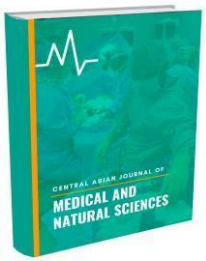

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Article

Evaluation of Klotho Protin Levels in Patient with Heart Disease in The City of Tikrit

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Abstract: This study investigated the relationship between soluble Klotho protein levels and acute coronary syndrome (ACS) in Iraqi patients. Klotho is an anti-aging protein with anti-inflammatory and cardiovascular protective properties, and reduced levels have been linked to several cardiovascular diseases. A case-control study was conducted between November 2023 and March 2024. The study included 120 participants, divided into four groups: healthy controls, patients with unstable angina (UA), ST-elevation myocardial infarction (STEMI), and non-ST-elevation myocardial infarction (NSTEMI). Blood samples were collected to measure serum Klotho levels using the ELISA technique, while inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were also evaluated. The results showed that serum Klotho levels were significantly lower in all ACS patients than in the healthy control group. Among the ACS subgroups, NSTEMI patients had the lowest Klotho levels. A significant negative correlation was found between Klotho and ESR only in the NSTEMI group, indicating that increased inflammation was associated with decreased Klotho concentrations. No significant relationship was observed between Klotho and CRP or other inflammatory markers in the remaining groups. The findings suggest that reduced Klotho levels are associated with acute coronary syndrome and may reflect increased inflammatory activity. Therefore, Klotho may serve as a promising biomarker for cardiovascular disease severity and progression. However, further large-scale, multicenter studies are recommended to confirm these findings and to investigate the potential therapeutic role of Klotho in patients with cardiovascular diseases.

Keywords: Klotho Protein, Acute Coronary, ELISA

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1. Introduction

The concept that the heart is the source of the body's heat, as well as the increasing use of human preparations for its heart, has led to Arvis's evidence that the heart is merely a muscular pump. Understanding the structure of the abnormal organ has allowed us to understand the pathogenesis of heart disease [1]. This anatomical knowledge was supplemented by pathological anatomy when pathology was established in the nineteenth century by Virchow. The development that occurred in the electron microscope in the twentieth century and new insights in the fields of biochemistry and biophysics led to a clearer understanding of prenatal diseases. Today, there are additional insights regarding the underlying structure and molecular basis of cardiovascular diseases due to the significant advances that have occurred in molecular biology.

The term that includes diseases and problems that occur in the heart is called heart disease. Some reports released by the World Health Organization state that about 12

million people die annually from heart disease. In addition to this, half of the deaths in the United States and developed countries of the world are due to cardiovascular disease, and it is the main cause of death for residents of developing countries. As for deaths in adults, it is the main cause. In various countries, including India, heart disease is the main cause of death. It has been noted that every 34 seconds a person dies from heart disease in the United States. Coronary artery disease, cardiomyopathy, and cardiovascular disease are some categories of heart disease. Conditions that affect the heart and blood vessels and the way blood is pumped and distributed throughout the body are included in the term "cardiovascular disease." Cardiovascular disease (CVD) causes various morbidities, disability, and death.

Despite advances in prevention, in many parts of the world, there is an increasing burden of cardiovascular disease. However, it is not possible to do more than that to address the risks of its development in society and in various specialized clinics. It is currently still fragmented and not linked to constructive lifestyle advice. Identifying and treating people who have an increased chance of developing cardiovascular diseases or diabetes. Or conditions such as fatty liver disease. From this perspective, a more consistent approach to weight control is what we advocate. Along with the comprehensive assessment of cardiovascular risk, we offer patients a variety of simple, evidence-based lifestyle choices. Or more intense, that is, sensitive and encouraging. Through repeated attempts and willingness to accept failure.

