

Implication of alpha-Klotho as the predictive factor of stress

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ABSTRACT

Stress is known as a risk factor for both mental and physical health problems. While stress is known as one of the major health problems in modern society, a biomarker of stress has not yet been well established. In the present study, we focused on the serum levels of α -Klotho (α Kl) as a possible objective biomarker of stress. Subjects included apparently healthy individuals who underwent a health examination in the Osaka University Health and Counseling Center. Physical and biochemical parameters were obtained from all subjects. Information regarding the lifestyle of each individual was obtained via questionnaires. Among male subjects, serum levels of soluble α Kl ($s\alpha$ Kl) were significantly elevated in subjects who had poor stress management and unsatisfactory sleep, suggesting that stress management and sleeping conditions influenced the serum levels of $s\alpha$ Kl. The total Kessler Screening Scale for Psychological Distress (K6) score was significantly increased in subjects who reported experiencing considerable stress, had poor stress management and unsatisfactory sleep. Since serum levels of $s\alpha$ Kl showed the same tendency as the K6 score in terms of the relationship between stress management and sleeping conditions in male subjects, increased $s\alpha$ Kl levels could be associated with considerable psychological stress in healthy men.

INTRODUCTION

Physiological and psychological stress affects mental health, and is associated with various diseases, such as cardiovascular disease, gastrointestinal disease and metabolic syndrome.^{1–3} However, the precise nature of the association remains unclear.

We have previously focused on smoking, which is known as a stress factor, and reported an interesting association that smoking increased serum levels of soluble α -Klotho ($s\alpha$ Kl) and inflammation-related cytokine interleukin-6 (IL-6) in male subject.⁴

α Kl is known as an antiaging gene, and gene-deficient mice have short life spans and multiple aging phenotypes, including skin atrophy, ectopic calcification, osteoporosis, atherosclerosis and pulmonary emphysema.^{5,6} α Kl is a membrane protein primarily expressed in the distal tubule, parathyroid gland and

choroid plexus. A soluble form of α Kl exists in serum and serum $s\alpha$ Kl levels are reported to decrease with age.⁷ $s\alpha$ Kl levels are also reported to change in some disease states.^{8,9}

Although stress has become a major health problem in modern society, an objective parameter of stress has not yet been well established. Since there was a study reporting the possibility of $s\alpha$ Kl as a biological link connecting stress, depression and risk for accelerated disease development,¹⁰ we here focused on the serum levels of $s\alpha$ Kl as a possible objective biomarker of stress. In the present study, we evaluated the association between stress and serum levels of $s\alpha$ Kl by using the Kessler Screening Scale for Psychological Distress (K6), which is widely used to screen mental health problems.^{11,12}

METHODS

Study subjects

This study included cross-sectional data obtained from the Osaka University employees. The subjects in the study were randomly selected individuals who underwent a health examination in the Osaka University Health and Counseling Center. One hundred two apparently healthy Japanese men between the ages of 40 and 60 years, never-smokers, who did not take any chronic or frequent medicine from at least 1 year before their health examination, and had not suffered acute illness within the previous 2 weeks were enrolled for the study. This information was obtained via questionnaires and was also confirmed in expert interviews. Age-matched women (n=100) were additionally selected in the last part of the study. Physical parameters, biochemical parameters and information regarding the lifestyle were obtained from all subjects between 09:00 and 11:00 hours.

Physical and biochemical parameters

Body mass index (BMI) and waist circumference (WC) were measured as anthropometric measurements. WC at the umbilical level was measured in the late exhalation phase in the standing position.

Serum was collected from subjects after overnight fasting and kept at $\leq -20^{\circ}\text{C}$ until assayed. The serum IL-6 levels were measured with a chemiluminescent enzyme immunoassay system



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(Fujirebio, Tokyo, Japan). α KI levels were measured with a sandwich ELISA system according to the manufacturer's instructions (Immuno-Biological Laboratories, Takasaki, Japan).

