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Research Paper

Serum Klotho Level and its Related Factors Among Male Opioids Addicts With Normal Renal Function Compared to Healthy Male Non-smokers and Smokers in Tabriz, Iran

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ABSTRACT

Background: Klotho is an aging-suppressor gene that encodes a single-pass transmembrane protein and acts as a hormone. In this study, we aim to investigate the serum α-Klotho level in male opioids addicts with normal kidney function compared to healthy male non-smokers and smokers in Tabriz, Iran.

Methods: Participnts were 87 men with normal kidney function referred to Sina Educational Research and Treatment Center in Tabriz, Iran (29 opioids addicts, 29 healthy non-smokers, and 29 healthy smokers). Blood samples were collected to measure the soluble a-Klotho level using an ELISA kit. Furthermore, blood creatinine (Cr) and hemoglobin (Hb) levels was measured. Body mass index (BMI) was also calculated for all participants.

Results: In addicts, BMI, Hb, and Cr levels were significantly lower than in healthy non-smokers and smokers, but their Klotho level was higher (P>0.05). The Klotho level in healthy smokers was significantly lower than in healthy non-smokers and addicts. The Klotho level of healthy smokers decreased as the pack year increased, but the duration of opioid addiction had no significant association with the Klotho level. There was no significant difference in the Klotho level between control groups (non-smokers and smokers) and men with addiction to different types of opioids.

Conclusion: The Klotho level in male opioid addicts is significantly higher than in smokers. There is a significant negative correlation between BMI and Klotho levels among men with normal BMI and overweight. Further studies are recommended in these fields.

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1. Introduction

Klotho is originally described as an aging-suppressor gene whose overexpression can increase life span [1]. According to the primary sequences, α-, β- and γ-Klotho are three identified klotho-related genes, among which α-Klotho is widely studied [2]. The Klotho gene encodes a single-pass transmembrane protein with a large extracellular domain. It is predominantly expressed in the renal tubules and other tissues, including the parathyroid gland, choroid plexus in the brain, skeletal muscles, pancreas, and sex organs [3, 4]. Ectodomain shedding of the extracellular domain of Klotho can release the soluble form of Klotho, which is the main functional form in the blood, urine, and cerebrospinal fluid [5]. Soluble α-klotho acts as a hormone-like substance once it enters the bloodstream. The α-klotho can protect cells against inflammation, oxidative damage, apoptosis, and aging, strengthen angiogenesis and vascularization, protect stem cells, and prevent kidney fibrosis and vascular calcification by inhibitory effect on phosphate absorption [6, 7]. Therefore, a defect in Klotho gene expression can cause premature aging, shortened lifespan, vascular calcification, cardiovascular disease, multiple organ degeneration, and frailty [5]. Aging is a complex phenomenon that is affected by various genetic and environmental factors. Smoking is the main environmental risk factor of aging that can cause premature death [8]. Several studies reported that the serum level of α-Klotho is significantly higher in smokers than non-smokers due to a compensatory response to smoking stress [8-11]. Cigarette smoking, sleep disorders, and psychological stress release various inflammatory markers, including interleukin 6 (IL-6), which is related to aging outcomes [9]. Based on previous studies, the soluble α-Klotho level is significantly higher in male smokers than male non-smokers, while its level is lower in female smokers than female non-smokers. Furthermore, the soluble α-Klotho level of female smokers is lower than that of male smokers. Due to the anti-inflammatory effect of soluble α-Klotho, it seems that its overexpression can be a compensatory response to inflammatory stress. Women are more vulnerable than men to the toxic effects of smoking [12, 13]. Nowadays, opioids are considered vital medications in palliative care and are used as the most common analgesics around the world. On the other hand, non-medical and recreational consumption of opioids has become a serious problem worldwide [14, 15]. Considering that the serum level of Klotho has not been evaluated in opioid addicts, this study aims to measure the serum level of α-Klotho in male opioid addicts with normal kidney function.

2. Materials and Methods

In this descriptive cross-sectional study conducted from January 2017 to January 2018, participants were 29 male opioid addicts with normal kidney function admitted to the emergency ward of Sina Educational Research and Treatment Center in Tabriz, Iran, due to opioid overdose (case group), as well as 29 healthy male non-smokers and 29 healthy male smokers with normal kidney function admitted to Sina Educational Research and Treatment Center for various complications (control groups). We included healthy smokers as the second control group because the majority of people with opioid addiction also smoke cigarettes. The sample size was determined based on a pilot study. The Mean±SD of Klotho value was 13±10 and 8±2 among male smokers and healthy men, respectively. Considering a test power of 0.80, α=0.05, and a one-sided hypothesis, the sample size was estimated at 29 for each group (total=87). The inclusion criteria were: Age 18-40 years, having an addiction to opioids (for the case group), being healthy (for smokers), no smoking (for non-smokers), and consent to participate in the study. The control groups were matched for age, and their physical health was approved by initial medical examinations. Those with abnormal kidney function, active neoplasia, history of alcohol consumption, addiction to other substances, and age >40 years were excluded.

