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Original article

Alteration in serum klotho levels in anorexia nervosa patients

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SUMMARY

Background & aims: Klotho is a trans-membrane protein which can be shed to act as a hormone; its blood levels may be regulated by the GH/IGF-1 axis. Klotho deficient mice exhibit short lifespan and characteristics of aging and malnutrition, including decreased fat and muscle mass, osteopenia, and impaired fertility. As anorexia nervosa (AN) is characterized by malnutrition and GH resistance, we hypothesized klotho levels would be altered in AN, and aimed to assess klotho levels in undernourished AN patients and changes in klotho following weight rehabilitation.

Participants and methods: 19 adolescent female AN inpatients (aged 16.1 ± 1.8 years) admitted to an inpatient service for eating disorders in a tertiary center were recruited. Blood samples were obtained on admission and after weight restoration (interval 4.0 ± 2.3 months) and analyzed for klotho, IGF-1, calcium, phosphorus, and alkaline phosphatase.

Results: Klotho levels on admission were lower than expected for age, and correlated with lumbar spine BMD Z-score ($r = -0.81$, $p < 0.001$) and alkaline phosphatase levels ($r = 0.66$, $p = 0.003$) but not with age, height-SDS, weight-SDS, BMI-SDS, or serum calcium, phosphorus and IGF-1 levels. Both IGF-1 and klotho levels increased significantly during hospitalization (IGF-1: 44 ± 17 nmol/l to 53 ± 11 nmol/l, $p = 0.008$; klotho: 1061 ± 421 pg/ml to 1519 ± 781 pg/ml, $p = 0.008$).

Conclusions: Klotho levels are low in the acute stage of AN and increase with nutritional rehabilitation. Low klotho on admission may be secondary to low IGF-1 levels and may contribute to the clinical manifestations of AN. The role of klotho in the pathophysiology of AN and as a novel marker of disease severity should be further explored.

1. Background

Anorexia nervosa (AN) is a chronic disorder with an onset usually during adolescence, characterized by self-imposed malnutrition, weight loss, and an intense fear of gaining weight, despite being underweight [1,2]. AN is characterized by reduced body fat mass and skeletal muscle mass and strength [3], and is associated with severe medical complications, including growth retardation [4], electrolyte disorders, hematologic disorders, cardiac dysfunction, gastrointestinal disturbances and severe bone loss [5,6]. Multiple endocrine abnormalities have been observed in AN, including hypothalamic amenorrhea, hypercortisolism, and growth hormone (GH) resistance leading to low concentrations of insulin growth factor-1 (IGF-1) despite increased GH levels [1].

α-Klotho (klotho) is a trans-membrane protein which can be cleaved and shed to the circulation. Circulating klotho can function as a hormone, and can be detected in the blood, urine, and cerebrospinal fluid [7]. Mice with homozygous mutated klotho allele (*kl*/*kl* mice) show a shortened lifespan and develop, by the age of four weeks, growth retardation and characteristics resembling human aging as well as malnutrition, including hypogonadism, muscle atrophy, osteopenia and paucity of abdominal fat tissue [7]. Conversely, overexpression of klotho increases lifespan and is associated with altered regulation of glucose homeostasis [8].

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Klotho is highly expressed in the distal convoluted tubules in the kidney and the choroid plexus, as well as in endocrine-related tissues including the testes, ovaries, the pituitary gland, and adipose tissue [7]. Several activities of klotho have been described to date. Klotho is an essential cofactor for the binding of fibroblast growth factor (FGF) 23 to its cognate receptor, and thus has a prominent role as a major regulator of phosphate homeostasis [9]. Another important activity of klotho is inhibition of the insulin and IGF-1 pathways [8,10,11]. Additionally, it plays a role in systemic glucose metabolism and has been shown to be involved in the maturation and differentiation of adipocytes [12,13].

In humans, klotho serum levels are higher in children and decrease with aging [14–16]. Among elderly people, reduced klotho levels may be associated with increased rate of cardiovascular disease and greater mortality [17,18]. An interaction between klotho, GH secretion and activity, and linear growth has been suggested: *kl*/*kl* mice are smaller compared to their wild-type counterparts, decreased klotho levels were observed in children with short stature and organic GH deficiency [19], and increased klotho levels were found in patients with GH-secreting pituitary adenomas [20,21]. Klotho may also serve as a direct regulator of GH secretion [22].

