, patients suffering from infectious diseases, patients on slimming diet or corticosteroid treatment were also excluded. Subjects on zinc or vitamin A treatments were also excluded.

Samples

Blood was collected between 6 and 8 a.m. from fasting subjects. Twenty ml of blood were collected into disposable plastic syringes (Becton Dickinson, Meylan, France) and transferred into three zinc-free polystyrene tubes (CML, Nemours, France). The first tube was without anticoagulant, the second tube contained zinc-free sodium heparin (Sigma, Saint Quentin Fallavier, France) and the third tube contained 1.25 ml of zinc-free 50 g/l Na₂EDTA, 2H₂O (Sigma, Saint Quentin Fallavier, France). Plasma, serum, leukocytes and red blood cells were separated from each sample within 30 min of the whole blood collection. Centrifugation of the first two tubes was performed at 1,600 × g for 10 min and at 10°C. Serum alkaline phosphatase activity and albumin were determined immediately. Plasma and erythrocytes were stored frozen at –18°C until zinc analysis. Hemolyzed samples were excluded. The 11.25 ml of the third tube was mixed with 3 ml of Plasmagel^R (Roger Bellon, Neuilly sur Seine, France). Differential sedimentation of the blood was allowed to take place for 1 h, after which 7 ml of leukocyte-rich plasma was placed in a zinc-free glass tube containing 14 ml of 8.7 g/l ammonium chloride (Prolabo, Paris, France). The tube was mixed by gentle inversion and centrifuged at 400 × g for 5 min. The supernatant was discarded and the leukocyte cells were washed twice by 10 ml of ringer – lactate – glucose infusion (Aguettant, Lyon, France). Leukocytes were resuspended in 1.6 ml ringer – lactate – glucose infusion. A 100 μl aliquot of the suspension was used for leukocyte counting (Coulter counter, Coultronic, Margency, France). The remaining suspension was centrifuged at 400 × g for 15 min and the cells were stored frozen at –18°C until zinc analysis. Hair was collected 7 days after shampoo from the occipital region as close to the scalp as possible using stainless steel scissors. The hair was cut into three sections (proximal, central, distal) of approximately 20 mg. It was washed with 10 ml acetone (Prolabo, Paris, France) to remove zinc surface contaminations and then with 10 ml of 20 g/l Triton X 100 (Prolabo, Paris, France). This procedure was then followed by four rinses with 10 ml of deionized water. The hair was then dried overnight at 50°C. The entire collection procedures were checked for zinc and were found to be free from contamination.

Analyses

To minimize analytical variations, zinc determinations of patient and matched control were performed in a batch. Zinc concentrations were

Table I. Age, body mass index and zinc status of the telogen alopecia group and the control group

Parameter	Telogen defluvium <i>n</i> = 16	Controls <i>n</i> = 16	Statistical significance
Age, years	30±7	31±7	NS
BMI, kg/m ²	21±3	20±2	NS
Plasma Zn, µmol/l	12.4±1.3	12.3±2.4	NS
Erythrocyte Zn, µmol/l	172±37	155±21	NS
Leukocyte Zn, µmol/10 ¹⁰ cells	1.5±0.6	1.6±0.5	NS
Hair Zn, µg/g			
Proximal	290±147	189±69	NS
Central	251±118	256±94	NS
Distal	261±108	241±71	NS
Albumin, g/l	47±5	46±4	NS
Alkaline phosphatase, U/l	103±57	81±26	NS

