



ISSN: 0067-2904

## Assessment of Malondialdehyde and Soluble $\alpha$ -Klotho Serum Levels in Iraqi Acromegaly Patients

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Received: 15/6/2023

Accepted: 24/11/2023

Published: 30/6/2024

### Abstract

Acromegaly has been associated with several metabolic health conditions that can increase risks of death and illness. These include heart disease, diabetes, and problems with how the body regulates insulin levels. Multiple studies have found that people living with acromegaly are two to four times more likely to pass away than others of the same age and sex in the general population. Research also suggests a link between acromegaly and higher levels of malondialdehyde (MDA) in the body. MDA is a byproduct formed during natural processes like prostaglandin and lipid peroxidation production that is able to damage genes and has been connected to cancer development. The transmembrane protein known as soluble  $\alpha$ -Klotho (SAKL) is named after a Greek deity. Numerous age-related illnesses, including osteoporosis, pulmonary emphysema, skin shrinkage, and atherosclerosis, have been shown to affect  $\alpha$ -Klotho-deficient mice. Sixty subjects (30 males and 30 female) Mean  $\pm$  SD was (53.2 $\pm$ 10.5). diagnosed with acromegaly and 30 (15 males and 15 female) healthy group Mean  $\pm$ SD was (52.1 $\pm$ 13.5). the age range between (30-60) years. Laboratory measurement including serum Insulin like growth factor-1 (IGF-1), Growth hormone (GH) Fasting serum glucose (FSG), lipid profile (Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL)), Soluble  $\alpha$ -Klotho (SAKL), Malondialdehyde (MDA). The results showed significantly higher  $P \leq 0.01$  IGF1, GH, FSG, TC, TG, LDL, VLDL, SAKL, and MDA levels in patients' group Compared to healthy group. In patients' group SAKL had a positive significant correlation with HDL, and negative significant correlation ( $P < 0.01$ ) with LDL, on the other hand, there was a substantial relationship  $P < 0.01$  between (TC, TG, and LDL) with MDA. The research findings suggest that levels of SAKL and MDA are increased in patients with acromegaly, potentially serving as novel biomarkers for assessing active acromegaly. The measurement of SAKL and MDA could prove valuable, particularly in cases where GH and IGF-I results are inconclusive or conflicting

**Keywords:** Acromegaly,  $\alpha$ -Klotho, Malondialdehyde, growth hormone, Lipid profile

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## تقييم مستويات Malondialdehyde و Soluble $\alpha$ -Klotho في مرضى ضخامة الاطراف العراقيين

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### الخلاصة:

ويرتبط مرض ضخامة الاطراف بالاضطرابات الأيضية، مثل أمراض القلب والأوعية الدموية والسكري ومقاومة الأنسولين، مما يؤدي إلى رفع معدلات الوفيات والمرض، أظهرت العديد من الدراسات أن معدل الوفيات لدى مرضى ضخامة الأطراف أعلى بمرتين إلى أربع مرات من معدل الوفيات لدى عامة السكان من نفس الجنس والعمر. Malondialdehyde هو منتج ثانوي مسبب للطفرات ومسبب للسرطان يحدث بشكل طبيعي من إنتاج البروستاجلاندين وبيروكسيد الدهون. تمت تسمية بروتين الغشاء المعروف باسم (SAKL) Soluble  $\alpha$ -Klotho على اسم إله يوناني. ثبت أن العديد من الأمراض المرتبطة بالعمر، بما في ذلك هشاشة العظام وانتفاخ الرئة وانكماش الجلد وتصلب الشرايين، تؤثر على الفئران التي تعاني من نقص  $\alpha$ -Klotho. تم جمع ستين عينة (30 رجل و 30 امرأة) مشخصين بمرض ضخامة الاطراف و 30 عينة (15 رجل و 15 امرأة) من مجموعة الاصحاء في هذه الدراسة. الفئة العمرية ما بين (30-60) سنة. تضمنت التحاليل المختبرية (MDA، SAKL، VLDL، LDL، HDL، HDL، TG، TC، FSG، GH، IGF-1) أظهرت النتائج ارتفاع ملحوظ في مستويات  $IGF1 P \geq 0.01$  في مجموعة المرضى مقارنة بمجموعة الاصحاء. في مجموعة المرضى SAKL كان هناك علاقة موجبة مع HDL، وعلاقة سالبة  $P < 0.01$  مع LDL، من ناحية أخرى، كان هناك علاقة معنوية  $P < 0.01$  بين TG، TC، و MDA. ونستنتج من الدراسة إلى أن مستويات SAKL و MDA مرتفعة لدى مرضى ضخامة الاطراف، ويمكن استخدامها كماركرات جديدة في تشخيص مرض ضخامة الاطراف من النوع النشط. قد يكون قياس SAKL و MDA مفيداً، خاصة عندما يعطي GH و IGF-1 نتائج متضاربة.