Like type 2 diabetes, cardiometabolic risk refers to the sum of risk factors that increase a person's risk of experiencing a cardiovascular event or developing metabolic disorders (see Figure 1). This risk arises from factors such as high blood pressure or smoking, which are traditional cardiovascular risk factors, from factors such as insulin resistance, which are non-traditional risk factors, and from factors that are not fully understood, both genetic and clinical. Cardiometabolic syndrome, an interesting collection of cardiovascular, renal, metabolic, prothrombotic, and maladaptive inflammatory abnormalities, is now recognized by the American Endocrine Association, the National Cholesterol

Education Program, and The World Health Organization and many other organizations recognize it as a disease entity. Individually and interconnected, cardiovascular and metabolic disorders significantly increase the incidence and mortality of cardiovascular disease (CVD), making CVD a well-established and important risk factor for premature cardiovascular disease, stroke, and critical illness. Well-established and advanced treatment strategies have proven that what helps in reversing these abnormal reactions and reducing the risk of infection with the MCV virus is adherence to moderate physical activity, weight loss, careful control of blood pressure, correction of dyslipidemia, and control of blood sugar.

Acute coronary syndromes Acute myocardial infarction (AMI) is the leading cause of death worldwide due to its rapid progression and high mortality rate. [2] Acute coronary syndrome is a group of conditions caused by reduced or closed blood flow through the coronary arteries. Acute coronary syndrome (ACS) is a medical crisis that requires immediate intervention. ACS includes any narrowing or blockage of the coronary arteries that leads to acute symptoms. Acute coronary syndromes include:

ST elevation myocardial infarction (STEMI)

Non ST elevation myocardial infarction (NSTEMI)

Unstable angina There are two main coronary arteries: the left coronary artery and the right coronary artery (Figure 1). The left side is divided into the anterior descending circumflex artery and the left circumflex artery, which supplies blood to the left side and front of the heart. The right coronary artery divides into the posterior border acute artery and the posterior border descending artery, the right atrium, right ventricle, SAN and AV nodes, and part of the left ventricle, they supplied by blood by it [3]

Figure 1. The coronary arteries Coronary arteries arise from the aorta and supply blood and oxygen to the heart muscle. The dominant vessel is the artery that supplies the posterior descending artery and one or more posterolateral branches. The main vessel in 80-85% of cases is the right coronary artery, and in some other cases, it contains a dominant or left system. This, in turn, may lead to narrowing or blockage in these arteries, reducing or preventing blood flow to parts of the heart outside the area of narrowing or blockage, which in turn may lead to the development of vascular syndrome [3]

Types of acute coronary syndromes Acute coronary syndrome (ACS) is the leading cause of death and disability worldwide.1–3 Of the three phenotypes that comprise ACS, ST-elevation myocardial infarction (STEMI), infarction non-ST elevation myocardial infarction (NSTEMI) and unstable angina (UA), the latter two diseases are widely believed to exist on a spectrum with common manifestations, pathophysiology and consequences in patients. Therefore, they are grouped to control compliments in

Practice Guideline which currently does not include ST-ACS Fractional Elevation. Angina is pain in or near the chest caused by severe but temporary pain. , absence of blood (ischemia) in a segment of myocardium, hence the term myocardial ischemia.For stable angina, the most important features are that the pain is caused by a specific stimulus and relief occurs within a few minutes after the stimulus ends. Angina can be classified as follows [4]:

- Stable angina
- Unstable angina
- Variant angina (Prinzmetal's), coronary artery spasm (CAS), a rare disorder[5]

Unstable Angina Unstable angina results from an imbalance between myocardial oxygen supply and demand. Perhaps the most common cause is myocardial hypoperfusion due to non-occlusive thrombosis on a fissured or eroded atherosclerotic plaque that previously typically caused only mild to moderate obstruction[6]. Clinical conditions in which unstable angina, defined as angina in the presence or absence of other conditions (such as anemia, fever, hypoxia, tachycardia, or thyrotoxicosis) or angina, develops within 2 weeks of acute myocardial infarction. The presence or absence of abnormalities in the electrocardiogram. Surprisingly, the prognosis is quite variable given the heterogeneity of the clinical manifestations of unstable angina. They often appear similarly to patients with unstable angina and those with non-Q wave myocardial infarction, and distinguishing between the two is only possible after several hours or days. When cardiac enzyme test results are available. Because many of the clinical trials discussed in this article enrolled patients before enzyme values were available, these studies were in fact examining both clinical entities.

Arterial lesions in patients with unstable angina often present with complex and eccentric morphological features on coronary angiography. These features are thought to represent plaque rupture with superimposed thrombus.

