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Clinical short communication

Multiple sclerosis influences on the augmentation of serum Klotho concentration

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ABSTRACT

We have already shown that the concentration of secreted form of Klotho decreases in the cerebrospinal fluid of patients with relapsing–remitting multiple sclerosis (RRMS). The current study aimed at assessing possible changes in the serum Klotho concentration of MS patients. Participants involved 15 new cases of RRMS patients in the relapse phase, 15 RRMS patients who had been suffering from the disease for more than three years and were under regular treatments (interferon beta-1a) and, finally, 15 non-MS patients who constituted the control group. Beside thorough neurological examinations, demographic and clinical data (e.g. gender, age, duration of disease and expanded disability status scale) were obtained. Serum Klotho concentration was measured using ELISA method. The results showed no statistically meaningful difference between new cases of RRMS (585.56 pg/ml ± 153.99) and control group (556.81 pg/ml ± 120.36; $P = 0.859$). The serum Klotho level, however, was significantly higher in patients with prolonged disease duration (696.94 pg/ml ± 170.52; $P = 0.037$) in comparison with the subjects in the control group. In conclusion, this study showed that serum Klotho concentration tends to be higher in MS patients when compared to control group. This finding might be attributed to treatment of MS patients with immunomodulatory drugs or a compensatory response to enhance CNS regeneration and/or vitamin D biosynthesis. Further studies are required to elucidate the role of Klotho in MS pathophysiology.

1. Introduction

Klotho is an anti-aging multifunctional protein accidentally discovered by Kuro-o et al. while generating a transgenic mice [1]. Several research groups have worked on this protein to elucidate its different roles in the human body [2,3]. There are two forms of Klotho, namely transmembrane and secreted, both of which are mainly produced in the kidneys and brain. It has been revealed that the secreted form of Klotho in the cerebrospinal fluid (CSF) is derived from the choroid plexus of brain, while the secreted Klotho in serum is mainly produced and released into the blood stream by kidneys [1,4]. The transmembrane form of Klotho, along with a member of fibroblast growth factor (FGF) superfamily which is called FGF23, is involved in the regulation of calcium, phosphate and vitamin D metabolism [2]. On the other hand, previous studies suggest that the secreted form of Klotho may play an important role in the modulation of oxidative stress [5]. In addition, recent investigations revealed that Klotho might act as a growth factor with the ability to differentiate oligodendrocyte progenitor cells (OPCs) [6].

Nowadays, Klotho is considered as the therapeutic target for some complicated and multifactorial disorders such as chronic kidney disease and neurological disorders, especially Alzheimer and multiple sclerosis (MS) [7,8]. MS is an autoimmune disease in which inflammatory cells attack the myelin sheath and, consequently, cause demyelination and axonal degeneration [9]. Despite many attempts that have been made to cure MS, the results are unsatisfactory and no absolute solution has been found yet [10,11].

We have recently reported for the first time that the CSF concentration of secreted form of Klotho decreases in patient with relapsing–remitting MS (RRMS) in comparison with the control group. It is noteworthy to mention that there was a significant negative correlation between the CSF Klotho concentration and the expanded disability status scale (EDSS) of the patients. Furthermore, we detected a significant positive correlation between CSF Klotho concentration and CSF total

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anti-oxidant capacity [12]. Our preliminary study showed that there is an association between Klotho alteration and MS pathophysiology. This finding results in several fresh questions that need to be addressed. For example, Do Klotho changes cause MS or they stem from it? In another word, does Klotho concentration change in MS patients prior to the onset of the disease or as the result of the MS pathogenesis? And, what is the status of secreted form of Klotho in the serum?

As a result, in the present study, we aimed to determine if there is any alteration in the serum Klotho concentration of MS patients.

2. Patients and methods

A retrospective observational case–control study was designed to understand the serum Klotho status in patients with RRMS. In order to obtain legal and ethical permission for sample collection, informed consents were taken from all individuals who participated in this research. Moreover, this project was approved by the ethics committee of Tehran University of Medical Sciences (ECTUMS; ethical code# 92-04-30-25660).

