Sudan University of Science and Technology (SUST)
College of Graduate Studies

Plasma Total Protein and Albumin levels among metabolic syndrome patients in Khartoum State

مستوي البروتين الكلي والألبيومين في بلازما الدم لدي المرضي بالمتلازمة الأيضية في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirement of master degree in Medical Laboratories Sciences - Clinical Chemistry

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November 2018

بِسْمِ ٱللّـهِ ٱلْـرَّحْمَـنِ ٱلْـرَّحِيـمْ

قال تعالي:

(وَيُسَبِحُ ٱلرَّعدُ بِحـَمدِهِ وَالْمَلائِكَةُ مِنْ خِيفَتِهِ وَيُرْسِلُ الصَّوَاعِقُ فَيُصِيبُ بِهَا مَن يَشَاء وَهُمْ يُجَادِلُونَ فِي اللَّهِ وَهُوَ شَدِيدُ الْمِحَالِ)

صدق الله العظيم

سورة الرعد

الايه(13)

Dedication

I dedicate this dissertation to my mother, whose words of encouragements were always there to support me while crossing this road.

To my family members, friends, who have had positive impacts on my life.

Acknowledgment

First thanks to ALLAH for blessing me. Then I would like to express my gratitude and ever last appreciation to my supervisor **Dr. Nuha Eljaili Abubaker** for this guidance, advice, support continues assistance through the whole process of this research.

Finally I am grateful to thank all patients participated in this study. And special thanks to Mohammed Adel for his great help and assistance.

Abstract

Background: The metabolic syndrome (Mets) has become one of the major public-health challenges worldwide. Total protein and albumin are two important parameters in the diagnosis and monitoring for diseases such as liver impairment and renal diseases in patients with metabolic syndrome.

Objectives: The study was done to measure plasma total protein and albumin in metabolic syndrome patients.

Materials and methods: The study was conducted in Khartoum state from Jun to September 2018. One hundred blood samples were collected, including 50 metabolic syndrome patients as case group and 50 healthy subjects as control group with age ranging between 42-85 years old. Plasma total protein and albumin were measured using semi-automated chemical methods, and data were analyzed using statistical package for social science computer program (SPSS version 20).

Results: The results demonstrated that, metabolic syndrome is most common among the age group (56 - 70) years (60%), and most abundant in females (66%) than males (34%).

The results showed that, there were significant increases in mean concentration of, total protein, albumin, Body Mass Index (BMI), and Waist Circumference (WC) in metabolic syndrome subjects compared to control group, mean± SD cases versus control group, (4.2 ± 0.6 versus 3.8 ± 0.3 g/dl, p- value 0.000), (7.2 ± 0.5 versus 6.8 ± 0.3 g/dl, p- value 0.000), (30.2 ± 7.0 versus 22.7 ± 1.2 Kg/m^2, p-value 0.000), (102.2 ± 15.2 versus 79.2 ± 2.7 cm, p-value 0.000), respectively.

The study demonstrated that, there was moderate positive correlation between albumin and total protein (r= 0.456, P-value =0.000).

The study also showed that, there were no correlation between total protein levels, duration of disease, Age, BMI, WC, among Mets group, (r=-0.188, P-value =0.190), (r = -0.01, P-value = 0.945), (r = -0.168, P-value = 0.243). (r = 0.025, P-value = 0.861), respectively. Also there were no correlation between albumin, age, BMI, WC, and duration of disease among Mets group, (r=-0.023, P-value =0.874), (r = -0.177, P-value = 0.218), (r = 0.191, P-value = 0.185), (r=-0.137, p-value=0.343), respectively.

Conclusion: The present study concluded that, patients with metabolic syndrome had high level of plasma albumin and total proteins. The metabolic syndrome is most abundant in females than males, and most common in age group (56 – 70) years old.

مستخلص الأطروحه

الخلفيه: المتلازمة الأيضية أصبحت احد التحديات الرئيسيه للصحه العامة فى جميع انحاء العالم. يعتبر البروتين الكلي و الألبيومين من اهم الوحدات في التحليلات الروتينيه فى تشخيص ومتابعه الأمراض، كامراض الكبد والكلي في مرضى المتلازمة الأيضية.

الأهداف: اجريت هذه الدراسه لقياس مستوى البروتين الكلي و الألبيومين في بلازما الدم لدى مرضى المتلازمة الايضية.

المواد والأساليب: تم إجراء هذه الدراسه في فتره زمنيه من يونيو إلي سبتمبر ٢٠١٨م في ولاية الخرطوم. تم جمع مئه عينه، ٥٠ عينة من مرضي المتلازمة الايضية، و ٥٠ عينة من الأشخاص الأصحاء كمجموعة ضابطة أو تحكم ، تتراوح اعمارهم مابين ٤٢-٨٥ عام. تم قياس مستوى الألبيومين والبروتين الكلي باستخدام طريقه كيميائيه نصف آليه، وتم تحليل البيانات باستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية علي الحاسوب (الإصدار ٢٠).

النتائج: اوضحت نتائج الدراسه ان المتلازمة الايضية أكثر شيوعا في مجموعة الأعمار (٥٦ - ٧٠) عام (٦٠٪)، واكثر شيوعا بين النساء مقارنه بالرجال.