Coronary atherosclerotic disease is the underlying cause of unstable angina in most patients with acute myocardial ischemia. The most common cause of unstable angina is narrowing of the coronary arteries due to a thrombus that develops on a ruptured and unoccluded atherosclerotic plaque. A less common cause is coronary artery spasm (variant of Prinzmetal's angina) [7]. Endothelial or smooth vascular dysfunction causes this vasoconstrictionAUs represent <10% of patients with acute coronary syndromes in the modern setting. Based on pathophysiological similarities, this is an ideal part of a comprehensive myocardial infarction (MI) endpoint. By adding UA as a component of the primary composite endpoint, the number of events and feasibility of the trial is increased, thereby reducing the size and cost of the trial [8]. Furthermore, UA has both economic and quality of life implications, at the societal and individual levels. However, using AU as an endpoint poses significant challenges.With the advent of high-sensitivity troponin, the

number of people diagnosed with UA has dropped to quite low levels, with a corresponding increase in the number of MIs [9].

ST-elevation myocardial infarction (STEMI) Non-ST-elevation myocardial infarction (NSTEMI) and persistent ST-elevation myocardial infarction (STEMI) are characterized by ischemic symptoms, electrocardiographic changes, elevated serum troponin, and markers Other biology indicates myocardial damage. ST elevation (STE) is considered to reflect acute transmural ischemia due to occlusion of the epicardial coronary artery by a blood clot. Therefore, it is recommended that patients with suspected acute STEMI and no contraindications [10] should be treated as soon as possible to recanalize the obstructed artery by primary percutaneous coronary intervention (pPCI) or thrombolysis. fibrin. In contrast, guidelines recommend conservative initial treatment for patients with suspected MI without STE, as ongoing ischemia may not be present and previous studies have not shown a benefit. benefit of reperfusion therapy in patients without ETS [11].In most people without pre-existing heart disease, the ST segment is at the level of the anterior P-R segment and/or the posterior T-P segment (called isoelectric). ST segment deviation (increase or decrease from baseline) may be a sign of myocardial ischemia. However, deviation of the ST segment from the isoelectric line due to non-ischemic causes is commonly observed. ST-segment elevation due to non-ischemic causes has been reported in to 15% of the general population. There are individuals with pre-existing ST-segment elevation secondary to non-ischemic causes (eg,for example, left ventricular hypertrophy or ―premature repolarization‖ may further develop acute MI (ST-segment elevation myocardial infarction or non-STEMI (NSTEMI)); therefore, the presence of benign NISTE forms does not always rule out acute coronary syndrome (ACS) and even STEMI[12]. ST-segment elevation myocardial infarction is a clinical syndrome that reduces the typical symptoms of acute myocardial ischemia associated with ST-segment elevation and increased concentrations of Blood biomarkers suggest myocardial necrosis. According to these guidelines, pPCI is recommended for people with symptoms suggestive of myocardial ischemia that began 12 hours or less before medical consultation and who present with ST elevation. Many patients with typical symptoms have ST-segment elevation due to causes other than ischemia [13].

Non-ST-elevation myocardial infarction (NSTEMI) Over the past 25 years, the diagnosis and treatment of patients with unstable coronary artery disease, or later defined as non-ST-segment elevation acute coronary syndrome, has changed significantly. In the 1990s, the disease was identified primarily based on symptoms of chest pain and signs of ischemia on the resting electrocardiogram (ECG). The definition gradually changed primarily based on a transient elevation of cardiac-specific troponin that meets the current definition of non-ST-elevation myocardial infarction (NSTEMI). Non-ST-elevation myocardial infarction (NSTEMI) and unstable angina (UA) are widely considered to exist along a spectrum with distinct physiological characteristics. disease and overall patient outcomes separately [14,4] ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) are types of AMI and comparison between the two is important. subject of extensive research. The pathophysiology and severity of STEMI and NSTEMI are different, so the prognosis is also different. For patients with non-ST-segment elevation myocardial infarction (NSTEMI), evidence from international studies shows that care, treatment and diagnosis following instructions is associated with better clinical outcomes [15,16]. Evidence from randomized controlled trials shows that the absolute efficacy of NSTEMI is greater in cases with high ischemic risk, reducing mortality and thus planned revascularization rates. , stroke, and hospitalization for heart failure were lower. However, it remains unclear whether, outside the trial setting, the effects of such interventions on NSTEMI (including both pharmacotherapy as well as invasive coronary treatment strategies) are evident. , and if so, whether these effects persist after discharge from the hospital. The NSTEMI group had a higher postdischarge mortality rate during the 3-year clinical follow-up period than the