### Introduction

Acromegaly is a chronic condition triggered by the excessive secretion of growth hormone (GH) and insulin-like growth factor 1 (IGF-1), with the primary underlying factor being the presence of a GH-secreting pituitary macroadenoma. Several studies showed that the mortality rate in acromegaly patients is two to four times higher than that in the general population with the same gender and age [1,2], which is mainly attributed to cardiovascular complications [3,4]. These complications likely stem from hypertension, glucose intolerance, and lipid irregularities, collectively representing the primary contributors to early atherosclerosis. Atherosclerosis, in essence, is a dynamic and advancing phenomenon resulting from endothelial dysfunction and inflammation. Oxidative stress (OS) is fundamental to its pathology, playing a crucial role in the process [5,6]. The OS is defined as an event resultant from the imbalance of magnitude between oxidizing substances and antioxidants [7,8]. Both substances (oxidants and antioxidants) are generated in oxidoreduction reactions, where oxidation and reduction correspond to gaining and losing electrons, respectively. Because generation and action of oxidants and antioxidants depend on this oxidoreduction system, many authors used the term redox system imbalance to refer to OS [9]. Malondialdehyde (MDA), oxidative reactions, were found to be an indicator of oxidative stress [10]. MDA is a very hazardous byproduct of the oxidation of lipids by free radicals. Malondialdehyde is harmful and has effects with proteins and phospholipids in both

reversible and irreversible ways [11]. Reactive species are identified as substances that perform lipid and glucose oxidation (lipoxidation and glycation, respectively). The products generated in lipoxidation are Malondialdehyde, glyoxal, acrolein, and 4-hydroxynonenal, and those generated in glycation are glyoxal and methylglyoxal. These compounds bind to amino acids, resulting in highly reactive final products of glycation and lipoxidation [12]. The SAKL, commonly known as Klotho, was first discovered to be a gene that prevents aging. In 1997, it has been found that, *α*-Klotho deficient mice have shorter life span than the normal mice [13]. The extracellular domain of transmembrane *α*-Klotho undergoes proteolytic cleavage and is released into circulation, despite the fact that it is mostly produced in the brain, kidney, choroid plexus, and parathyroid gland [14]. Up to now, many *α*-Klotho functions have been discussed. Klotho protein is known to exist in two variations with distinct functions. As a co-receptor for the fibroblast growth factor-23 (FGF-23), membrane-bound *α*-Klotho plays a crucial role in the renal function of FGF-23 and is a key regulator of phosphate homeostasis [15]. Traditionally, SAKL protein is a co-factor of FGF23, which controls the balance of phosphorus and vitamin D [16]. In this study, the concentration of IGF1, GH, lipid profile Malondialdehyde and soluble *α*-Klotho in patients suffering from acromegaly was estimated. Also, the correlation between MDA and SKL with biochemical parameters was investigated.

### Materials and Methods

This study was performed at National Diabetes Center / Mustansiriyah University from October to December 2022. The study included (90) subjects who were divided into two groups acromegaly patients (60) 30 male and 30 female undergoing treatment of (Sandostatin injection, Octreotide Acetate) the dose 20 mg once monthly and 30 subject healthy control (15 male and 15 female). The age range of the patients was between 30 to 60 years. Clinically, these patients were diagnosed with GH-secreting pituitary adenomas or pituitary adenomas, which was then confirmed pathologically. The SAKL was measured in the serum samples by the (enzyme-linked immunosorbent assay (ELISA) using Kit (MyBioSource, USA). The thiobarbituric acid technique of Buege and Aust was used to test MDA [17].

