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Article

Estimation of the Level of Klotho Protein, Hemoglobin, and D- Dimer Factor in Obese Persons in Salah Al-Din Governorate

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Abstract: This study was conducted to determine the effect of obesity on vital biomarkers (cloth protein, MCHC, and D-dimer) by using 60 blood samples consisting of 40 samples from an obese group and 20 samples from a healthy group. Whether it was significantly lower in obese than in healthy individuals clofaslan D-dimer was assayed, and appeared to be higher (all p These changes suggest that obesity is associated with increased inflammation and oxidative stress, impaired oxygen transport, and a higher tendency for thrombosis. Hence, this study validates that obesity has a negative impact on various key physiological functions and is associated with an increased risk for cardiovascular diseases and blood disorders.

Keywords: Obesity, Hemoglobin, Klotho Protein, D- Dimer.

Introduction

Obesity is a chronic, complex, multifactorial metabolic disease that develops from the confluence of genetic, biological, environmental, behavioral socio-cultural and economic factors. Obesity is a chronic disease that calls for life-long treatment and has many health consequences. Direct effects arise from excess adipose tissue and increase in body weight per se, leading to orthopedic disease (osteoarthritis), low back pain, obesity hypoventilation syndrome with or without obstructive sleep apnoea and asthma. Indirect damage is correlated with the metabolic disorders of obesity, which can manifest as hypertension, dyslipidaemia, cardiovascular disease (CVD), type 2 diabetes, metabolic-associated fatty liver disease (MASLD), chronic kidney disease (CKD), gallbladder disease, gout and certain cancers and reproductive problems. Importantly, obese patients were more vulnerable to severe infection, as it was rather well illustrated during the COVID-19 pandemic and are at increased risk of post-COVID syndrome due to their chronic systemic inflammation associated with obesity-related cardiac and metabolic disorders [1], [2].

Obesity is a medical condition in which the excess body fat accumulates. It adversely affects health and is evaluated by the Body Mass Index (BMI), which quantifies fat distribution based on the waist-hip ratio [3]. Directly due to obesity or indirectly related as in case of poor diet, may lead to complication like diabetes [4], hypertension, dyslipidemia (high cholesterol and triglyceride level).

The prevalence of overweight among children as well as adolescents increased nearly four-fold from 1990 to 2022, and the prevalence of obesity in adults more than doubled. The disease burden attributable to overweight and obesity also increased (annual percentage change 0.48%) during this

time:173 million disability-adjusted life years (DALYs) were attributed to overweight and obesity in 2021, corresponding to 9·5% of all global DALYs and with an interestingly similar numbers between high-Middle-Income countries (63 m). In light of the increasing prevalence of obesity in low- and middle-income countries (LMICs), the problem of undernutrition has not been completely solved, resulting in a double burden of malnutrition emerging whereby populations experience both undernutrition and obesity [5 – 6]. In fact, the authors highlight that obese people are at 37% increased chance of being hospitalized and their life expectancy is decreased by approximately 3 years, while severe obesity can threaten life expectancy like cigarettes: reducing it by a factor of about seven to eight to ten years. Also, people with obesity are more likely to suffer from depression and anxiety, have fewer employment opportunities, and experience higher discrimination and social stigma levels [7].

Klothoprotein is either a solubilized or membrane-bound anti-aging protein that is critical to the protective activity necessary for organ function, the incidental incorporation of a mutant gene produced genetically modified mice manifesting multiple features associated with aging-related disorders [8]. Klotho-deficient mice express phenotypes that resemble human aging. The Klotho protein is a receptor for fibroblast growth factor and predominantly found in the kidneys and brain, participating in endocrine homeostasis. Moreover, Klotho inhibition of glycemic insulin/insulin-like growth factor signalling pathway regulates oxidative stress and attenuates cells death [9].

Correlations have been reported between serum Klotho and classic cardiovascular risk factors, as well as a clinical history of cardiovascular disease [10]. Klotho deficiency suggest that these processes are critical in CVD, CAD, atherosclerosis, myocardial infarction and left ventricular hypertrophy. Thus, Klotho may be key either through its signaling pathways or through regulation of appropriate cellular metabolism for cardiovascular protection [11]. Klotho, a potent inhibitor of apoptosis, fibrosis and cellular senescence is also a strong autophagy promotor [12]. Klotho suppresses insulin and lipid-growth factor signaling [13]. Klotho exerts protective actions on the cardiovascular system and has been associated with perivascular fibrosis [14].