Demographic information, including age, gender, weight, height, history of smoking, underlying diseases, medication history, and clinical data (such as creatine and hemoglobin levels), was collected using a questionnaire. Body mass index (BMI) was calculated by dividing weight (in kilograms) by height (meter squared) [16]. History of smoking (pack year) was calculated as follows:

(Number of cigarettes smoked per day/20)×Number of years smoked [17].

Blood samples were collected to measure the serum level of Klotho using a human Klotho ELISA kit (ZellBio GmbH, Germany; Cat. No: ZB-12781S-H9648 /2016).

The SPSS software, version 23 (Chicago, IL, USA) was used for data analysis. The normality of data was tested by one-sample Kolmogorov–Smirnov test. Analysis of Variance (ANOVA) was used to compare the means of Klotho level among the three groups. A multiple linear regression model was used to estimate the adjusted coefficients (B) at a 95% confidence interval (CI) for the association between Klotho level and opioid addiction. $P<0.05$ was statistically significant.

3. Results

Characteristics of participants

There was no significant difference in age and Klotho level among the three groups (P>0.05). The participants in the case group were in the age range of 21-40 years. In this group, those aged 21-30 years had a higher Klotho level (18.68±17.98 ng/mL) than those aged 31-40 years (10.19±3.32 ng/mL) (P<0.05). In addition, the Klotho level of smokers was lower (8.32±2.11 ng/mL) compared to non-smokers and addicts. The baseline characteristics of participants in the three groups are presented in Table 1. Twenty-seven (out of 29) opioid addicts had a history of smoking. Moreover, a high association was reported between duration of opioid addiction and pack year (F=43.135; B=0.784, P<0.001).

In terms of clinical characteristics in the case group, 10.3% (n=3) had high addiction (21-30 years of addiction, Klotho level=8.80±0.71 ng/mL), 27.6% (n=8) had moderate addiction (11-20 years of addiction, Klotho level=9.99±4.90 ng/mL), and 62.1% (n=18) had mild addiction (1-10 years of addiction, Klotho level=15.17±13.69 ng/mL). No significant difference was found in Klotho level among addicts with three severities of addiction (P=0.620). The BMI of opioid addicts was significantly lower than that of smokers and non-smokers (P=0.01). On the other hand, the hemoglobin (Hb) and creatine (Cr) levels of addicts were significantly lower level than that of healthy smokers and non-smokers (P<0.001 and P=0.03, respectively).

Association of Klotho level with the duration of opioid addiction and smoking

The mean duration of opioid addiction was 11.01±7.7 years. It showed no significant correlation with Klotho level (r=-0.177, P=0.359). The mean pack year was 23.57±13.69 years. There was a negative correlation between pack year and Klotho expression level (r=-0.222. P=0.038).

Klotho level based on different types of opioids

Table 2 compares Klotho level between men with addiction to different types of opioids (methadone, opium, mixed opioid, and other) and control groups (healthy smokers and non-smokers). According to the results of ANOVA, there was no significant difference in the mean level of Klotho among the groups (F=1.56, P=0.192).

Klotho level among different study groups

Based on the research findings, the study groups were categorized into four groups: Healthy, smokers and addicts, only smokers, and only addicts. The mean level of Klotho was 13.45±11.65 ng/mL in the group consisted of smokers and addicts (n=27), 8.60±1.83 ng/mL in only addicts (n=2), and 8.32±2.11 ng/mL in only smokers (n=29). Without judging the reliability of the history, one 26-year-old patient with 1.5 years of addiction to opioids without smoking history had a serum Klotho level of 7.30 ng/mL. The patient with two years of addiction to opioids without a history of smoking had a serum Klotho level of 9.90 ng/mL. Among 87

Table 1. Baseline characteristics of participants (n=87)