determined by electrothermal atomic absorption spectrometry. We used a Perkin Elmer (Norwalk, CT, USA) Model 560 atomic absorption spectrometer, equipped with an HGA 500 graphite furnace and AS 40 autosampler. The wavelength and slit settings were 213.9 nm and 0.7 nm. All reagents used were free from zinc contaminations. All plasticware and glassware for analyses were previously cleaned by soaking in 1.6 M nitric acid for 16 h, then in 1 M hydrochloric acid for 16 h and finally by rinsing with deionized water. Working standard solutions were prepared by appropriate dilution of the stock standard solution (Titrisol Merck, Darmstadt, Germany) with 1 g/l Triton X100 (Prolabo, Paris, France) for zinc determination in red blood cells or with deionized water for other zinc measurements. Plasma samples were diluted 10-fold with deionized water before 10 µl injection in a standard graphite furnace. Red blood cells were diluted 200-fold in 1 g/l Triton X100 before zinc measurement. Leukocytes and hair were digested at 50°C in 1.5 (leukocytes) or 2 (hair) ml of concentrated nitric acid (Merck, Darmstadt, Germany). Digests were diluted 5-fold (leukocytes) or 30-fold (hair) in deionized water. The furnace heating procedure was: dry for 50 s, with a 5 s ramp to 110°C; char for 20 s, with a 1 s ramp to 700°C and atomize for 6 s, with a 1 s ramp to 2200°C. With these methods, the coefficients of variation for ten determinations were less than 7.7% and the recoveries of standard additions varied from 94 to 109%. Moreover, zinc values in Serum Seronorm (Nycomed, Oslo, Norway) were within the ranges of assigned values for this element.

Albumin and alkaline phosphatase activity were measured using a RA1000 autoanalyzer (Bayer, Tournai, Belgique). Alkaline phosphatase activity was determined according to the recommendations of the A.A.C.C. Measurement of albumin involved bromocresol green.

Statistical methods

Means and standard deviations were calculated. Differences between the two groups were tested using the paired Student *t*-test. Results were considered significant at *p*<0.05.

RESULTS

Table I lists the age, BMI, zinc concentrations in plasma, erythrocytes, leukocytes and hair, albumin concentration in plasma and alkaline phosphatase activity obtained in the telogen defluvium group and the control group. No significant differences between the two groups were noted.

DISCUSSION

All the patients included in this study had a telogen defluvium of unknown aetiology, and subjects with the more common presentations of telogen defluvium were excluded (4). Only one patient had hypozincemia (<10.7 µmol/l) and no patient had hair zinc under the deficiency cut-off of 70 µg/g or leukocyte zinc under the deficiency limit of 0.7 µmol/10¹⁰cells (5, 6). The involvement of zinc in the aetiology of telogen defluvium is therefore questionable. However, laboratory diagnosis of zinc deficiency is not easy (1). Indeed, human beings are able to maintain zinc homeostasis by increasing efficiency of zinc absorption and decreasing zinc excretion when they are subjected to dietary zinc restriction (7). Therefore, zinc concentration in biological tissues or fluids may remain within the reference ranges (1). The functional index, i.e. zinc-dependent alkaline phosphatase or zinc-binding proteins, could also remain within the reference ranges (1). The results obtained do not establish any zinc status change in telogen defluvium, which is the same as those reported in alopecia areata without other disorders (8).

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REFERENCES

1. Van Wouwe JP. Clinical and laboratory diagnosis of acrodermatitis enteropathica. *Eur J Pediatr* 1989; 149: 2–8.
2. Prasad AS. Discovery of human zinc deficiency and studies in an experimental human model. *Am J Clin Nutr* 1991; 53: 403–412.
3. Kligman AM. Pathologic dynamics of reversible hair loss in humans. 1. Telogen effluvium. *Arch Dermatol* 1961; 83: 175–198.
4. Bertolino AP, Freedberg IM. Disorders of epidermal appendages and related disorders. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. *Dermatology in general medicine*. New York: Mc Graw-Hill, Inc., 1993: 671–696.
5. Pilch SM, Scuti FR. Assessment of the zinc nutritional status of the US population based on data collected in the second national health and nutritional examination survey. Bethesda: Life science research office, Federation of American societies for experimental biology, 1984.
6. Aggett PJ, Favier A. Zinc. *Int J Vit Nutr Res* 1993; 63: 301–307.
7. Lee DY, Prasad AS, Hydrick-Adair C, Brewer G, Johnson PE. Homeostasis of zinc in marginal human zinc deficiency: role of absorption and endogenous excretion of zinc. *J Lab Clin Med* 1993; 122: 549–556.
8. Momcilovic B, Handl S, Oremovic L, Zjajic-Rotkovic V. Zinc, iron and copper status in patients with alopecia areata. In: Mills CF, Bremner I, Chesters JK, eds. *Trace elements in man and animals – TEMA 5*. London: Commonwealth Agricultural Bureaux, 1985: 785–788.

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