Materials and Methods

collection Samples

Between December 3rd, 2025 and March 20th, 2026, sixty blood samples were taken. Samples were obtained from Tikrit Teaching Hospital and private medical laboratories in Tikrit after correctly diagnosed by specialist doctors depending on clinical signs and other pathological tests. The samples were separated into two groups based on the diagnosis of the patients: patient (40 samples) age 22-75 years old including obesity cases and control group (20 samples) age 22-75.

Collection and preservation of blood samples

Ten milliliters of blood were extracted from each group via a medical syringe and pooled into 6 ml gel-lined plastic tubes. The blood was subsequently subjected to centrifugation at 4000 x g for 15 minutes. A fraction of the serum that was separated immediately after, was put in a plastic tube (Bendroff tube). The concentration of hormone and protein was measured using this tube stored at −20 °C. The remaining 2 ml of blood was collected in tightly sealed plastic tubes containing EDTA to perform a blood count. Finally, a further 2 ml of blood was added to the tube dedicated for D-dimer analysis.

Body Mass Index (BMI) [15]

Calculations

Use the following mathematical relationship:

$$\text{BMI} = \frac{\text{Kg}}{(\text{m})^2}$$

D – Dimer [16]

Assay Procedure

1. Set the device and at least 60 minutes before this test (serum) samples to room temperature, but serum in a capillary is also heated to room temperature.
2. Hold the capillary tube from both ends and shake, thus mixing the sample.
3. Inject the reagent in the cuff containing aminomethane buffer (Tris(hydroxymethyl)) 30 mM/L.
4. Hold the rootlessiscel ReAgent in a single capillary tube with your sample for at least 3 to 5 seconds, as this is time required to prevent evaporation.
5. Insert the capillary tube in apparatus Results will be shown on screen 7 mins later and printed automatically.

Quantitative measurement of Klothoprotein in serum: [17]

A- Basic Principle

Klothoprotein levels were measured using a commercially available assay kit purchased from Bioassay Technology Laboratory China. They are known to be enzyme-linked immunosorbent assays (ELISAs). The plate was first coated with an anti-human Klothoprotein antibody. Next, the klothoprotein from the sample was added and attached to antibodies coated on wells. Then biotinylated human Klothoprotein antibody was then added and bound to the observed Klothoprotein in sample. After the biotinylated Klothoprotein antibody, streptavidin-HRP was added. Five washes that follow incubation. Substrate solution was subsequently added and colour developed. The higher the concentration of Klothoprotein in the sample, the more intense was the color. An acidic stop solution was added to stop the reaction and absorbance is read at 450 nm.

B-Solutions used

Table 1. Shows the instruments and materials used in measuring the concentration of Klotho Protein.

Components	Quantity (96T)
Standard solution(64Pg/mL)	0.5ml x1
Pre-coated ELISA Plate	12*8well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate(25x)	20ml x1
Biotinylated Human ADP Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

C-Assay Procedure

Make all the reagents, standard solution and samples as instructed and let them acclimatize at room temperature prior to the test run.

1. Measure adiponectin concentration with all the wells on the plate.
2. For the standard solution, add a volume of 50 μL to each well where the standard solution is added.
3. 40 μL serum is added to the wells, followed by a mixture of 10 μL KL-antibodies (specific or non-specific) and 50 μL HRP-streptavidin.
4. Seal the plate.

5. Incubate the plates at 37 degrees for 60 minutes.
6. Next, remove the cover and wash the plate 5 times with the wash solution (soaking each well in 300 μL of wash solution for 30 seconds to 1 minute per wash).
7. Drain the plate using paper towels or some kind of absorbent material after washing.
8. 200 μL of substrate solution A was added to each well, then 50 μL of substrate solution B was added to each well.
9. Wrap the plate with a fresh cover and incubate for over 10 minutes at 37°C in the dark.
10. Add 50 11. Measure each hole optical density at (10) minutes after stopping solution was added, using a plate reader.
11. ELISA instrument reads the values to be tested.

Calculations

Construct a standard curve by plotting the mean O.D. for each standard solution (ABS) (Y) against the concentration (X) and plotting a best-fit curve using the points on the graph.