Variables	Non-smokers (n=29)		Smokers (n=29)		Addicts (n=29)		Total (n=87)		P
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	
Age (y)	36.14±4.64	38	35.07±4.93	36	34.31±6.39	37	35.17±5.37	37	0.53
Klotho level (ng/mL)	13.03±10.32	9.50	8.32±2.11	8.3	13.12±11.3	9.30	11.49±9.10	8.80	0.07
BMI (kg/m^2)	24.70±0.90	25	24.74±0.62	25	24.2±0.8	24	24.55±0.82	25	0.01
Hg (mg/dL)	13.02±0.64	13	14.27±0.76	14.4	12.35±2.13	11.6	13.21±1.56	13	<0.001
Cr (mg/dL)	1.17±0.25	1.2	1.21±0.11	1.2	1.6±0.24	1	1.15±0.22	1.20	0.03
Addiction duration (per year)	-	-	-	-	11.01±7.73	8	11.01±7.73	8	-
Pack year	-	-	32.79±10.00	35	12.72±9.89	10	15.17±15.77	10	-

Abbreviations: SD: Standard deviation; BMI: Body mass index; Hg: Hemoglobin; Cr: Creatinine.

Table 2. Comparison of Klotho levels between men with addiction to different types of opioids addicts and controls using one-way ANOVA

Group		No. (%)	Mean±SD	F	P
Controls		58(66.6)	10.67±7.76	1.56	0.192
Types of opioids	Methadone	1(1.1)	6.90±0		
	Opium	17(19.5)	12.52±10.87		
	Mixed opioid	9(10.3)	12.05±8.62		
	Other	2(2.3)	26.05±26.51		
Total		87(100)	11.49±9.10		

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participants, the highest Klotho level (52.20 ng/mL) was seen in a 28-year-old man with five years of smoking and opioid addiction. However, no significant difference was found in the mean Klotho level among the study groups (control, addicts only, smokers only, smokers+addicts), P=0.120 (Table 3).

Predictors of Klotho level in opioid addicts

The factors affecting Klotho level in opioid addicts were examined in Table 4. According to univariate regression analysis, the increase in pack year could significantly reduce the Klotho level (P=0.038). Based on both univariate and multivariate regression analyses, the increase in BMI could significantly reduce the Klotho level (P=0.001). The multivariate regression analysis results showed that when other factors were constant, with every one unit increase in opioid addiction and pack year, the Klotho level decreased by 0.088 and 0.083, but it was not statistically significant (P=0.549 and P=0.216, respectively).

4. Discussion

The findings of the present study showed that the serum level of Klotho in male smokers was lower than in male non-smokers and opioids addicts. Nakanishi et al. also showed that the serum level of Klotho was high in healthy male smokers [8]. Verde et al. indicated that the serum level of Klotho was associated with smoking intensity. In addition, a high level of serum Klotho was observed in healthy heavy smokers [10]. Abnormal kidney function can affect the α-Klotho level. A study showed a positive correlation between the estimated glomerular filtration rate and Klotho serum level [18]. The Klotho gene is involved in the aging process in mammals for over 30 years, which regulates the fibroblast growth factor activity and phosphate homeostasis [5]. Previous studies have indicated that overweight/obesity and high BMI are significantly associated with low Klotho level [19-21].Yao et al. showed that the serum level of α-Klotho was low in female smokers with singleton gestations between 20 and 34 weeks. By increasing the

Table 3. Comparison of Klotho levels among the study groups (ANOVA results)

Groups		No. (%)	Mean±SD	F	P
Healthy		29(33.3)	13.03±10.32	2.002	0.120
Smokers (n=56)	Smokers+addicts	27(31.03)*	13.45±11.65		
	Smokers only	29(33.3)	8.32±2.11		
Addicts (n=29)	Addicts+smokers	27(31.03)*	13.45±11.65		
	Addicts only	2(2.30)	8.60±1.83		
Total		87(100)	11.49±9.10		

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*No. of 27 participants were considered once because those are the same participants who were both addicts and smokers.

Table 4. Results of regression analyses for finding the predictors of Klotho level and opioid addiction

Variables	Univariate Regression			Multivariate Regression			
	Standardized Coefficient (B)	95% CI	P	Unstanderdized Coefficient	Standardized Coefficient (B)	95% CI	P
Age (y)	-0.204	-0.56, 0.26	0.267	0.183	0.112	-0.21, 0.58	0.370
Cr (mg/dL)	-3.590	-12.42, 5.24	0.421	2.943	0.072	-5.76, 11.64	0.503
Hb (mg/dL)	-1.199	-2.42, 0.031	0.056	-0.812	-0.139	-2.21, 0.58	0.252
Addiction time (per year)	0.022	-0.26, 0.30	0.881	-0.088	-0.071	-0.38, 0.20	0.549
Pack year	-0.128	-0.25, -0.007	0.038	-0.083	-0.139	-0.21, 0.05	0.216
BMI (kg/m^2)	-4.37	-6.58, -2.16	0.001	-4.958	-0.445	-7.59, -2.32	<0.001

Abbreviations: CI: Confidence interval; BMI: Body mass index; Hg: Hemoglobin; Cr: Creatinine

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smoke exposure, the α-Klotho level first decreased and then increased. Furthermore, there was no significant relationship between smoking and α-Klotho level [22]. The discrepancy between our results and the findings of these studies may be related to difference in the study population. In the present study, only males with normal kidney function were included; females and those with chronic kidney disease were excluded. To our knowledge, this is the first study that examined the serum level of Klotho in male opioids addicts with normal kidney function. Therefore, there is no similar study to compare their results with our findings.