### Statistical Analysis

The data analysis was conducted by the software version (20.0 of the SPSS) program. The data represented as mean  $\pm$  SD. A t-student's test was calculated to determine the p-value.

### Results and Discussion

The data in Table 1 shows that acromegaly patients had higher levels of IGF-1, GH, FSG, TC, TG, LDL, VLDL, SAKL, and MDA compared to the control group. These differences were statistically significant, with a p-value less than 0.0001. However, it merits noting that HDL levels were higher in the control subjects, also with a p-value less than 0.0001.

**Table 1:** Comparison of assessed parameters among patient group and Control

Variables	Acromegaly patients (60) Mean $\pm$ SD	Control (30) Mean $\pm$ SD	P value
IGF-1 (ng/ml)	477.24 $\pm$ 263.82	261.23 $\pm$ 111.3	<i>P</i> < 0.01
GH (ng/ml)	5.82 $\pm$ 2.46	2.60 $\pm$ 1.25	<i>P</i> < 0.01
FSG (mg/dL)	146.93 $\pm$ 85.71	87 $\pm$ 13.4	<i>P</i> < 0.01
TC (mg/dL)	178.8 $\pm$ 40.31	154.95 $\pm$ 43.94	<i>P</i> < 0.01
TG (mg/dL)	145 $\pm$ 75.52	108 $\pm$ 23.6	<i>P</i> < 0.01
HDL (mg/dL)	40.23 $\pm$ 9.25	48.7 $\pm$ 7.22	<i>P</i> < 0.01
LDL (mg/dL)	94 $\pm$ 35.01	60.25 $\pm$ 25.26	<i>P</i> < 0.01
VLDL (mg/dL)	29.35 $\pm$ 14.87	21.49 $\pm$ 10.61	<i>P</i> < 0.01
Soluble $\alpha$ -Klotho (SAKL) (pg/ml)	1036.36 $\pm$ 195.09	788.21 $\pm$ 271.70	<i>P</i> < 0.01
MDA ( $\mu$ mol/l)	2.48 $\pm$ 1.19	1.25 $\pm$ 0.37	<i>P</i> < 0.01

IGF-1: Insulin like growth factor-1, GH: Growth hormone, FSG: fasting serum glucose, TC: Total Cholesterol, TG: Triglyceride, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low-Density Lipoprotein  
\*Highly significant *P* < 0.01

Table 2 shows that soluble  $\alpha$ -Klotho correlated negatively but not significantly with IGF-1, GH, FBG, TC, TG, LDL, and VLDL, with correlation coefficients of -0.162, -0.14, -0.089, -0.1, -0.09, -0.33, and -0.062 respectively. However, the correlation between soluble  $\alpha$ -Klotho and LDL was statistically significant, with a p-value less than 0.01. The only positive correlation found was between soluble  $\alpha$ -Klotho and HDL, with a correlation coefficient of 0.34, and this correlation was statistically significant, with a p-value of 0.0079. MDA correlated positively with IGF-1 and GH, with correlation coefficients of 0.147 and 0.045 respectively, but these positive correlations did not reach statistical significance, with p-values of 0.2621 and 0.7312 respectively. The MDA correlated with all the remaining variables (FBG, TC, TG, HDL, LDL and VLDL) was negative thus the correlation coefficients are (-0.059, -0.34, -0.26, -0.02, -0.36, and -0.016) respectively, the negative correlation were statistically significant for (TC, TG, and LDL) p value are (0.0079, 0.0448, and 0.0047) respectively but the negative correlation did not reach statistically significance between MDA and (FBG, HDL, and VLDL) p value were (0.6534, 0.8794, 0.9027) respectively.