STEMI group; however, older age and renal dysfunction were identified as major risk factors for long-term mortality in the STEMI group. These results suggest that patients with NSTEMI should receive more intensive medical treatment and undergo regular clinical monitoring to prevent ischemic heart failure compared with those with STEMI [17,18]

Erythrocyte sedimentation rate (ESR) ESR measures the rate at which red blood cells decrease or deposit in the plasma of an anticoagulated blood sample randomly collected over a specified period of time (usually 60 minutes) in millimeters (mm)/hour; however, newer methods that involve centrifugation can produce results in about 5 minutes. This phenomenon was first observed in 1897 by Dr. Edmund Faustyn Biernacki, who discovered that the rate at which blood was delivered varied between individuals and red blood cells (erythrocytes) stabilized more rapidly in the presence of increased fibrinogen concentrations. Erythrocyte sedimentation rate (sedimentation rate, sedation rate and abbreviated ESR) is a common hematology test that can indicate and monitor increased inflammatory activity in the body due to one or more conditions. conditions such as autoimmune diseases, infections or tumors. ESR is not specific to any particular disease, but is used in combination with other tests to determine the presence of increased inflammatory activity. ESR has long been used as a ―disease indicator‖ due to its reproducibility and low cost.Over the decades, several methods have evolved tperform the test. However, the reference method for measuring ESR proposed by the International Committee for Standardization of Hematology (ICSH) is based on results described by Westergren a century ago. Newer automated systems using sealed blood collection tubes and automated readers have been introduced into the laboratory to reduce biological risks to the operator and reduce the time required to perform ESR. The ESR test measures the rate at which red blood cells (RBCs) or red blood cells in a whole blood sample fall to the bottom of a Westergren tube. This ―falling‖ process is called deposition red blood cells often decrease more rapidly in people with inflammatory diseases such as infections, cancer, or autoimmune diseases. These conditions lead to an increase in the amount of protein in the blood. This increase causes red blood cells to stick together (cluster) and stabilize more quickly. A group of red blood cells gathered together will form a pile (similar to a stack of coins) called a roller (meaning pleural roller). Roll formation is possible due to the special disk shape of the red blood cell.The flat surface of red blood cells allows them to come into contact with other red blood cells and stick together [19]. Elevation in ESR in severe forms of coronary heart disease (CAD) can be considered as an indicator to predict coronary heart disease mortality. . However, few studies shed light on the added value of ESR to improve risk prediction and risk-based treatment in STEMI. ESR is a strong predictor of cardiovascular mortality and adverse clinical outcomes in STEMI. patients undergoing primary PCI after adjustment for traditional cardiovascular risk factors.Additionally, ESR improved the ability to predict GRS in relation to mortality of STEMI patients. Clinical studies show that ESR and hs-CRP are associated with an increased risk of myocardial infarction and stroke in the general population [20] especially in patients with chronic disease, chronic inflammation. [21]. Notably, a large amount of published data indicates that hs-CRP is significantly associated with the severity of coronary artery stenosis and the prognosis of patients with ACS and may even improve some risk stratification[22].

Soluble Klotho The systemic effects of α-Klotho appear to be primarily mediated by its circulating form, soluble Klotho protein (s-Klotho), which is expressed primarily in the kidney and to a lesser extent in the brain, skeletal muscle, bladder, aorta, gonads, and thyroid [23]. The α-Klotho gene has been described as a ―suppression of aging‖ gene in mice, encoding a protein, s-Klotho, with multiple pleiotropic effects. A defect in mouse α-Klotho gene expression results in shortened lifespan and a progeroid phenotype associated with many aging-like disorders in humans [23]. This phenotype includes organ and skin atrophy, infertility, atherosclerosis, vascular endothelial dysfunction, vascular

calcification, cardiac hypertrophy, decreased bone density, sarcopenia, impaired glucose metabolism and cognitive disorders. In contrast, overexpression of the α-Klotho gene significantly extended the lifespan and health of mice [24]. S-Klotho functions as a humoral factor by binding to its cell surface receptor and as an active enzyme on the cell surface. activating (e.g., calcium channel proteins) or inhibiting (e.g.,g. phosphate transporters), cell surface receptors, and enzymes in some tissues and organs [25].

