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Klotho dysfunction: A pathway linking the aging process to bipolar disorder?

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ABSTRACT

Aim: Although accelerated aging profile has been described in bipolar disorder (BD), the biology linking BD and aging is still largely unknown. Reduced levels and/or activity of a protein named Klotho is associated with decreased life span, premature aging and occurrence of age-related diseases. Therefore, this study was designed to evaluate plasma levels of Klotho in BD patients and controls.
Methods: Forty patients with type 1 BD and 30 controls were enrolled in this study. After clinical evaluation, peripheral blood samples were drawn and plasma levels of Klotho were measured using enzyme-linked immunosorbent assay.
Results: Patients with BD and controls presented similar age and sex distribution. The mean ± SD length of illness was 24.00 ± 12.75 years. BD patients presented increased frequency of clinical comorbidities in comparison with controls, mainly arterial hypertension, diabetes mellitus, and hypothyroidism. Both patients with BD in remission and in mania exhibited increased plasma levels of Klotho in comparison with controls. There was no significant difference between patients in mania and patients in remission regarding the levels of Klotho.
Conclusion: Klotho-related pathway is altered in BD. Contrary to our original hypothesis, our sample of patients with BD presented increased plasma levels of Klotho in comparison with controls. Elevated levels of Klotho in long-term BD patients may be associated with the disorder progression. Further studies are needed to better understand the role of Klotho in BD and other mood disorders.

1. Introduction

Despite the corollary that bipolar disorder (BD) is a chronic psychiatric disorder, a large body of evidence has proposed BD as a multisystemic condition presenting with several medical conditions, such as cardiovascular and metabolic diseases, and cognitive impairment (Barbosa et al., 2014).

Recently, it has been suggested that BD is accompanied by an accelerated aging process. The high prevalence of age-related medical conditions and mortality in BD patients corroborates this hypothesis (Goldstein et al., 2015; Osborn et al., 2007; Rizzo et al., 2014). Aging is a natural process that involves psychological, social and biological aspects of an individual. In the biological context, aging is defined as a series of cellular and metabolic changes leading to increased susceptibility to age-related diseases (Hayflick, 2000).

Although the pathophysiology of BD is not completely understood, inflammatory molecules and pathways may play a significant role on it (Barbosa et al., 2014; Munkholm et al., 2013). Increased inflammatory activity is also a marker of the aging process, characterized mainly by elevated levels of proinflammatory cytokines, telomeres shortening and increased oxidative stress and

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apoptosis (Minciullo et al., 2016; Singh and Newman, 2011; Zhang et al., 2016). Recently, a protein named Klotho has been associated with the aging process. The *KLOTHO* gene encodes a transmembrane protein capable of suppressing multiple aging phenotypes (Ersoy, 2014). Reduced levels and/or activity of Klotho is associated with decreased life span, premature aging and occurrence of age-related diseases, such as chronic renal failure, cardiovascular diseases, diabetes, osteoporosis and Alzheimer's disease (Carracedo et al., 2012; Ersoy, 2014; Izquierdo et al., 2012; Keles et al., 2015; Koyama et al., 2015). Secreted Klotho may exert anti-aging and organ-protective effects through pleiotropic actions. For instance, Klotho is able to downregulate inflammatory cytokines and attenuate the generation of reactive oxygen species, acting as an anti-inflammatory protein (Kim et al., 2015).

Although accelerated aging profile has been described in BD and the chronic low-grade inflammation is a feature of both BD and aging, the biology linking BD and aging is still unknown. Reduced circulating levels of Klotho might explain why patients with BD are more prone to age-related diseases. Therefore, this study was designed to evaluate plasma levels of Klotho in patients with BD and controls.

2. Methods

2.1. Subjects

The present study included 40 patients with type 1 BD and 30 controls. Patients were recruited at the Hospital Governador Israel Pinheiro, Belo Horizonte, Brazil. BD diagnosis was independently performed by two psychiatrists using the Mini-International Neuropsychiatric Interview (MINI-Plus) (Sheehan et al., 1998). Psychiatric comorbidities were also evaluated by the MINI-PLUS (Sheehan et al., 1998). The Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967) were used to evaluate the severity of manic and depressive symptoms, respectively. Remission was defined by YMRS score <7 and HDRS score <7 for at least 8 consecutive weeks (Beyer, 2008). The control group was recruited from the local population. Controls were required to not present any psychiatric disorder (excluded by the MINI-Plus interview), family history of psychiatric disorder, or cognitive deficits. Subjects with medical history of renal disease, autoimmune disease or dementia were excluded. Individuals were also excluded if they presented infectious disease or have used anti-inflammatory, corticosteroids or antibiotics drugs in the four weeks previous the study. All subjects provided written informed consent before admission to the study. The Research Ethics Committee of the Federal University of Minas Gerais, Brazil, approved this study. The research protocol was in accordance with the provisions of the Declaration of Helsinki.