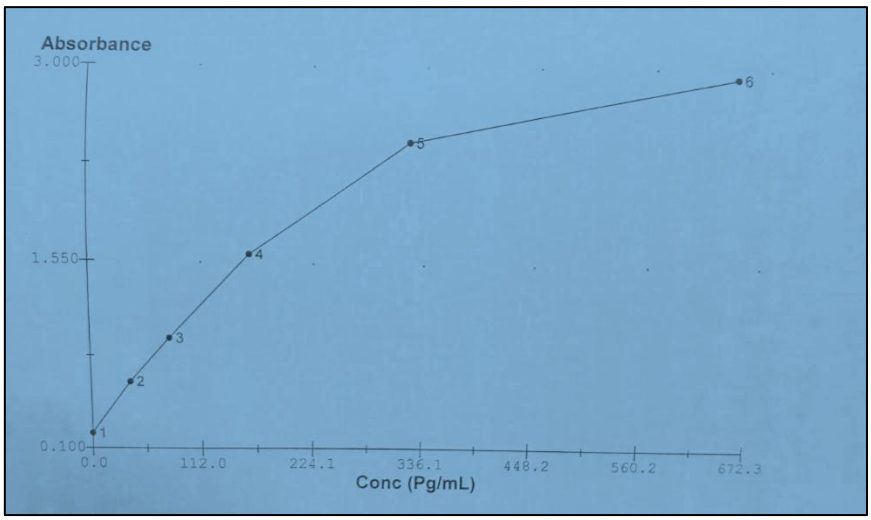

Figure 1. Illustrates the standard curve for Klotho Protein and shows the relationship between absorbance and concentration.

Blood Picture

Appropriate characterization of all blood component including mean corpuscular hemoglobin (MCH) using Sysmex XP-300 Hematology Analyzer. Both tubes were EDTA (K3) anticoagulant-pigment. 3 tubes were each filled with 2.5 mL of blood and carefully shaken to prevent clot formation. Anticoagulant tubes are used to prevent clotting since EDTA aggregates blood components. This is the basis for any diagnosis because an increase or decrease suggests the existence of a particular disease. [18].

Statistical Analysis

The data were statistically analyzed (SPSS). The t-test was performed to compare differences between two groups. The means of their standard deviation can be found in the values in tables over here. P 0.05, means not significant.

Results and Discussion

Serum Klotho Proteinconcentrations were measured as follows

The mean ± SD values of gluten protein of different samples from obese patients were (22.1 ± 132) pg/mL(Table 2), while the mean ± SD in control group(healthy individuals) was (290 ± 25.3)pg/mL.

Table 2. Shows the mean ± standard deviation in the concentration of Klotho Protein in the study samples.

Group Parameter	Control	Patient	P ≤
	Mean ± S.D		
No.	20	40	….
Klotho protein (pg/ml)	290±25.3	22.1±132	0.001

The results indicate that the level of Klotho Protein shows a highly significant decrease at the probability level (P ≤ 0.001) in obese patients compared to the control group.

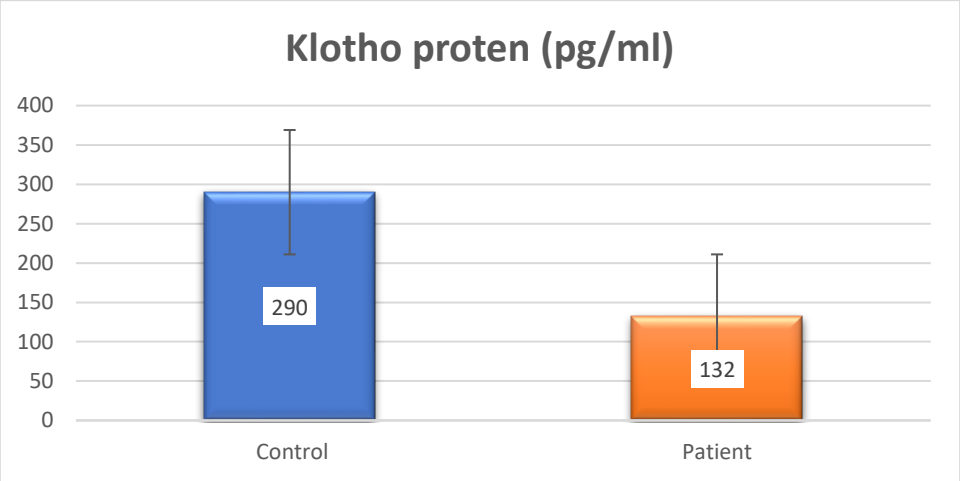

Figure 2. Shows the mean ± standard deviation in the concentration of Klotho Protein in the study samples.