Lifestyle assessments

Information on medical history, use of medicines, smoking status, stress awareness, stress management, relaxation from sleep and K6 scores were obtained via questionnaires. Stress awareness, stress management and relaxation from sleep, during the past 30 days were semi-quantified using the following scales. Stress awareness: 1=almost no stress, 2=sometimes feel stressed, 3=often feel stressed, 4=always feel stressed; stress management: 1=well managed, 2=mostly managed, 3=hardly managed, 4=not managed; relaxation from sleep: 1=well obtained, 2=mostly obtained, 3=hardly obtained, 4=not obtained. The K6 contains six questions asking about the frequency of the following feelings: sad, nervous, restless or fidgety, hopeless, everything is an effort and worthless, during the past 30 days. All questions are scored on a 5-point scale, ranging from 0 (none of the time) to 4 (all of the time). The total K6 score ranges from 0 to 24.¹¹

Statistical analysis

All statistical analyses were performed using STATA .14 (STATA, Texas, USA). The distribution of continuous variables was tested by Shapiro-Wilk test. Normally distributed variables were presented as mean \pm SD; non-normally distributed variables were reported as median (IQR). Kendall's rank correlation coefficient and multiple regression analysis were used to analyze variables. Analysis of variance with Bonferroni post hoc test or Kruskal-Wallis test was used to assess the differences between groups. Statistical significance was set at $p < 0.05$.

RESULTS

Serum levels of α KI increased in subjects with poor stress management and unsatisfactory sleep

The median age, mean α KI levels and median IL-6 levels were 50 (43–52) years, 539 ± 126 pg/mL and 1.2 (0.9–1.4) pg/mL, respectively. The median K6 score of the study subjects was 1 (0–4).

The associations of serum levels of α KI and IL-6 with stress awareness, stress management and relaxation from sleep are shown in figure 1. Serum levels of α KI tended to increase in subjects who reported experiencing considerable stress (figure 1A), and were significantly increased in subjects who reported poor stress management (figure 1B). Moreover, α KI levels were significantly increased in subjects who reported that they did not feel relaxed after sleep (figure 1C). In addition, we found significant correlations between α KI levels and stress management ($\tau = 0.151$, $p = 0.013$), and relaxation from sleep ($\tau = 0.116$, $p = 0.024$). These results suggest that stress management and sleeping conditions affected the α KI levels. As serum levels α KI is reported to relate with age, BMI and renal function,^{7 13} multivariable regression analysis was performed for further analysis (table 1). We found that stress management remained as the independent variable for serum levels of α KI increase, suggesting that α KI might be a marker of

considerable psychological stress in apparently healthy subjects.

Serum levels of IL-6 exhibited no significant association between stress awareness (figure 1D), stress management (figure 1E) or relaxation from sleep (figure 1F).

Stress awareness, stress management and sleeping conditions influenced the K6 score

As K6 is known to reflect mental health problems,¹¹ we verified the association of the K6 score with psychological stress and sleeping conditions. The K6 score was significantly increased in subjects who reported experiencing considerable stress ($p = 0.002$) and in those with poor stress management ($p < 0.0001$). Moreover, the K6 score was significantly increased in subjects with unsatisfactory sleep ($p < 0.0001$). Furthermore, there were significant correlations between the K6 score and stress awareness ($\tau = 0.270$, $p < 0.0001$), stress management ($\tau = 0.301$, $p < 0.0001$) and relaxation from sleep ($\tau = 0.216$, $p < 0.0001$). Therefore, it is suggested that stress awareness, stress management and sleeping conditions influence the K6 score. We confirmed the correlation between the K6 score and serum levels of α KI; however, significant correlation was not shown.

α KI levels did not associate with stress awareness, stress management or sleeping conditions in female subjects

In the following studies, we evaluated the association of serum levels of α KI with psychological stress and sleeping conditions in female subjects. The median age, α KI levels, and K6 score were 47 (44–53) years, 618 (528–746) pg/mL and 2 (0–4), respectively. However, unlike in male subjects, α KI levels exhibited no correlation between stress awareness, stress management or relaxation from sleep in female subjects (see online supplementary table S1).

DISCUSSION

As stress affects both mental and physical health, stress is regarded as one of the major risks of health disorders in modern society. In the present study, we analyzed the association between stress and serum levels of α KI. α KI was originally established as an antiaging gene and is reported to relate with various diseases.⁹ In this study, we found increased α KI levels in subjects with poor stress management and unsatisfactory sleep. To verify these findings, we evaluated the K6 score and the association between K6 score and α KI levels in the study subjects. The K6 has been used to assess the degree of non-specific psychological distress and is known as a reliable tool for evaluating the severity of mental health problems.^{11 12 14} We found that the K6 score was increased in subjects who reported experiencing considerable stress, poor stress management and unsatisfactory sleep. Since 'severe mental illness' is defined as $K6 \geq 13$ and 'moderate mental distress' is considered as $K6 \geq 5$, perceived psychological stress level in our study subjects was supposed to be mild.¹⁵ Although stress level was mild, serum levels of α KI showed the same tendency as the K6 score in terms of the relationship between stress management and sleeping conditions. These results suggest that α KI levels might be a predictive factor to detect psychological stress.