أظهرت الدراسه ايضا ان هناك زياده ملحوظه فى متوسط تركيز البروتين، الألبيومين ، وارتفاع في متوسط كتلة الجسم ومحيط الخصر علي التوالي، المتوسط ± الإنحراف المعياري لدي المرضى مقارنة بمجموعة التحكم: (٤,٢± ٠,٦ مقابل ٣,٨ ± ٠,٣ جرام/ديسيليتر، الإحتمال الإحصائي للمقارنه ٠,٠٠٠)، (٧,٢ ± ٠,٥مقابل ٦,٨±٠,٣ جرام/ديسيليتر، الإحتمال الإحصائي للمقارنه٠,٠٠٠)، (٣٠,٢ ± ٧,٠ مقابل٢٢,٧ ±١,٢كجم/متر مربع، الإحتمال الإحصائي للمقارنة ٠,٠٠٠)، (١٠٢,٢± ١٥,٢ مقابل ٧٩,٢± ٢,٧سنتيميتر، الإحتمال الإحصائي للمقارنه٠,٠٠٠) علي التوالي.

كما اوضحت نتائج الدراسه ايضا انه لايوجد إرتباط بين مستوي البروتين الكلي، فترة الاصابه بالمتلازمة الايضية، العمر، كتلة الجسم، ومحيط الخصر لدى مرضى المتلازمة الايضية، (معامل بيرسون = ٠,١٨٨-، ومستوى المعنوية =٠,١٩٠)، (معامل بيرسون = ٠,٠١-ومستوى المعنوية =٠,٩٤٥)، (معامل بيرسون =٠,١٦٨-ومستوى المعنوية=٠,٢٤٣) (معامل بيرسون =٠,٠٢٥ومستوى المعنوية ٠,٨٦١) على التوالي. كذلك لايوجد إرتباط بين مستوي الالبيومين،العمر، كتلة الجسم، محيط الخصر، وفترة الاصابه لدى مرضى المتلازمة الايضيه، (معامل بيرسون = ٠,٠٢٣-، ومستوى المعنوية=٠,٨٧٤)، (معامل بيرسون = ٠,١٧٧-ومستوى المعنوية =٠,٢١٨)، (معامل بيرسون = ٠,١٩١-ومستوى المعنوية = ٠,١٨٥) (معامل بيرسون = ٠,١٣٧-ومستوى المعنوية ٠,٣٤٣) على التوالي.

اظهرت نتائج الدراسة كذلك انه يوجد إرتباط إيجابي متوسط بين مستوى الالبيوين والبروتين الكلى (معامل بيرسون = ٠,٤٥٦ ومستوى المعنوية =٠,٠٠٠).

الخلاصه: خلصت الدراسه إلي أنه توجد زياده ملحوظه في مستوى الالبيوين والبروتين الكلي في مرضى المتلازمة الأيضيه. وان المتلازمة الأيضية اكثر شيوعا بين النساء مقارنة بالرجال، واكثر شيوعا في مجموعة الأعمار (٥٦-٧٠) عام.

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List of abbreviations

BMI	Body Mass Index
Cm	Centimeter
CDS	China Diabetes Society
DBP	Diastolic Blood Pressure
FBG	Fasting Blood Glucose
HDL	High Density Lipoprotein
2 HPP	2 Hours Post Prandial
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
SBP	Systolic Blood Pressure
TG	Triglycerides
Mets	Metabolic Syndrome
WC	Waist Circumference

Chapter one

Introduction

Rationale

Objectives

Chapter One

Introduction, Rationale and Objectives

1.1 Introduction:

Metabolic Syndrome (Mets) defined according to the Criteria proposed by " China Diabetes Society " (CDS), were three or more of the following risk factors : overweight or obesity , BMI ≥ 25.0 kg/ m^2 , abdominal obesity was defined as elevated waist circumference ≥ 85 cm in male , ≥ 80 cm in female , hypertention ,systolic blood pressure SBP ≥ 140 mmHg , or diastolic blood pressure (DBP) ≥ 90 mmHg , or previous diagnosis of hypertention , dyslipidemia, TG ≥ 1.7 mmol / l (150 mg / dl) or low HDL – C ≤ 0.9 mmol / l in men, ≤1.0 mmol/l (40 mg /dl) in women, and hyperglycemia, FBG ≥ 6.1 mmol /l (110 mg / dl) or 2 HPP ≥ 7.8 mmol / l (140 mg /dl). Or previous diagnosis with hyperglycemia. (Alberti *et al.*, 2006; Krithika *et al.*, 2016; Shumei *et al.*, 2017).

Protein (Greek- proteios, primary) is the class of macromolecules containing nitrogen that are essential for the survival of life. It is now well known that proteins are at the center of action in biological processes and are essential structural components of the cells. Proteins are composed of smaller units called amino acids. All proteins are composed of 20 different amino acids. Each polypeptide has free ∝. Amino group (from the first amino acid) represented on the left hand side of the protein chain. It is called an amino terminal or N.terminal end. A polypeptide also has free ∝. carboxylic group (from the last amino acid), which is present on right hand side of the chain and is called as the carboxy terminal or C-terminal end. (Harbans and Rajesh., 2011). Total protein measurements can reflect nutritional status, kidney disease, liver disease, and many other conditions. (Bishop *et al.*, 2010).

