



Correlation between the changes of Klotho protein with calcium and phosphate concentrations in the serum at early stages of multiple sclerosis

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Original Article

Abstract

BACKGROUND: There are several studies indicating that an anti-aging protein, namely Klotho protein, participates in the regulation of calcium and phosphate metabolism. In addition, we showed that Klotho protein was involved in the pathogenesis of multiple sclerosis (MS). Hence, we hypothesized that Klotho protein changes in patients with multiple sclerosis might lead to alteration of calcium and phosphate metabolism. Accordingly, the aim of the present study was to evaluate the alteration of calcium and phosphate levels together with the concentration of Klotho protein in the serum of patients with multiple sclerosis.

METHODS: In this case-control study, 14 patients with newly diagnosed relapsing-remitting multiple sclerosis (RRMS) along with 14 control individuals with noninflammatory neurological disorders were enrolled. The serum concentrations of Klotho protein, calcium, and phosphate were measured in serum of participants using commercial kits. The data were analyzed at the significant level of $P < 0.050$.

RESULTS: There were no significant changes in serum concentrations of Klotho protein, and phosphate in patients with multiple sclerosis when compared to controls. However, the serum calcium concentration was significantly lower than the control group. Regarding patients with multiple sclerosis, there was a significant positive correlation between changes in serum concentrations of Klotho protein and calcium ($r = 0.604$, $P = 0.022$), whereas the other correlations were not statistically significant.

CONCLUSION: To our knowledge, this is the first study demonstrating a positive correlation between serum concentrations of secretory Klotho protein and calcium in patients with multiple sclerosis.

KEYWORDS: Klotho Protein, Calcium, Phosphorus, Multiple Sclerosis

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Introduction

There are several studies indicating that an anti-aging protein, namely Klotho protein, participates in the regulation of vitamin D,

calcium, and phosphate metabolism.^{1,2} Klotho protein is highly expressed in the kidneys and choroid plexus of brain. The secretory Klotho protein in the plasma comes mainly from kidneys, and in the cerebrospinal fluid (CSF) derives from choroid plexus.^{1,3} It has been reported that transmembrane form of Klotho protein acts as a co-receptor for fibroblast

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growth factor receptors (FGFRs). In fact, transmembrane form of Klotho protein in the kidneys increases the affinity of FGFRs toward fibroblast growth factor 23 (FGF 23). FGF 23 downregulates vitamin D biosynthesis and increases phosphate excretion from the kidneys.⁴ In addition, the secretory form of Klotho protein in kidney can increase the stability of potassium [such as renal outer medullary potassium channel-1 (ROMK1)] and calcium channels [such as Transient receptor potential cation channel subfamily V member 5 (TRPV5)] which leads to calcium retention in the human body. Furthermore, secretory Klotho protein increases phosphate excretion from kidneys through sodium-phosphate co-transporters [such as type II sodium-dependent phosphate co-transporter a (Npt2a)].⁵

Recently, we showed that Klotho protein may participate in the pathogenesis of multiple sclerosis (MS).⁶⁻⁸ We found that secretory form of Klotho protein decreased in the CSF of patients with MS when compared to control individuals,⁶ while serum Klotho protein concentration was comparable to control group at early stages of MS.⁷ We hypothesized that Klotho protein changes in patients with MS may lead to alteration of calcium and phosphate metabolism. Accordingly, the aim of the present study was to evaluate the changes of calcium and phosphate levels along with Klotho protein concentration in the serum of individuals with newly diagnosed MS.

Materials and Methods

This case-control study was the continuation of our previous works which were established at the Department of Neurology, Imam Khomeini hospital, Tehran, Iran.⁶⁻⁸ This study was approved by the Ethics Committee of Tehran University of Medical Sciences (ECTUMS; ethical code# 92-04-30-25660). In addition, informed consent was obtained from all the participants.

Altogether, 28 individuals were recruited in this study. Fourteen patients were selected among patients who definitely diagnosed for the first time (new cases) as having active relapsing-

remitting MS (RRMS) using the revised McDonald's criteria,⁹ and thus had no experience of receiving immunomodulatory drugs or supplementation. The remaining patients, comprised of 14 individuals with non-inflammatory neurological diseases as a control group. The inclusion/exclusion criteria for the present study have been described before.⁶

Demographic and clinical data were obtained. Serum samples were collected. The concentration of Klotho protein was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit with high accuracy and precision (human soluble α -Klotho protein assay kit, IBL Co., Japan, Code No. 27998 and Lot No. 1L-303). Furthermore, serum levels of calcium and phosphate were assayed on an auto-analyzer instrument (Hitachi 917, Tokyo, Japan) using available kits (Pars Azmoon Co; Tehran, Iran).

Data analysis was performed using GraphPad Prism software (version 6.01, GraphPad Software, La Jolla, CA, USA). Data were presented as mean \pm standard deviation (SD). Comparison between the groups was performed using independent-samples t test. The chi-square test was applied in order to compare the sex distribution between the case and control groups. The significance was considered as less than 0.05 ($P < 0.050$).

Results

As indicated in table 1, the case and control groups were good sex- and age-matched. There were no significant changes in serum concentrations of Klotho protein and phosphate in patients with MS when compared to controls ($P > 0.050$).

Among the patients with MS, serum calcium concentration was significantly lower than the control group ($P = 0.028$).

Regarding the patients with MS, there was a significant positive correlation between the changes in serum concentrations of Klotho protein and calcium ($r = 0.604$, $P = 0.022$), whereas the other correlations were not statistically significant ($P > 0.050$ for all).

Table 1. Demographic, clinical and biochemical data of control individuals and multiple sclerosis (MS) patients

	Control individuals (n = 14)	MS patients (n = 14)	Statistical test (P)
Gender (Female/Male ratio)	12/2	12/2	Chi-square test (P = 1.000)
Age (year) (mean ± SD)	33.28 ± 14.14	28.07 ± 8.54	Independent-samples t-test (P = 0.249)
Duration of disease EDSS (mean; range of scores)	---	newly diagnosed patients 2.85; 1.5-4.5	---
Serum Klotho protein concentration (mean ± SD)	552.23 (pg/ml) ± 123.54	587.43(pg/ml) ± 159.63	Independent-samples t-test (P = 0.520)
Serum calcium concentration (mean ± SD)	10.51 (mg/dl) ± 0.70	9.90 (mg/dl) ± 0.67*	Independent-samples t-test (P = 0.028)
Serum phosphate concentration (mean ± SD)	4.06 (mg/dl) ± 0.71	3.73 (mg/dl) ± 0.53	Independent-samples t-test (P = 0.168)

EDSS: Stands for expanded disability status scale; MS: Multiple sclerosis; SD: Standard deviation; * means significantly different from control group (P < 0.05)

Discussion

The results of the present study showed that serum calcium concentration decreased in patients with MS. This result corroborates the finding of Ellidag et al.,¹⁰ but contradicts their results regarding serum concentrations of phosphate and Klotho protein. In the present study, patients were new cases, while they enrolled patients with prolonged disease duration.

Ellidag et al.,¹⁰ indicated that serum Klotho protein and phosphate were elevated in patients with MS, whereas we previously showed that serum Klotho protein concentration increased in patients with prolonged MS duration.⁷ This contradictory results may be attributed to the duration of the disease regarding Klotho protein expression as a time-dependent factor. Interestingly, we found a significant positive correlation between Klotho protein changes and calcium level in the serum of patients with MS.

Conclusion

It is plausible to speculate that secretory Klotho protein is involved in the homeostasis of serum calcium concentration in patients with MS.

Conflict of Interests

Authors have no conflict of interests.

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