Taking into account the results of the statistical analysis, we found a significantly lower concentration of klotho protein in patients with obesity compared to that in the control group. Also in agreement with previous investigations, klotho was only a mortality marker among individuals with a high level of obesity and lesser levels of physical exercise and that physical activity modulates the effect of blood klotho concentrations on mortality [19]. Loss of klotho protein functions normally results in cardiovascular disease with advanced age in adult mice and an overexpression of klotho causes excessive loss of endothelial dysfunction via oxidative stress exposure [20], [21].

Mean Creatine Hemoglobin Concentration (MCHC)

The mean ± standard deviation (± SD) MCHC values of some samples from obese patients are shown as (0.757 ± 30.768 g / dL), while the mean ± SD in the control group (healthy individuals) is accounted as [35.990 ± 0.787 g/dL]. Table (3): the mean ± SD of MCHC concentration in study samples.

Table 3. Mean ± Standard Deviation MCHC values in a number of samples of obese patients.

Group Parameter	Control	Patient	P ≤
	Mean ± S.D		
No.	20	40	….
MCHC g /dl	35.990±0.787	0.757±30.768	0.05

Data in table(3) shows a significant (P≤0.05) decrease compared to control group (Healthy group) for mean Creatinine hemoglobin concentration in Obese patients as shown in figure 3.

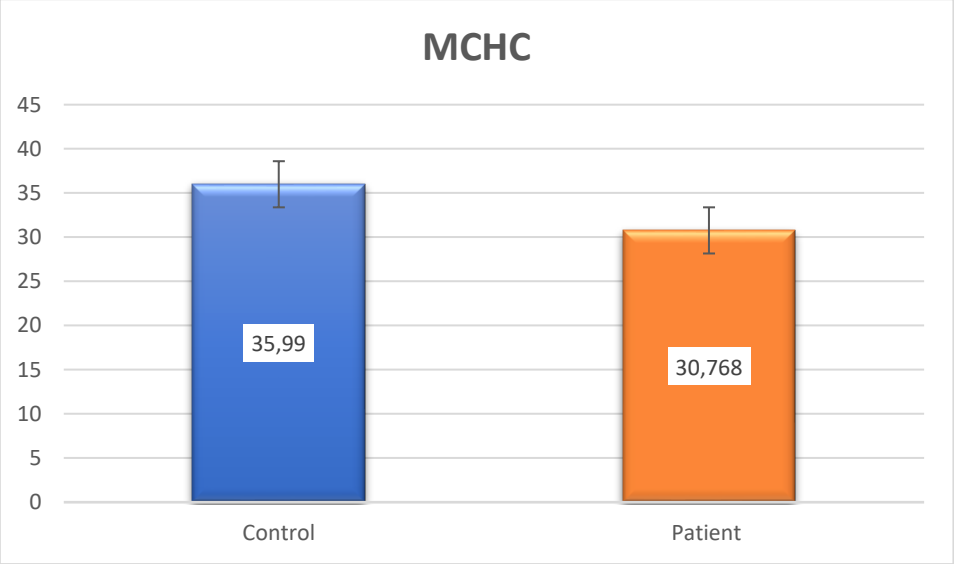

Figure 3. Mean ± Standard Deviation (MCHC) values in a number of samples of obese patients in the study samples.

Obesity correlates with "inflammatory-associated anemia", because high levels of inflammatory cytokines (IL-6 is a good example) induce the release of hepcidin hormone which blocks, in turn, iron absorption and decreases its availability for hemoglobin synthesis. As a result, hemoglobin levels might drop even without overt nutritional deficiency. Our findings were consistent with other studies, and can account for this association in patients with low hemoglobin levels in circulating blood who present with anemia: the role of Hb as an oxygen transporter to affect organs through blood flow. When hemoglobin concentration in the bloodstream is low, oxygen transport to different organs is impaired; as a result, hypoxia occurs and multi-organ dysfunction followed, especially respiratory dysfunction. This multi-organ dysfunction is increased in causing [22] and performed that obesity was linked with decreased functional iron and inflammatory-associated anemia5733 for inhibition of red blood cell indices [23].