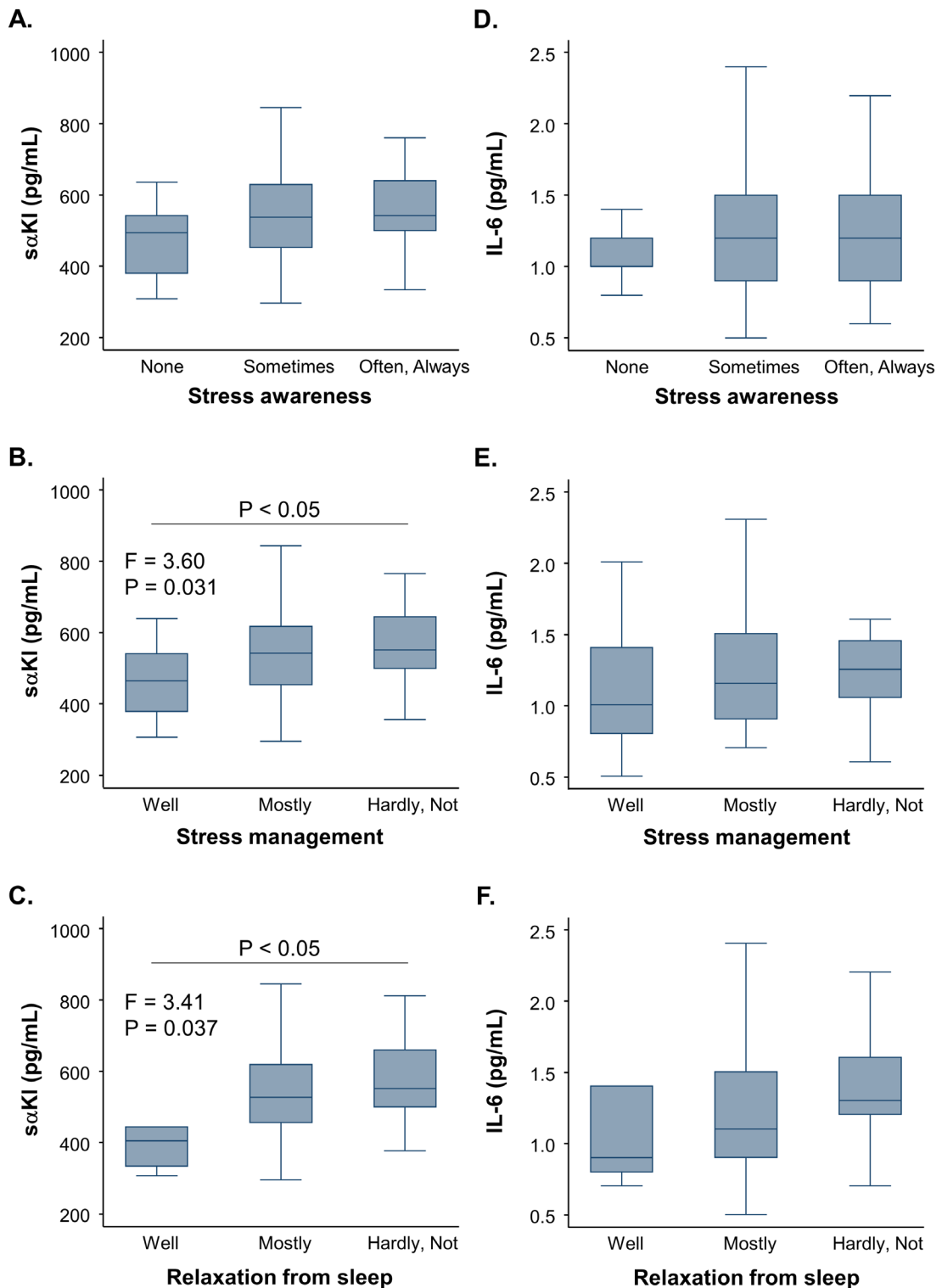


Figure 1 Relationship of serum levels of soluble α -Klotho (s α KI) and interleukin-6 (IL-6) with stress awareness, stress management and relaxation from sleep. Relationship between serum levels of s α KI and (A) stress awareness, (B) stress management, (C) relaxation from sleep and relationship between serum levels of IL-6 and (D) stress awareness, (E) stress management and (F) relaxation from sleep. Stress awareness was classified into three stages: almost no stress (none), sometimes feel stressed (sometimes), often or always feel stressed (often, always). Stress management was classified into three stages: well-managed (well), mostly managed (mostly), hardly or not at all managed (hardly, not). Relaxation from sleep was classified into three stages: well-obtained (well), mostly obtained (mostly), hardly or not at all obtained (hardly, not). Data are shown as the sample minimum, lower quartile, median, upper quartile and sample maximum.

Table 1 Multivariable analysis for serum levels of soluble α -Klotho

	β	P value
Age	-4.544	0.050
BMI	-1.271	0.731
Cr	134.646	0.256
Stress management	43.128*	0.046
Relaxation from sleep	33.334	0.242

Adjusted R²=0.089.

*P<0.05.

BMI, body mass index; Cr, creatinine.

We previously reported that the serum levels of α Kl and IL-6 were upregulated by smoking in healthy male smokers.⁴ Smoking is a stress factor that stimulates the release of IL-6 and promotes inflammation.¹⁶ Psychological stress and sleep restriction are also reported to elevate the levels of inflammatory markers, including IL-6.^{17,18} Since α Kl has an anti-inflammatory effect,¹⁹ we suppose that increased α Kl levels might be a compensatory response to counteract the inflammatory stress.⁴ However, IL-6 levels exhibited no significant association with psychological stress or sleeping conditions. IL-6 levels also did not correlate with α Kl levels. As psychological stress level was mild in these subjects, IL-6 levels might not increase obviously. To evaluate the involvement of inflammation, other pro-inflammatory marker or anti-inflammatory marker should be assessed in our further study. As α Kl is also known to regulate other pathways, including insulin/IGF-1 and Wnt,²⁰ it is quite possible that these pathways may also be involved in the mechanism of upregulated serum levels of α Kl.

Although α Kl levels increased in subject with poor stress management and unsatisfactory sleep, direct correlation between α Kl levels and K6 score was not shown. Mild psychological stress level and unclear inflammatory response might result in this relationship between α Kl levels and K6 score. To reveal this problem, we need to investigate high-stressed or inflammation-obvious population in the further study. Moreover, as various complex mechanism regulates psychological stress, interaction with α Kl levels might be a part of this complex mechanism.²¹