**Table 2:** Correlation between (SAKL and MDA) with other variables in acromegaly patients

Variables	Soluble $\alpha$ - Klotho R	P-value	MDA R	P-value
IGF-1 (ng/ml)	-0.162	0.2175	0.147	0.2621
GH (ng/ml)	-0.14	0.2819	0.045	0.7312
FBG (mg/dL)	-0.089	0.495	-0.059	0.6534
TC (mg/dL)	-0.1	0.4546	-0.34	0.0079
TG (mg/dL)	-0.09	0.4807	-0.26	0.0448
HDL (mg/dL)	0.34	0.0079	-0.02	0.8794
LDL (mg/dL)	-0.33	0.0100	-0.36	0.0047
VLDL (mg/dL)	-0.062	0.6376	-0.016	0.9027

Acromegaly is characterized by high mortality mainly owing to cardiovascular complications, but it has been increasingly associated with other neoplasia that, together, may contribute to a higher mortality in people suffering from these complications. Up to 50% of individuals with acromegaly also have hyperlipidemia, which is primarily characterized by hypertriglyceridemia and decreased HDL values. The demonstrated (LDL) concentrations were found to be elevated or similar to those of normal patients [3]. The OS and endothelial dysfunction were fundamental mechanisms of atherosclerosis, and therefore contributed to an increase in cardiovascular disease incidence [18]. The MDA in the current study increased in acromegaly patients when compared with healthy control. These results agree with a study conducted by Abass *et al* [19], which found malondialdehyde higher levels in sera of Iraqi patients when compared with control. But, Bonini *et al.* found decreased MDA in acromegaly patients when compared with healthy control [20], the observed difference in antioxidant levels could be justified by a richer diet in these vitamins in the acromegaly group. Another potential explanation is that octreotide, which was administered to most patients (76%) in this study, possesses antioxidant properties. Previous research induced acute pancreatitis in Wistar rats using sodium taurocholate and then treated them with octreotide. This resulted in a significant decrease in MDA levels and significant increases in the activity of antioxidant enzymes (glutathione peroxidase and superoxide dismutase), indicating octreotide's ability to attenuate oxidative stress in pancreatitis. Therefore, octreotide usage in the current study's acromegaly patients may have conferred antioxidant effects, influencing the observed MDA and SKL levels [21]. Acromegaly is connected to elevated levels of OS, along with a decreased ability of antioxidants, and endothelial dysfunction, which is shown by low levels of nitric oxide (NO)

[22]. Insulin signaling is reduced by SAKL, which also controls calcium homeostasis. The FGF-23, a phosphaturic hormone that is co-receptor on the membrane -Klotho, elevated in acromegaly (despite increasing GFR and phosphate), indicating a condition of FGF-23 resistance [23]. Although full characterization is still pending, preliminary, data indicate that (SAKL) may have endocrine function [24]. The study found significant increases of SAKL in acromegaly patient when compared with control group, this result agrees with Coopmans *et al* [25] and Neidert *et al* [26], found significantly increased the levels of SAKL when compared acromegaly patients with control. The Neidert *et al* explain increased SAKL the A Disintegrin and Metalloproteinase' (ADAM) family members ADAM10 and ADAM17 (a-secretases) have been proposed as responsible enzymes. It is hypothesized that GH may control these enzymes' activity directly or indirectly through other factors or proteolytic activity that GH induces [26]. Recent research indicates that following a successful surgical procedure, soluble a-Klotho levels return to normal in patients with active acromegaly. This study, which is the first to include acromegaly patients from Iraq, supports prior results that there is a very significant difference in SAKL levels between individuals with active acromegaly and other groups [27-28]. The study conducted by Przybyłowska showed SAKL was the highest in the (active acromegaly group (AA) and decrease in a (surgically cured acromegaly group (SCA)) [29].

## Conclusions

The study found that in acromegaly patients, levels of SAKL and MDA were elevated. The oxidative stress (malondialdehyde) may be useful in identifying endocrine disorders such as acromegaly. The SAKL and MDA may potentially serve as novel biomarkers for active acromegaly. Measuring levels of SAKL and MDA could be useful, particularly when GH and IGF-I tests provide conflicting or ambiguous results. Increased level of lipid profile (TC, TG, LDL, and VLDL) in acromegaly patients

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