Albumin is small globular protein with a molecular mass of 66.3 KDa. It is the most abundant protein found in plasma, accounting for approximately one half the plasma proteins mass. Because of its high plasma concentration and relatively small size, albumin is also the major protein component of most extravascular body fluids, including CSF, interstitial fluid, urine, and amniotic fluid. Approximately 60%of the total body albumin is in the extravascular space. It is highly soluble in water due to its high net negative charge at physiological ph. (Carl *et al.*, 2008).

1.2 Rationale:

Metabolic syndrome is a serious medical condition increases the incidence of multiple cancers, community stigma, chronic depression, and effect of the life quality. Mets prevalence is about 20-25% of the world's adult population with increased prevalence in advanced ages. (Carr e*t al.*, 2004; Alberti *et al.*, 2006). This problem is found all over the world, and it's fatal unless controlled and treated properly. Albumin is considered as a potent component in human body in order to function properly. Any reduction or increase in total protein or albumin levels can carry rough consequences as it can be an indication for drastic prognosis. Plasma albumin and total protein are biomarkers for metabolic syndrome risk for liver cancer. The present study help to spot light on this side of metabolic syndrome regarding the biochemical changes in blood. There is no pervious study published in Sudan.

1.3 Objectives:

1.3.1 General Objective:

To assess plasma total protein and albumin levels among metabolic syndrome patients.

1.3.2 Specific Objectives:

1. To estimate and compare plasma albumin, total protein concentrations, BMI, WC among metabolic syndrome patients with control group.
2. To correlate plasma albumin and total protein concentrations with BMI, WC, age, and duration of disease among metabolic syndrome patients.

Chapter two

Literature review

Chapter two

Literature Review

2.1 Metabolic syndrome:

The term "metabolic syndrome" dates back to at least the late 1950s, but came into common usage in the late 1970s. The terms "metabolic syndrome," "insulin resistance, syndrome "syndrome X", Dysmetabolic syndrome X, mixed metabolic syndrome. (Sarafidis *et al.*, 2006; Falkner *et al*., 2014).

The metabolic syndrome (visceral obesity, dyslipidaemia, hyperglycaemia, and hypertension), has become one of the major public-health challenges Worldwide. The clustering received scant attention until 1988 when Reaven described syndrome X: insulin resistance hyperglycemia, hypertension, low HDL-cholesterol, and raised VLDL-triglycerides. (George *et al*., 2005).

2.1.1 Signs and symptoms of metabolic syndrome:

- Metabolic syndrome has no symptoms; although a large waist circumference (central obesity) is a visible sign. Blood sugar is very high, might have signs and symptoms of diabetes (including increased thirst and urination, fatigue, and blurred vision.)
- Impaired fasting glucose, insulin resistance, or prediabetes.
- High blood pressure.
- Decreased fasting serum HDL cholesterol. Elevated fasting serum triglyceride level. (Knowler *et al*., 2002).

2.1.2 Causes of metabolic syndrome:

The following are causes of metabolic syndrome:

(A)Stress: Recent research indicates prolonged chronic stress can contribute to the hypo thalamic-pituitary-adrenal axis (HPA-axis), high cortisol levels to circulate, which results in raising glucose and insulin levels, dyslipidemia and hypertension. (Knowler *et al*., 2002; Chiasson *et al*., 2003; Carr *et al*., 2004).

(B)Central obesity: Central obesity is a key feature of the syndrome, being both a symptom and a cause of it in that the increasing adiposity often reflected in highs waist circumference both often results from and often contributes to insulin resistance. However, despite the importance of obesity, patients who are of normal weight may also be insulin-resistant and have the syndrome.

(C)Sedentary lifestyle: Many components of metabolic syndrome are associated with a sedentary lifestyle, including increased adipose tissue (Predominantly central); reduced FUR cholesterol; and a trend toward increased triglycerides, blood pressure, and glucose in the genetically susceptible.

(D)Aging: Metabolic syndrome affects 60% of the U.S population older than age 50. With respect to that demographic, the percentage of women having the syndrome is higher than that of men. The age dependency of the syndrome's prevalence is seen in most populations around the world.

(E) Psychiatric illnesses.

(F) Alcohol Abuse. (Knowler *et al.*, 2002; Chiasson *et al.*, 2003; Carr *et al.*, 2004).

2.1.3 Diagnosis of metabolic syndrome:

Several organizations have criteria for diagnosing metabolic syndrome. According to guidelines used by the National Institutes of Health, have metabolic syndrome if have three or more of these traits or are taking medication to control them:

1- Large waist circumference:

a waistline that measures at least 35 inches (89 centimeters) for women and 40 inches (102 centimeters) for men.

2- High triglyceride level:

150 milligrams per deciliter (mg/dL), or 1.7 millimoles per liter.