In humans, s-Klotho has been shown to improve renal function [26] systemic phosphate homeostasis and glucose metabolism [27], to inhibit the insulin/IGF-1 signaling pathway, which acts as a co-receptor for fibroblast growth factor 23 [28], to reduce intracellular oxidative stress and chronic inflammation [24,29]. Therefore, the effects of s-Klotho on lifespan and lifespan may stem from its metabolic functions such as increasing Blood phosphate, oxidative stress, chronic inflammation, unbalanced glucose metabolism, decreased IGF-1 levels. accelerated the aging process in people [30]. Thus, s-Klotho levels have emerged as a biomarker of healthy aging, inversely associated with chronological age and several age-related diseases in model organisms and in humans and is an attractive target for intervention studies [29]. This is especially important because life expectancy has increased significantly in developed countries over the past century, leading to increasing rates of chronic diseases [31]. Oxidative stress and chronic inflammation are positively associated with age-related conditions such as type 2 diabetes and cardiovascular disorders [32], common metabolic diseases worldwide, with prevalence expected to increase. Due to its anti-inflammatory and antioxidant properties, s-Klotho is expected to play a protective role against cardiometabolic diseases [33], for example by maintaining homeostasis of the endothelial wall. and integrity, improving vascular calcification, improving kidney function or insulin sensitivity.However, the possible role of s-Klotho as an early marker of cardiometabolic risk remains unproven [34].

Previous research has shown that Klotho gene polymorphisms in humans are associated with multiple cardiovascular events and metabolic syndrome as well as cardiometabolic risk factors, such as reduced HDL levels and hypertension. Furthermore, s-Klotho levels have been shown to be inversely associated with the incidence of cardiometabolic disease in adults aged 24–102 years or with stress-induced cardiac hypertrophy and remodeling [24]. On the other hand, one study found no relationship between s-Klotho levels and hypertension or arterial stiffness in the general Chinese population [16]. Recently, another study showed that s-Klotho levels were inversely associated with cardiovascular risk levels and markers of insulin resistance in a group of healthy men and women. strong from 40 to 65 years old, but not young people from 18 to 25 years old. This suggests that s-Klotho may serve as a marker of cardiometabolic status in middle-aged but not very young individuals [35].S-Klotho levels have also been shown to be associated with early signs of atherosclerosis such as carotid intima-media thickness, flow-mediated dilatation of the brachial artery, and epicardial fat thickening in healthy adults with a mean age of 32 years. , however a relatively small sample (N = 50). [36]. S-Klotho levels were also shown to be inversely associated with the likelihood of developing high blood sugar and hyperglycemia in 80 men aged 40 to 80, years [37]. To date, it has been shown that s-Klotho levels are lower in individuals with cardiometabolic disease or are associated with cardiometabolic risk in individuals with the disease. cardiovascular metabolism. but to our knowledge, no studies have investigated the relationship between s-Klotho levels and physiological markers of cardiometabolic risk in young, middle-aged men. If s-Klotho levels are a reliable marker of current and future health in relatively young people, it could help identify individuals at higher risk of age-related disorders and can be used as a measure of relative fitness, predicting disability later in life. Studies of early signs of age-related disorders are especially important for early interventions in Western populations, where life expectancy has increased in recent decades, but The basic aging process remains unchanged. Studies of anti-aging factors in middle-aged or elderly populations are influenced by the fact that the majority of participants already have some