Calculation of D-Dimer Concentration in Blood Serum:

The results obtained showed that the mean ± standard deviation of blood clot concentration in obese individuals was 1601.0 ± 50.3 ng/ml, while the mean ± standard deviation for the control group was 30.4 ± 200.5 ng/ml, as shown in Table 4.

Table 4. Mean ± Standard Deviation of Blood Clot Concentration in Study Samples.

Group Parameter	Control	Patient	P ≤
	Mean ± S.D		
No.	20	40	….
D - dimer ng / ml)(	200.5±30.4	50.3±1601.0	0.001

The study revealed a very significant difference (P ≤ 0.001) in the level of blood clotting factor (D-dimer) between obese groups and control group after performing statistical analysis. D-dimer is elevated in the obese group compared with control as shown in figure 4.

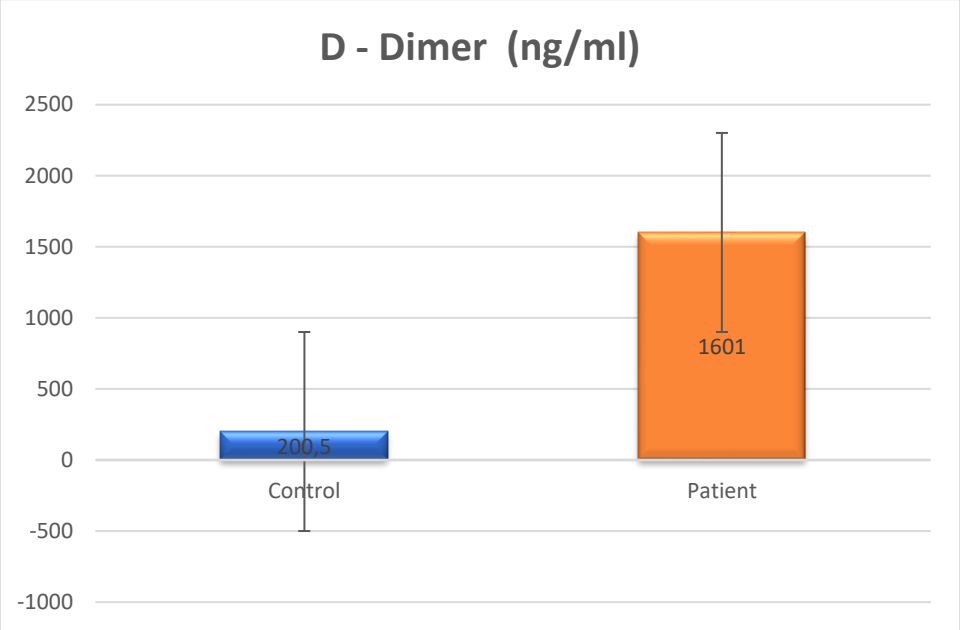

Figure 4. Mean ± Standard Deviation (D-Dimer) in the study samples.

In our study of obese patients (men and women) the level of blood clotting was significantly higher in the obese than in healthy controls. These results are in line with previous studies showing that excess BMI and waist circumference are independent predictors of higher D-dimer levels, as well as increased risk for other changes in coagulation factors, indicating changes toward a prothrombotic state due to obesity. This study showed that high D-dimer levels are a consequence of increased fibrin turnover; probably reflecting activation of the coagulation system as well as impaired fibrolysis. Accordingly, the marked elevation of D-dimer levels observed in obesity versus controls ($P \leq 0.001$) in our study is consistent with recent population-based observations confirming that at a level there is an effect of obesity on the activation of the coagulation system [24].

Conclusion

Findings from this study prove that obesity is a serious complex chronic disease which has impact on a number of important parameters in the body. The Klotho Protein was decreased in obese compared with healthy individuals to a greater extent, suggesting that the increase would be relevant for cardiovascular disease and accelerated cell aging. A decline in mean corpuscular hemoglobin (MCHC) concentration was also observed indicating an association between obesity, inflammatory anemia and diminished oxygen transport to tissues. On the other hand, they found that obese patients had significantly higher D-dimer levels indicating hypercoagulability and increased risk for thrombosis. This can be concluded as obesity not just a condition of over weight but it extends itself to various physiological problems involving the circulatory, hematological and metabolic disorders thus act as one of the most prominent risk factors for several chronic diseases.

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