Several key characteristics of malnutrition and AN were noted in the *kl*/*kl* model, including decreased fat mass, growth retardation, reduced skeletal muscle mass, osteopenia, abnormalities in glucose metabolism, disturbances in gastric motility, and impaired fertility. Furthermore, low klotho levels were recently noted in a group of 12 AN patients [23]. We therefore aimed to study the association between klotho serum levels, anthropometric measurements, and GH/IGF-1 axis activity in adolescent female inpatients with AN at the time of hospitalization and following weight rehabilitation.

2. Methods

2.1. Patients

The study group included 19 female adolescents hospitalized because of AN at the Pediatric Psychosomatic Department of the Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Israel. This is a tertiary referral center admitting patients from all over Israel, and patients were referred for hospitalization after outpatient or other inpatient treatment has failed.

The diagnosis of AN was established according to the criteria of the DSM-IV-TR [2] using the Eating Disorders Family History Interview (EDFHI) [24,25]. All 19 patients would have also been diagnosed with AN according to the criteria of the DSM-V [26]. The diagnosis was established independently by two highly experienced child and adolescent psychiatrists, with a correlation coefficient (r) of 0.92 between the two raters, and was finalized in clinical meetings of the department's team. None of the patients included in the study had evidence of an organic brain disorder, schizophrenic-spectrum disorders, substance use disorders, or any significant medical or neurological disorder.

Patients and parents (in the case of minors) signed a written informed consent. The study was approved by the Human Investigations (Helsinki) Committee of the Sheba Medical Center.

2.2. Clinical evaluation and management

Demographic and clinical data, including age, country of birth, years of schooling, anthropometric measurements, and results of pertinent laboratory tests were obtained from the patients' medical charts. Standing height was measured to the nearest 0.1 cm, using a wall mounted stadiometer, and body weight was obtained to the nearest 0.1 kg, with the patient wearing a hospital gown and without any footwear. For the sake of reducing internal variability, all measurements were taken during the morning hours, using standardized procedures. Body mass index (BMI) was calculated based on the formula: weight (kg)/height $(m)^2$. Height, weight, and BMI standard deviation scores (SDS) were calculated using age and gender-specific growth data (based on the Centers for Disease Control and Prevention's Year 2000 Growth Charts) (www.cdc.gov/growthcharts). These data have been found adequate for assessing Israeli children and adolescents [27].

Assessment of nutritional status and nutritional history was performed on admission by registered dietitians. The multi-modal program carried out in this department focuses on the treatment of the eating disorder, comorbid psychiatric disorders, and relevant psychosocial difficulties. A nutritional rehabilitation program geared toward weight-gain of 0.5–1.0 kg/week was constructed, and target weight was established according to age and the estimated potential height. In order to be discharged from inpatient treatment, patients with restrictive AN (AN-R) were required to have reached their required weight and maintain it for two weeks. Patients with anorexia nervosa binge/purge type (AN-B/P) were additionally required to abstain from binge/purge behaviors for two weeks, as assessed with daily food monitoring charts.

2.3. Bone mineral density

Bone mineral density (BMD) at the lumbar spine (L1-L4) was evaluated using DXA (Lunar Prodigy; GE Medical Systems, Madison, WI, USA). BMD is expressed in grams per square centimeter and in terms of z-scores (i.e. the difference between the BMD of the patient and the average BMD of age- and sex-matched controls divided by the standard deviation of the control group).

2.4. Laboratory measurements

Blood samples were obtained on admission and before discharge, upon the attainment of target weight. All samples were drawn in the early morning hours after overnight fasting.

Serum biochemical analysis, including calcium, phosphorus, and alkaline phosphatase (ALP), was conducted immediately using the AU5800 Chemistry Analyzer (Beckman Coulter).

Samples for klotho and IGF-1 measurements were immediately centrifuged for 15 min at 2700 rpm, separated, and frozen at −70 °C until use. Klotho levels in the serum were analyzed using an α-klotho ELISA kit (Immuno-Biological Laboratories Co, Japan) as described before. The kit has been validated and has been widely used for the measurement of klotho levels [15,16,28,29]. The intra-assay coefficiency of variation (CV) ranged from 3% to 3.5%, and the inter-assay CV ranged from 3.7 to 8.1%. . IGF-1 was measured by a chemiluminescent immunometric method (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The analytical sensitivity of the assay was 2.6 nmol/L and the inter-assay CV ranged from 3.7 to 8.1%. IGF-1 levels were transformed to natural logarithm (ln) in order to achieve normal distribution, and standard deviation scores (IGF-1-SDS) for each subject were calculated as explained elsewhere [30].

2.5. Data analysis

The initial analysis included estimations of means, standard deviations (SDs), and frequency distribution. Comparisons between values on admission and discharge were made using the paired t-test. Spearman correlation coefficient was used to determine the relation between continuous variables. Results were considered

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