form of age-related disease, which can affect levels of these factors [38]. The age of onset of age-related cardiometabolic disease is decreasing in Western countries, increasing in the thirties. The onset of these disorders can occur even 9 to 12 years before clinical diagnosis. It is therefore important to identify early signs of these diseases (and the risk of their development) for prevention purposes, as they may no longer be diseases that primarily affect older people. Cardiometabolic risk was assessed using biomarkers. because of the risk of developing cardiovascular problems, impaired glucose tolerance and liver dysfunction. Because s-Klotho levels are reduced in people with kidney damage [4], the possibility of kidney dysfunction based on estimated glomerular filtration rate (eGFR) and creatinine levels should also be monitored. There were controls for factors that appear to be related to s-Klotho levels and cardiometabolic risk, such as chronological age [39], body composition [40], physical activity, testosterone levels , alcohol consumption [41].

2. Materials and Methods

The Study Population

During the time period of novembe 14, 2023, through march 12, 2024, the Department of Chemistry and Biochemistry at the College of Medicine/Al-Nahrain University collaborated with the Department of Biochemistry Laboratory at Al Imamain Alkadhimain Teaching Hospital and the Hospital of Cardiology in Missan to conduct a case-control study. A total of 120people who had ACSs participated in the research. The study group divided into:

1: 30 patients with UA

2: 30 Patients with STEMI.

3: 30 Patients with NSTEMI

Inclusion Criteria All adult patients were diagnosed with acute coronary syndrome. A primary positive ECG is used to diagnose ACS. and clinical diagnosis by internal medicine and cardiology specialists.

Exclusion Criteria

1. Pregnancy
2. Chronic kidney disease
3. Liver disease.
4. Stroke.
5. Sepsis.
6. Pulmonary embolism.
7. Cancer.
8. Diabetes mellitus.

Blood Samples

10 ml of venous blood was collected from each participant; 3 ml of which was kept in EDTA tube and the other 7ml after separation to serum, serum resulted was divided into two tube. The latter was preserved at -20 oC until be used. As in table 1

Materials

Laboratory Instruments and Equipment

The laboratory instruments equipment used in this study are listed in table 1.

Table 1. Equipment and Instruments Used in the Study

Equipment and instrument	Company/country
Apple spectrophotometer	Japan
Copas C11 Auto analyzer	Hitachi/Netherlands

Deep Freezer (-20°C)	Japan
ELISA Reader	SINNOWA, Germany
Eppendorf tubes	Germany
ESR analyzer	Vital / Netherland
Micropipette	Dragon/Germany
Nanodrop/UVS-99	Germany
Ohaus centrifuge	Germany
Safety cabinet	China
Shaker	MX/China
U. V. transilluminator and camera	Bioneer/Korea
Vortex	Germany
Water bath	HH/2 / China

Diagnostic Kits

The main diagnostic kit that used throughout this study are shown in table 2.

Table 2. Kits used in the study

Kits	Company
Klotho kit	Cloud – clone corp. /USA

Methods

Detection of Klotho Klotho-specific antibodies are pre-coated on the microchip included in this kit. Then, a klotho-specific biotin-conjugated antibody was added to the relevant microplate wells after addition of standards or samples. Then, to each microplate well, we added horseradish peroxidase (HRP)-conjugated avidin and incubated the plates. Addition of TMB substrate solution caused a color change only in wells containing klotho, a biotin-conjugated antibody, and enzyme-conjugated avidin . Sulfuric acid solution was used to block the enzyme-substrate reaction The resulting substance and color change were detected spectrophotometrically at wavelengths from 450 nm to 10 nm.The amount of klotho in the samples was calculated by comparing their optical density with the reference curve.

3. Results

Calculation of results

Sex: A total of 120 people participated in our study, divided into four groups; control group, unstable angina, STEMI and NSTEMI, each group 40 people. The studied group range had the same male/female ratio (1:1) without statistical significance (p value > 0.05). Table 3-1 and Figure 3 show the gender distribution between groups.

Table 3. Sex distribution of the study population

Sex		Control	Unstable angina	STEMI	NSTEMI	Total	P value
Female	Count	15	17	14	13	60	0.977
	%	25 %	30%	23.3%	21.6%	100%	
Male	Count	15	13	16	17	60	
	%	25%	21.6%	28.3%	26.6%	100%	
Total	Count	30	30	30	30	120	
	%	25.0%	25.0%	25.0%	25.0%	100%	

Age

The mean age of the study population was 56 year (± 9.7 SD) and it is not significantly varied among the groups (p value >0.05) as table 4 shows.