3- Reduced high-density lipoprotein (HDL) cholesterol:

Less than 40 mg/dI (1.04 mmol/L) in men or less than 50 mg/dL (1.3 mmo1/1.) in women of this "good" cholesterol. (Knowler *et al.*, 2002; Chiasson *et al.*, 2003; Carr *et al.*, 2004; Krithika *et al.*, 2016).

4- Increased blood pressure:

130/85 millimeters of mercury (mm Hg) or higher.

5- Elevated fasting blood sugar:

100 mg/dL (5.6 mmol/L) or higher. (Knowler *et al.*, 2002; Chiasson *et al.*, 2003; Carr *et al.*, 2004; Krithika *et al.*, 2016).

2.1.4 Prevention and Treatment of metabolic syndrome:

Various strategies have been proposed to prevent the development of metabolic syndrome.

2.1.4.1 A healthy lifestyle as:

(**1)Eat better:** Adopt a diet rich in whole grains, fruits, vegetables, lean meats and fish, and low-fat or fat-free dairy products and avoid processed food, which often contains partially hydrogenated vegetable oils, and is high in salt and added sugar.

(2)Get active: Incorporate at least 150 minutes of moderately vigorous physical activity into weekly routine. Walking is the easiest place to start, but may want to experiment to find something else like to do that gets heart rate up. If needed, break exercise up into several short, 10-minute sessions throughout the day to reach goal. (Knowler *et al.*, 2002; Chiasson *et al.*, 2003; Carr *et al.*, 2004).

(3)Lose weight: Reduce risk by successfully losing weight and keeping it off. Learn recommended calorie intake, the amount of food calories consuming, and the energy calories burring off with different levels of physical activity. Balance healthy eating with a healthy level of exercise when changes in lifestyle alone do not control the conditions related to metabolic syndrome, health practitioner may prescribe medications to control blood pressure, cholesterol, and other symptoms. Carefully following practitioner's instructions can help prevent many of the long term effects of metabolic syndrome. Every step counts and hard work and attention to these areas will snake a difference in health.

(4)Stopping smoking: Smoking cigarettes worsens the health consequences of metabolic syndrome.

(5)Managing stress: Physical activity, meditation, yoga and other programs can help handle stress and improve emotional and physical health. (Knowler *et al.*, 2002; Chiasson *et al.*, 2003; Carr *et al.*, 2004).

2.1.5 Risk factors of metabolic syndrome:

Risk increases when more components of metabolic syndrome are present. The following factors increase chances of having metabolic syndrome:

(A)Age: risk of metabolic syndrome increases with age.

(B)Race: In the United States, Mexican-Americans appear to be at the greatest risk of developing metabolic syndrome.

(C)Obesity: Carrying too much weight, especially in abdomen, increases risk of metabolic syndrome. (knowler *et al.*, 2002; Krithika *et al.*, 2016).

(D)Diabetes: more likely to metabolic syndrome if had diabetes during pregnancy (gestational diabetes) or if have a family history of type 2 diabetes.

(E)Other diseases: risk of metabolic syndrome is higher if ever had non-alcoholic fatty liver disease, cardiovascular disease, polycystic ovary syndrome.

2.1.6 Management: Food and Drug administration:

The first line treatment is changing of lifestyle a healthy lifestyle, drug treatment is frequently required. Diuretics and ACE inhibitors may be used to treat hypertension. Cholesterol drugs may be used to lower LDL cholesterol and triglycerides levels, if they are elevated, and to raise HDL levels if they are low. Use of drugs that decrease insulin resistance, e.g., rnetformin and thiazolidinediones. (knowler *et al.*, 2002; Krithika *et al.*, 2016).

2.2 Liver:

The liver is a large and complex organ weighing approximately 1.2–1.5 kg in the healthy adult. It is located beneath and is attached to the diaphragm, is protected by the lower rib cage, and is held in place by ligamentous attachments. (Bishop *et al.*, 2010).

The liver has a central and critical biochemical role in (1) metabolism, (2) digestion, (3) detoxification, and (4) the elimination of substances from the body. All blood from the intestinal tract initially passes through the liver, where products derived from digestion of food are processed, transformed, and stored. It also has a central role in protein, carbohydrate, and lipid metabolism and synthesizes bile acids from cholesterol to facilitate dietary fat and vitamin absorption. The liver metabolizes both endogenous and exogenous compounds, such as drugs and toxins through biotransformation, allowing their elimination. The liver performs endocrine functions as it catabolizes thyroid hormone, cortisol, and vitamin D, and synthesizes insulin-like growth factor 1, angiotensinogen, and erythropoietin. Many of these hepatic functions may be assessed by laboratory procedures to gain insight into the integrity of the liver. (Carl *et al.*, 2008).

The liver has a significant reseme capacity, preventing protein concentrations from decreasing unless there is extensive liver damage. In addition, many liver proteins have relatively long half-lives, such as albumin at approximately 3 weeks. The patterns of plasma protein alterations seen in liver disease depend on the type, severity, and duration of liver injury. For example, in acute hepatic dysfunction, there is usually little change in the plasma protein profile or the total plasma protein concentration; with fulminant hepatic failure or severe liver injury, concentrations of

short-lived hepatic proteins (such as transthyretin and prothrombin) will fall quickly and become abnormal, whereas proteins with longer half-lives will be unchanged. In cirrhosis, concentrations of all liver-synthesized plasma proteins decrease, while immunoglobulins increase (related to impaired Kupffer cell function). (Carl *et al.,* 2008).