2.2. Klotho levels measurement

Peripheral blood samples (10 mL) were drawn from each subject by venipuncture into a heparin-containing tube at the moment of clinical interview. The blood was immediately centrifuged twice at 1800 *g* for 10 min, plasma samples were collected and kept frozen at −70 °C until assayed. Plasma levels of Klotho were measured using enzyme-linked immunosorbent assay (ELISA) kit (DuoSet, R&D Systems, Minneapolis, MN, USA), in accordance with manufacturer instructions. Concentrations were expressed as pg/mL. The detection limit was 10 pg/mL.

2.3. Statistical analysis

All variables were tested for normality of distribution by means of the Kolmogorov-Smirnov test. Descriptive statistics were used to report socio-demographic and clinical features of the sample. Comparisons between dichotomous variables were assessed with the chi-square or Fisher's exact test as appropriate. Between-group differences (patients vs. controls) were assessed with the Mann-Whitney *U* test. Differences among the three groups (patients in mania *vs.* patients in remission *vs.* controls) were calculated with the Kruskal-Wallis test. Multiple comparisons among levels were checked with Dunn's post-hoc test. Spearman's correlation analysis was performed for Klotho levels, age, length of illness, and YMRS and HDRS scores. All statistical tests were two-tailed and performed at a significance level of $p < 0.05$. Outliers defined as values higher than two standard deviations from the mean were excluded from the analysis. Statistical analyses were performed using SPSS version 22.0.

3. Results

Clinical and socio-demographic characteristics of the sample according to BD mood state (mania and remission) and controls are shown in Table 1. Patients with BD and controls presented similar age and sex distribution ($p > 0.05$). The mean ± SD age of BD patients was 50.28 ± 12.80 years. The mean ± SD length of illness was 24.00 ± 12.75 years. Twenty-four out of 40 BD patients (60.0%) were women. In the control group, the mean ± SD age was 45.87 ± 9.61 years and 22 out of 30 (73.3%) participants were women. In comparison with controls BD patients presented increased frequency of clinical comorbidities, mainly arterial hypertension ($p = 0.03$), diabetes mellitus ($p = 0.04$), and hypothyroidism ($p = 0.004$).

BD patients presented increased plasma levels of Klotho in comparison with controls: medians (interquartile ranges) were 165.89 (86.34–241.13) pg/mL for BD patients *versus* 73.95 (44.86–111.07) pg/mL for controls ($p < 0.005$, Mann-Whitney test). We then stratified patients with BD according to the mood state. Both patients with BD in remission and in mania exhibited increased plasma levels of Klotho in comparison with controls: median (interquartile range) were 180.42 (100.16–245.41) pg/mL for BD patients in remission; 125.39 (84.51–224.49) for BD patients in mania; and 73.95 (44.86–111.07) for controls ($p < 0.01$, Kruskal-Wallis test with Dunn post doc analysis, Fig. 1). There was no significant difference between patients in mania and patients in remission ($p > 0.05$; Kruskal-Wallis test with Dunn post doc analysis, Fig. 1). Circulating levels of klotho were similar in female and male subjects.

Among patients with BD, plasma levels of Klotho correlated negatively with age ($\rho = -0.38$, $p = 0.02$). Klotho levels did not correlate with YMRS or HDRS scores. Klotho levels were not associated with the presence of clinical co-morbidities, or the use of any mood-stabilizing drug (lithium, anticonvulsants or atypical antipsychotics).

4. Discussion

To the best of our knowledge, this is the first study that evaluated circulating levels of Klotho in BD. Contrary to our original hypothesis, patients with BD presented increased plasma levels of Klotho in comparison with controls regardless of mood state (mania or remission), clinical comorbidities or medication. The increase in the levels of Klotho in BD sample might be associated with the disorder progression.

One of the mechanisms proposed to explain the antiaging activity of Klotho is its ability to induce oxidative stress resistance