This study built on our previous study [12] which was carried out at the Department of Neurology, Imam Khomeini hospital. The revised McDonald's criteria were used to diagnose MS patients [13]. A total number of 45 individuals were recruited and categorized into three groups: the first group comprised of 15 individuals which were definitely diagnosed for the first time (new cases) as having active RRMS and thus had no experience of receiving immunosupressor/immunomodulator or/and vitamin D treatment. The second group consisted of 15 patients who had been suffering from active RRMS for more than 3 years (the chronic group) and were under regular treatments (interferon beta-1a). The third group involved 15 non-MS patients, hence the control group. The complete exclusion criteria for control and new case groups have been previously described [12]. In addition to complete neurological examinations, demographic and clinical data, including gender, age, duration of disease and EDSS, were collected. Serum samples were obtained and kept at −80 °C until assayed. The concentration of secreted form of Klotho was measured using a sandwich ELISA assay kit (human soluble α-Klotho assay kit, IBL Co., Japan, Code No. 27998 and Lot No. 1L-303). The sensitivity of kit was 6.15 pg/ml. The assay was performed according to the instruction provided by the manufacturer. In brief, 100 μl of each test sample blank, serum samples (without dilution) and dilutions of Klotho standard were poured into the appropriate coated wells (Anti-Human Klotho Mouse IgG MoAb) and incubated for 1 h at room temperature. After washing the plates for 7 times with washing buffer, 100 μl of adequately diluted secondary antibody (HRP conjugated Anti-Human Klotho Mouse IgG MoAb) was added to each well and incubated for 30 min at room temperature. The plates were washed 9 times, then 100 μl of peroxidase substrate (TMB) was added to each well and plates were incubated for 30 min at room temperature in a dark place. For stopping the reaction, 100 μl of stop solution (1 N H_2SO_4) was added to each well. The absorbance of blank, serum samples and standards were measured at 450 nm by a microplate reader (TECAN, Austria). The absorbance of standards plotted against their respective concentration. The concentration of serum samples were obtained from standard curve. The ELISA test was run in duplicate.

Statistical analyses were performed by GraphPad Prism (version 6.01, September 21, 2012) and SPSS software (version 16, September 13, 2007). To determine if the data were normally distributed, One-Sample Kolmogorov–Smirnov test was used. The mean age and Klotho concentrations were compared across the three groups using One-Way ANOVA. Tukey test was further conducted in order to detect significant differences among the three means (each belonging to a group). Fisher's exact test was chosen to compare sex ratio. The mean difference of EDSS between new cases and chronic group was compared using Student T-test. Data are shown in graph and tables as mean ± SD. The level of significance was set at $P < 0.05$.

3. Results

As illustrated in Table 1, the groups were comparable in terms of sex and age, with no significant difference among them ($P > 0.05$). Fisher's exact test showed that the sex ratio between the control group and new cases was not statistically significant ($P = 1$). The same results were obtained for the control and chronic groups ($P = 0.080$) and also the new cases and chronic group ($P = 0.214$).

Furthermore, the EDSS of the new cases and chronic group ranged from 1 to 4.5 and from 1 to 3.5, respectively. Table 1, therefore, indicates that there was no considerable difference between the mean of EDSS in the new cases and chronic group ($P = 0.343$). The duration of disease in chronic cases ranged from 3 to 16 years with the mean ± SD equals to 7.86 ± 3.79 (Table 1).

The histogram in Fig. 1 indicates that serum Klotho concentration in new cases (585.56 pg/ml ± 153.99) was higher than that of the control group (556.81 pg/ml ± 120.36). This difference, however, was not statistically significant ($P = 0.859$). On the other hand, there was a significant increase in the concentration of serum Klotho of the chronic group (696.94 pg/ml ± 170.52) when compared to the control group ($P = 0.037$). No significant difference was observed between the new cases and the chronic group in this regard ($P = 0.116$).

4. Discussion

Soluble (secreted) form of Klotho in the serum is considered as a biomarker for some complicated diseases such as chronic kidney disease, cardiovascular disease and diabetes [14]. Our previous studies show significant changes in the serum and CSF protein content of MS patients [15,16]. This study set out to determine the serum Klotho concentration in patients with RRMS. We had already found that the secreted form of Klotho in CSF of patients with RRMS was reduced in comparison with the control group [12]. In contrast to our expectations, the most obvious finding to emerge from this study is that serum Klotho concentration tends to be higher in RRMS cases especially patients with prolonged duration (Fig. 1).

We believe this finding might be due to the treatment of MS patients with immunomodulatory drugs or a compensatory response to enhance CNS regeneration and/or vitamin D biosynthesis. Several studies have indicated that oxidative stress and inflammatory conditions, as main

Table 1
Demographic and clinical features of cases and controls.

	Controls (n = 15)	New cases (n = 15)	Chronic cases (n = 15)	Statistical test (*P* value)
Gender (female/male ratio)	14/1	13/2	9/6	Fisher's exact test* ($P = 0.080$)
Age (mean years ± SD)	30.93 ± 9.72	28.86 ± 8.04	35.33 ± 6.70	One-way ANOVA ($P = 0.103$)
Duration of disease (mean years ± SD)	–	–	7.86 ± 3.79	–
EDSS (mean ± SD)	–	2.40 ± 1.03	2.06 ± 0.84	Student T-test ($P = 0.343$)

New cases were selected from patients who are newly diagnosed with relapsing–remitting multiple sclerosis (RRMS).
* The *P* value which is shown in the table has been calculated between controls and chronic cases.