Clinical and epidemiologic studies have associated non – alcoholic fatty liver with the metabolic syndrome, with insulin resistance as the pivotal pathogenic factor. Obesity, type 2 diabetes mellitus, dyslipidemia, and hypertension contribute to risk for liver disease and to disease progression.

The presence of multiple metabolic abnormalities is associated with the severity of liver disease. (Marchesini and Marzocchi., 2007).

There is higher risk of hepatocellular carcinoma among patients with metabolic syndrome. (Raxitkumar *et al.,* 2014).

2.3 Plasma protein:

Proteins are present in all body fluids, but it is the proteins of the blood plasma that are examined most frequently for diagnostic purposes. Over 100 individual proteins have a physiological function in the plasma. Quantitatively, the single most important protein is albumin. With the exception of fibrinogen, the other proteins are known collectively as globulins. Changes in the concentrations of individual proteins occur in many conditions and their measurement can provide useful diagnostic information. (Marshal *et al.*, 2012).

In very general terms, variations in plasma protein concentrations can be due to changes in any of three factors: the rate of protein synthesis, the rate of removal and the volume of distribution. The concentration of proteins in plasma is affected by posture: an increase in concentration of 10–20% occurs within 30 min of becoming upright after a period of recumbency. Also, if a tourniquet is applied before venepuncture, a significant rise in protein concentration can occur within a few minutes. In both cases, the change in protein concentration is caused by increased diffusion of fluid from the vascular into the interstitial compartment. These effects must be borne in mind when blood is being drawn for the determination of protein concentration. Only changes in the more abundant plasma proteins (i.e. albumin or immunoglobulins) will have a significant effect on the total protein concentration. (Bishop *et al.,* 2010).

The major measured plasma proteins are divided into two groups: albumin and globulins. There are four major types of globulins, each with specific properties and actions. A typical blood

panel will provide four different measurements—total protein, albumin, globulins, and the albumin/globulin ratio.

A fasting specimen is not needed. Interferences in some of the methods occur in the presence of lipemia; hemolysis falsely elevates the total protein result because of the release of RBC proteins into the serum. The reference interval for serum total protein is 6.5–8.3 g/dL (65–83 g/L) for ambulatory adults. In the recumbent position, the serum total protein concentration is 6.0–7.8 g/dL (60–78 g/L). This lower normal range is a result of shifts in water distribution in the extracellular compartments. The total protein concentration is lower at birth, reaching adult levels by age 3 years. There is a slight decrease with age. Lower total protein levels are also seen in pregnancy. (Bishop *et al.*, 2010).

2.3.1 Protein synthesis:

Most plasma proteins are synthesized in the liver and secreted by the hepatocyte into the circulation. The immunoglobulins are exceptions because they are synthesized in plasma cells. It is the information encoded in genes, specified by the nucleotide sequence that provides each protein with its own unique amino acid sequence. This amino acid sequence of a polypeptide chain is determined by a corresponding sequence of bases (guanine, cytosine, adenine, and thymine) in the DNA contained in the specific gene. This genetic code is sets of three nucleotides known as codons with each three-nucleotide combination standing for a specific amino acid. (Bishop *et al.*, 2010).

2.3.2 Properties of protein:

Many properties of proteins are used for their separation, identification, and assay. The following five properties are among them:

1. Molecular size: Most proteins are macromolecules of high molecular mass. Because of their sizes and differing molecular masses, it is possible to separate proteins from smaller molecules by (1) dialysis, (2) ultrafiltration, (3) gel filtration chromatography, and (4) density-gradient ultracentrifugation. (Carl *et al.*, 2008).

2. Differential solubility: Protein solubility is affected by the (1) pH, (2) ionic strength, (3) temperature, and (4) dielectric constant of the solvent. When these parameters are varied, individual proteins become either more or less soluble. For example, through variations in the ionic strength of a solution, proteins become either more soluble ("salting-in") or less soluble ("salting-out").

3. Electrical charge: The effect of pH to introduce, enhance, or change the surface charges on a protein creates various species of different charges that migrate at different rates in an electrical field. Separation by electrophoresis and isoelectric focusing are based on this behavior. Ionexchange chromatography is based on electrostatic interactions of charged proteins with oppositely charged solid media.

4. Adsorption on finely divided inert materials: These materials offer large surface areas for interactions with proteins. These interactions may be (1) hydrophobic, (2) absorptive, (3) ionic, or (4) molecular (hydrogen bonding). (Carl *et al.,* 2008).

2.3.3 Structural organization of proteins:

Variation in the number and order of different amino acids or the manner in which these amino acids are arranged in a polypeptide chain, reflects the type of protein structure.

There are 4 levels of structural organization of proteins:

2.3.3.1 Primary structure of proteins:

Primary structure of protein refers to the unique order and sequence (dicated by the codon sequence) of covalently linked amino acid residues in polypeptide chain.