A previous study reported opposite result to our study regarding the association between psychological stress and α Kl levels among mothers.¹⁰ Although a different scale of stress was used, high-stressed mother in the previous study were supposed to be experiencing higher psychological stress than in our female subjects. Since stress level was relatively mild, α Kl levels might not show significant association with psychological stress in our female subjects. We also considered the involvement of menopause in this association (see online supplementary table S2); however, involvement of menopause was not clear. In this study, serum levels of α Kl showed a different reaction to psychological stress between men and women. We and others previously reported the different reaction of α Kl between men and women,^{22,23} however, underlying mechanism of this different reaction is still unclear. Further study is necessary to reconcile this difference.

This study has several limitations. The study was single-center study and the numbers of subjects were small.

Multicenter study and large numbers of subjects are preferable in the future study. There are several measures of psychological stress, including serum cortisol level, startle reflex and heart rate variability.²⁴⁻²⁶ In the present study, we used only K6 score to assess the psychological stress, however, these other measures should be evaluated in our further study.

In the present study, we elucidated an association between psychological stress and serum levels of α Kl. α Kl levels increased and showed the same tendency as the K6 score in male subjects with poor stress management and unsatisfactory sleeping conditions, suggesting that serum levels of α Kl could be a predictive factor of stress.

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Contributors KN, MN and KY-T designed the study. KN performed the experiments, analyzed the data and wrote the manuscript. MN, HA and KY-T provided appropriate suggestions during the development of this study. Valuable comments on a first draft were received from MT, RY and TM. All authors have given approval to the final version of the manuscript.

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Competing interests No, there are no competing interests for any author.

Patient consent for publication Not required.

Ethics approval All procedures in this study were performed in accordance with the Declaration of Helsinki and the ethics guidelines for clinical research from the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology, Japan. All experimental protocols in this study were approved by the Ethics Committee of Health and Counseling Center, Osaka University.

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Data sharing statement All data relevant to the study are included in the article.

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