In the present study, younger addicts (aged 21-30 years) had higher Klotho level than older addicts (aged 31-40 years), but there was no significant difference in Klotho level between the age groups. Yazici et al. in Turkey indicated a significant negative correlation between age and Klotho level among those with chronic cannabis use in the withdrawal period [23], which suggests the detrimental effect of cannabis on Klotho level in the body. According to our results, the serum level of Klotho was almost the same in male addicts and healthy men.

Our findings also showed that the severity of opioid addiction had no significant effect on the serum level of Klotho. Similar to our results, Yazici et al. reported no association between the severity of cannabis abuse, cannabis use time, and Klotho level. Also, they showed a relationship between Klotho level and the age at onset of cannabis use, indicating that if the use of synthetic cannabinoids starts at adolescence, a time-specific effect on Klotho level would be observed [23]. In the present study, the BMI and Hb level of male addicts were significantly lower than that of male non-smokers and smokers, while the Hb level in smokers was at the highest level. The relationship of smoking and opioid addiction with Hb level has been reported in other studies [9, 20, 21]. The increase in Hb concentration is due to exposure to carbon monoxide, which is higher in smokers than in non-smokers, and can be considered a compensatory mechanism in smokers. In Haghpanah et al.'s study, Hb concentration was lower in opioid-dependent groups such as heroin addicts, than in non-addicts [24], which is consistent with our study.

The findings of the present study also showed that the serum level of Klotho and Cr among male opioids addicts was higher than in smokers. The relationship between BMI and Klotho level in humans is unclear. However, in a recent review study, Landry et al. showed that overweight/obese individuals had significantly lower Klotho level in the cerebrospinal fluid than their lean counterparts. High BMI or obesity is attributed to the secretion of inflammatory mediators such as TNF-α, IL-6, and adiponectin reduction [20]. However, more studies are needed on the effect of Klotho on fat accumulation or the effect of BMI on the reduction of Klotho level through increased inflammatory response or oxidative stress.

Despite the effort to select the individuals based on the same inclusion criteria in all three groups, the differences in Hb, Cr and BMI of men in three groups were statistically significant, which can be considered as the confounding factors. The smoking history is another confounding factor in the present study. After eliminating these confounding factors, there was no significant

correlation between opioid addiction history and Klotho level. Many effective factors and confounders were associated with the serum level α-Klotho in the study groups. To solve this issue, we carried out multiple linear regression to estimate the adjusted measure of association after controlling all confounders and effective factors. Furthermore, we only examined male participants.

5. Conclusion

Despite the low level of Cr, Hb, and BMI in male opioids addicts, their Klotho level is higher than in healthy non-smokers and smokers. After removing the effect of confounding factors, a significant difference was observed between the Klotho level and the history of opioid addiction. A significant negative correlation was observed between BMI and Klotho levels among men with normal BMI and overweight. Further studies are recommended in this field.

Ethical Considerations

Compliance with ethical guidelines

All methods were carried out in accordance with relevant guidelines and regulations. Ethical clearance was obtained from the Research Ethics Committees of Tabriz University of Medical Sciences (Code: IR.TBZMED.REC.1395.1011). Informed consent was obtained from all participants.

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Authors' contributions

Conceptualization and study design Ali Banagozar Mohammadi, Maryam Zaare Nahandi, and Ahad Banagozar Mohammadi; Data collection and sampling: Rasoul Estakhri, Ali Banagozar Mohammadi, Maryam Zaare Nahandi and Ali Ostadi; Data analysis and interpretation: Ali Banagozar Mohammadi, Elghar Soltani, and Hosein Azizi; Preparation of the initial draft: Ali Banagozar Mohammadi, Maryam Zaare Nahandi, Ahad Banagozar Mohammadi, Elghar Soltani, Masoomeh Kashef Nejad, and Saiedeh Razi Soofiyani; Editing: Ali Banagozar Mohammadi, Maryam Zaare Nahandi, and Ahad Banagozar Mohammadi and Rasoul Estakhri; Supervision and review: Ali Banagozar Mohammadi, Maryam Zaare Nahandi, and Elghar Soltani, Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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