2.3.3.2 Secondary structure of proteins:

Secondary level of protein structure includes folding or twisting patterns of the polypeptide chain in a protein. Secondary structures include $\propto.helix$ and β.pleated sheets. Certain compinations of secondary structure are also observed in different folded protein structures. They are referred to as structural motifs. These longer pattern lengths of secondary structure may include multiple structural motifs and when commonly observed in more than one protein is referred to as supersecondary structures. (Harbans and Rajesh., 2011).

2.3.3.3 Tertiary structure of proteins:

Tertiary structure of protein involves the intra –molecular folding of the polypeptide chain into compact three dimentional structures with a specific shape. Tertiary structure is maintained by electrostatic linkages, hydrogen bonds, disulphide bridges, vander woal forces and hydro phobic interactions. (Harbans and Rajesh., 2011).

2.3.3.4 Quaternary structure of proteins:

Multisubunit or multimeric proteins contain several identical and \ or different chains where each of the polypeptide chain is called a subunit. These polypeptide subunits associated with specific geometry. The spatial arrangement of these subunits refers to the quaternary structure. (Harbans and Rajesh., 2011).

2.3.4 Functions of protein:

Proteins demonstrate numerous biological functions, for example:

- Enzymes are proteins that catalize biochemical reactions.
- Protein, polypeptide, and oligopeptide hormones regulate metabolism; and antibodies and components of the complement system protect against infection.
- Plasma proteins maintain the osmotic pressure of plasma.
- They transport hormones, vitamins, metals, and drugs, often serving as reservoirs for their release and use.
- Apo lipoproteins solubilize lipids; hemoglobulin carries oxygen; and protein coagulation factors affect hemostasis. (Carl *et al*., 2008)

2.3.5 Total protein abnormalities:

The total protein test is a rough measure of all of the proteins in the plasma. Total protein measurements can reflect nutritional status, kidney disease, liver disease, and many other conditions. If total protein is abnormal, further tests must be performed to identify which protein fraction is abnormal, so that a specific diagnosis can be made. (Bishop *et al.*, 2010).

2.3.5.1 Hypoproteinemia:

Hypoproteinemia, a total protein level less than the reference interval, occurs in any condition where a negative nitrogen balance exists. One cause of a low level of plasma proteins is excessive loss. Plasma proteins can be lost by excretion in the urine in renal disease; leakage into the gastrointestinal tract in inflammation of the digestive system; and the loss of blood in open wounds, internal bleeding, or extensive burns. Another circumstance producing hypoproteinemia is decreased intake either because of malnutrition or through intestinal malabsorption as seen in sprue. Without adequate dietary intake of proteins, there is a deficiency of certain essential amino acids and protein synthesis is impaired. A decrease in serum proteins as a result of decreased synthesis is also seen in liver disease (site of all nonimmune protein synthesis) or in inherited immunodeficiency disorders, in which antibody production is diminished. Additionally,

hypoproteinemia may result from accelerated catabolism of proteins, such as occurs in burns, trauma, or other injuries. (Bishop *et al.*, 2010).

2.3.5.2 Hyperproteinemia:

Hyperproteinemia, an increase in total plasma proteins, is not an actual disease state but is the result of the underlying cause, dehydration. When excess water is lost from the vascular system, the proteins, because of their size, remain within the blood vessels. Although the absolute quantity of proteins remains unchanged, the concentration is elevated due to a decreased volume of solvent water. Dehydration results after a variety of conditions, including vomiting, diarrhea, excessive sweating, diabetic acidosis, and hypoaldosteronism. In addition to dehydration, hyperproteinemia may be a result of excessive production, primarily of the _-globulins. Some disorders are characterized by the appearance of a monoclonal protein or paraprotein in the serum and often in the urine as well. (Bishop *et al.*, 2010).

2.4 Albumin:

Albumin is the highest concentration protein in plasma. It's produced from 585 amino acids at the rate of 9 -12 grams per day with no reserve or storage. (Bishop *et al.*, 2010). It's synthesized primarily by the hepatic parenchymal cells. The synthetic rate of albumin is controlled by colloid osmotic pressure (COP) and secondarily by protein intake.

Albumin catabolism occurs primarily by pinocytosis in all tissues. The normal plasma half-life of albumin is 15 to 19 days. (Carl *et al.*, 2008).

2.4.1 Functions of albumin:

Albumin's primary function is the maintenance of COP in both the vascular and extravascular spaces. Albumin also binds and transports a large number of compounds, including thyroid hormones, iron, and fatty acids. For example, albumin binds unconjugated bilirubin, salicylic acid (aspirin), fatty acids, calcium and magnesium ions, and many drugs. (Carl *et al.*, 2008)

2.4.2 Clinical significance of albumin:

2.4.2.1 Hypoalbuminemia:

Hypoalbuminemia is defined as decreased concentrations of serum albumin. It can be seen in a multitude of clinical conditions such as:

- Analbuminemia: Individuals with this rare genetic deficiency have plasma albumin concentrations less than 0.5 g/L but mild if any edema. Major clinical manifestations are related to abnormal lipid transport. (Carl *et al.*, 2008).