Table 4. Age distribution of the study population

Age (years)	No.	Mean	Std. Deviation	P value
Control	30	54.2	8.5	0.121
Unstable angina	30	57.7	11.5	
STEMI	30	55.5	10.1	
NSTEMI	30	56.7	8.9	

The average zero standard optical density was removed from the average of the duplicate standard, control, and sample readings. The concentration of klotho was plotted against absorbance in the form of a standard curve. Using regression analysis, we were able to draw a curve that best fits the data. As in table 5 and figure 1

Table 5. Klotho Standard curve

STD.(ng/ml)	OD -1
10	1.2
5	0.78
2.5	0.57
1.25	0.452
0.625	0.419
0.312	0.367
0.156	0.295

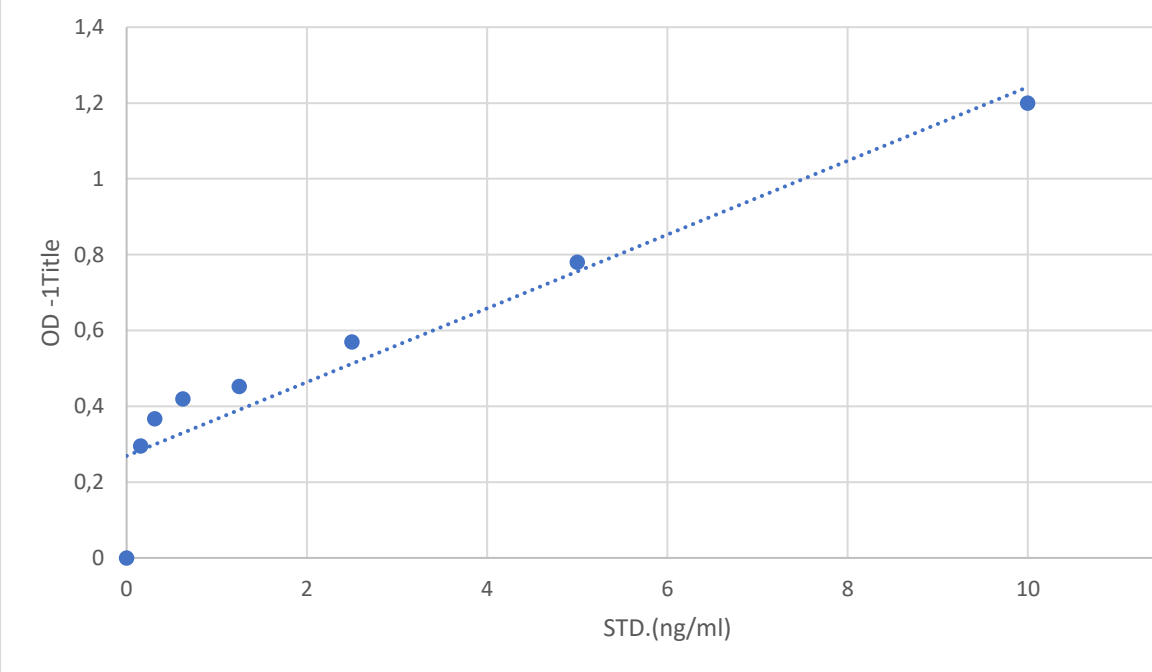

Figure 1. Standard Curve for Klotho

Detection Range

(0.156-10ng/mL).

The standard curve concentrations used for the ELISA's were 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156 ng/mL.