- Inflammation: Acute and chronic inflammations are the most common causes of Hypoalbuminemia.p
- Hepatic disease: in which hypoalbuminemia is a result of increased immunoglobulin concentrations, loss into the extravascular space, and direct inhibition of synthesis by toxins and alcohol.
- Urinary Loss.
- Gastrointestinal Loss.
- Protein Energy Malnutrition. (Carl *et al*; 2008).

2.4.2.2 Hyperalbuminemia:

Hyperalbuminaemia can be either an artefact, for instance as a result of venous stasis during blood collection or overinfusion of albumin, or be a result of dehydration. Albumin synthesis is increased in some pathological states as a response to protein loss, but this never causes hyperalbuminaemia. (Marshal *et al.*, 2012).

Chapter three

Materials and methods

Chapter Three

Materials and Methods

3.1 Materials:

3.1.1 Study approach:

A quantitative method was used to measure the levels of plasma total proteins and albumin in metabolic syndrome patients during the period from Jun to September 2018.

3.1.2 Study design:

This is case control study.

3.1.3 Study area:

The study was conducted in Zenam and Samir medical center.

3.1.4 Study population:

The study included patients with metabolic syndrome, and apparently healthy individual.

3.1.5 Sample size:

The study included 50 patients with metabolic syndrome as case subjects and 50 apparently healthy individual as control subjects (age and gender were matched).

Inclusion criteria:

Mets patients –individuals with central obesity (WC $\geq$ 85 cm in male, $\geq$ 80 cm in female), hypertension and hyperglycemia. And healthy individuals as control were enrolled in this study.

Exclusion criteria:

Individuals suffering from liver disease, renal failure, any type of inflammations, recent burns, malnutrition or thyroid disease were excluded from this study.

3.1.6 Ethical considerations:

Verbal consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

3.1.7 Data collection:

A structured interviewing questionnaire was designed to provide personal and medical information about the study subjects.

3.1.8 Sample collection and processing:

About 3 ml of venous blood was collected from each enrolled subject. The blood samples were dispensed into containers with lithium heparin anticoagulant, mixed well and centrifuged immediately after collection at 3000 rpm for 5 minutes, and the plasma from it was dispensed into a clean dry plain container.

3.2 Methods:

The plasma sample from each subject was stored at $4^{o}C$ until analysis by Biuret method for total protein and BCG method for albumin estimation.

3.2.1 Estimation of plasma albumin:

Plasma albumin estimations were performed using bromocresol green method. The kits supplied by (Biosystems, Spain)

3.2.1.1 Principle of the method:

Albumin in the sample reacts with bromocresol green in acid medium forming a coloured complex that can be measured by spectrophotometry

3.2.1.2 Procedure of plasma albumin:

1. Reagents were pipetted into labelled test tubes as follow:

	Blank	Standard	Sample
Albumin Standard(S)	——	10 µl	——
Sample(plasma)	——	——	10µl
Reagent(A)	1.0 ml	1.0 ml	1.0 ml

2. Mixed thoroughly and incubated for 1 minute at room temperature.

3. The absorbance (A) of the Standard and the Sample was read at 630 nm against the blank. (The color is stable for 30 minutes). (**Appendix II)**.

3.2.2 Estimation of plasma total protein:

Plasma proteins were measured using biuret method. The kits supplied by (biosystems, Spain).

3.2.2 .1 Principle of the method:

Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry.

3.2.2.2 Procedure of plasma total protein:

1. Reagents were pipetted into labelled test tubes as follow:

	Blank	Standard	Sample
Distilled water	20 µl	——	——
Protein Standard(S)	——	20µl	——
Sample(plasma)	——	——	20µl
Reagent(A)	1.0 ml	1.0 ml	1.0ml

2. Mixed thoroughly and and incubated for 10 minutes at room temperature.

3. The absorbance (A) of the Standard and the Sample was read at 545 nm against the blank. The colour is stable for at least 2 hours. (**Appendix III)**.

3.3 Quality control Method:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of tests and control samples.

3.4 Statistical analysis:

Data obtained from this study were analyzed using statistical package for the social science (SPSS version 20), and the results were presented into figures and tables using the Microsoft Excel computer program. Independent t test was used for comparison and person correlation was used for correlation.

Chapter four

Results

Chapter four

4. Results:

A total of 100 volunteers were enrolled in this study, half of which (50 subjects) were metabolic syndrome patients as a case group with ages range from 42 – 85 years old and the other half (50 subjects) were healthy volunteers served as a control group.

Table (4.1): Represent the comparison mean ± SD of albumin, total protein, BMI, and WC in case versus control group. The result showed (4.2 ± 0.6 versus 3.8 ± 0.3 g/dl, p- value 0.000), (7.2 ± 0.5 versus 6.8 ± 0.3 g/dl, p- value 0.000), (30.2 ± 7.0 versus 22.7 ± 1.2 Km/m^2, p-value 0.000), (102.2 ± 15.2 versus 79.2 ± 2.7 Cm, p-value 0.000), respectively.