The main study outcome

The mean of klotho in all ACS groups; UA (472.8 ±238.3), STEMI (371.8 ±198.5 SD), NSTEMI (355 ±167.9 SD) was lower than normal range and that among control (686 ±125.9 SD) (p value <0.05). as in table 6,7

Table 6. Difference of biochemical markers means among groups by ANOVA test

Biomarkers	Status	No.	Mean	Std. Deviation	p value
Klotho	Control	40	686.0	125.9	<0.05
	Unstable angina	40	472.8	538.3	
	STEMI	40	371.8	398.5	
	NSTEMI	40	355.0	167.9	
	Total	160	471.4	361.6	

Table 7. Post hoc test for biochemical markers, mean difference within groups

Dependent Variable	Status	Status	P value
Klotho	Control	Unstable angina	0.011
		STEMI	0.176
		NSTEMI	0.249
	Unstable angina	STEMI	0.681
		NSTEMI	0.565
	STEMI	NSTEMI	0.998

4. Discussion

Relationship of Klotho with inflammatory markers among ACS patients:

Linear regression analysis shows significant correlation only between klotho value and ESR values among NSTEMI group. For each point increase in ESR, there was about 7 points decrease in klotho value and this model explains 23.4% of the noticed correlation (p value 0.007) (figure 1). No statistical significance was noticed in inflammation markers among other ACS groups (p value > 0.05) as in Table 8.

Table 8. Correlation of klotho with inflammation markers among ACS groups

Dependent Variable: Klotho

Dependent Variable: Klotho		R square	P value
Unstable angina	CRP	0.079	0.123
	ESR	0.000	0.961
STEMI	CRP	0.008	0.639
	ESR	0.014	0.527
NSTEMI	CRP	0.019	0.466
	ESR	0.234	0007

Some study recently studied the relation of Klotho with ACS [42].

Preset case--control study will deal with the relationship of Klotho with ACS in a sample of Iraqi people.

Demographic and clinical characteristics of the study groups

Age The mean value of age in the present study between the ACS and control groups, respectively, was 56 years (±9.7 SD) and did not differ significantly between groups (p--value > 0.05), as presented in Table 4. This is consistent with previous research . The risk of coronary artery disease is high at ages > 45 years old. also performed a comprehensive study of ACS patients from 25 European and Mediterranean countries, showing that older age remains an important predictor of higher in--hospital mortality . for all types of ACS.In particular, heart failure significantly complicates the hospital stay of elderly people with ACS. Four out of five deaths occurred in patients under 65, suggesting that better hospital treatment for older patients could save a significant number of lives. Young people have a relatively low mortality rate. To determine the extent of this possible benefit, more precise information on comorbidities and contraindications is needed [43].

Klotho By comparing klotho levels between control and ACS patients, it was found that klotho in the control group was significantly lower than that in the ACS group, and this result was consistent with previous research showing: low-grade inflammation can serve as a substrate for the onset and progression of atherosclerotic damage characterized by blood concentrations of various acute-phase proteins and inflammatory cytokines, as well as as infiltration of immune cells into vascular tissue. Tumor necrosis factor alpha (TNF) and interleukins are two examples of inflammatory cytokines involved in the initiation and progression of vascular damage. Interestingly, inflammatory cytokines can inhibit Klotho levels. S-Klotho was significantly inferior to the control group in a case-control study of 110 people with a history of cardiovascular disease [42]. When it comes to albuminuria and reduced kidney function, people with advanced diabetic kidney disease have the lowest Klotho levels. Additionally, significantly lower serum Klotho levels were observed in patients with acute coronary syndrome compared with controls [44]. Soluble Klotho deficiency is a new risk factor for cardiovascular disease in people with chronic kidney disease. More severe cardiac hypertrophy and fibrosis are associated with hyperphosphatemia and reduced Klotho solubility.Surprisingly, pathological cardiac remodeling was associated with higher FGF23 levels only in the presence of Klotho deficiency. However, PLC signaling activates exogenous FGF23 in cardiomyocytes and, independently of Klotho, produces ventricular hypertrophy. Klotho, independently of FGF23, has been proposed to prevent cardiac hypertrophy by inhibiting transient receptor potential channel 6 activity in cardiomyocytes.

5. Conclusion

Conclusion Klotho is reduced in patients with ACS and this is because this marker has an anti-inflammatory effect and when reduced it allows an increase in inflammatory factors and may lead to a prognostic effect on atherosclerotic effects .

Recommendation

1. Increase the number of patients from different centers to ensure the results of SNP research.
2. Introduce a therapeutic study to demonstrate the role of drugs in the effects of SNPs.
3. Comparison of outcomes with and without therapy in the ACS study.

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