Table (4.2): Correlation between total protein levels, duration of disease, Age, BMI, WC, and albumin, among MetS group, (r=-0.188, P-value =0.190), (r = -0.01, P-value = 0.945), (r = -0.168, P-value = 0.243), (r = 0.025, P-value = 0.861). (There were no correlations). (r= 0.456, P-value =0.0000), respectively. (There were moderate positive correlation between albumin and total protein.

Table (4.3): Correlations between albumin levels, Age, BMI, WC, and duration of disease among MetS group, (r=-0.023, P-value =0.874), (r = -0.177, P-value = 0.218), (r = -0.191, P-value = 0.185), (r = -0.137, P-value = 0.343).). (There were no correlations).

Figure (4.1): Age distribution among case group, the result showed that, (60%) of patients group (56 - 70), (26%) group (40 - 55), and (14%) group (71 - 85) years.

Figure (4.2): Gender distribution among case group, the result showed that, (66%) were females while (34%) were males.

Table (4.1): Mean concentrations, and values of albumin, total protein, BMI, and WC in case and control group:

Variable	**Case (n=50) Mean ± SD**	**Control(n=50) Mean ± SD**	*P*. value
Albumin (g/dl)	4.2 ± 0.6 (2.9 – 5.1)	3.8 ± 0.3 (3.5 – 4.6)	0.000**
Total protein (g/dl)	7.2 ± 0.5 (6.1 – 7.9)	6.8 ± 0.3 (6.4 – 7.4)	0.000**
BMI (Kg/m^2)	30.2 ± 7.0 (21.7 - 50.0)	22.7± 1.2 (20 – 24.8)	0.000**
WC (Cm)	102.2 ±15.2 (80 - 149)	79.2 ± 2.7 (74 – 84)	0.000**

**: Significant at 0.0 level, ranges between bracket. Analyzed by independent T-test.

Table (4.2): Correlations between total protein levels, duration of disease, Age, BMI, waist circumference and albumin among MetS group:

Variables	Coefficient	Total protein
Duration of disease	R	-0.188
	P	0.190^{ns}
Age	R	-0.01
	P	0.945^{ns}
BMI	R	- 0.168
	P	0.243^{ns}
Waist circumference	R	0.025
	P	0.861^{ns}
Albumin	R	0.456
	P	0.000^{**}

Ns: No significant difference, **: significance at a level less than 0.01.

Table (4.3): Correlations between albumin level, Age, BMI, WC and duration of disease among MetS group:

Variables	Coefficient	Albumin
Age	R	-0.023
	P	0.874^{ns}
BMI	R	- 0.177
	P	0.218^{ns}
Waist circumference	R	- 0.191
	P	0.185^{ns}
Duration of disease	R	-0.137
	P	0.343^{ns}

Ns: No significant difference.

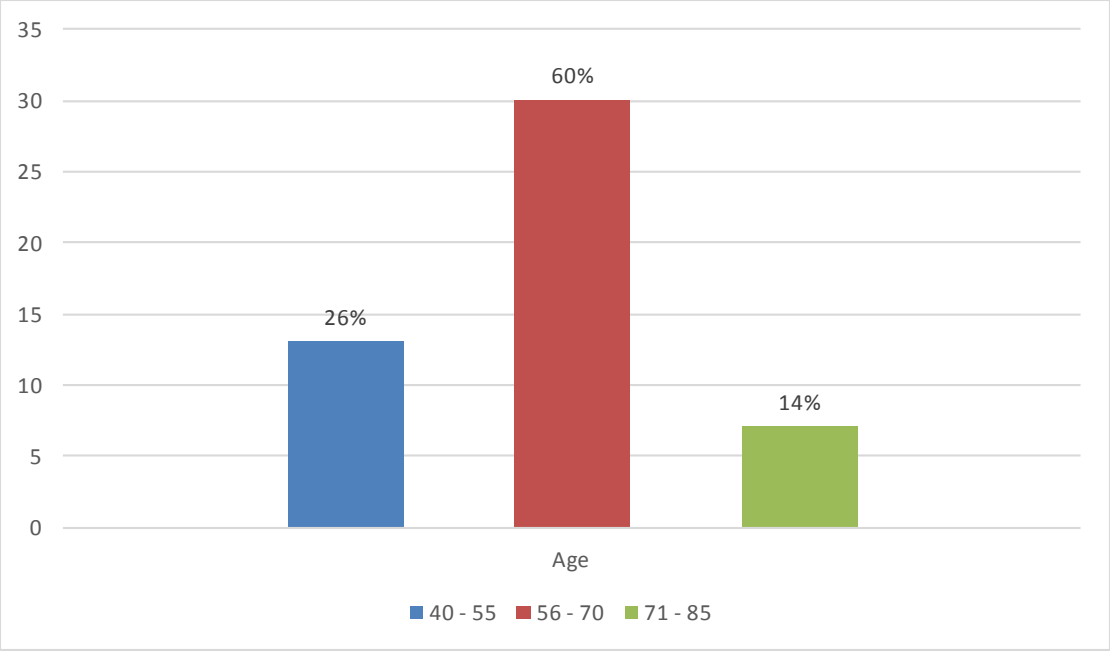

Figure (4.1): Age distribution among metabolic syndrome patients.

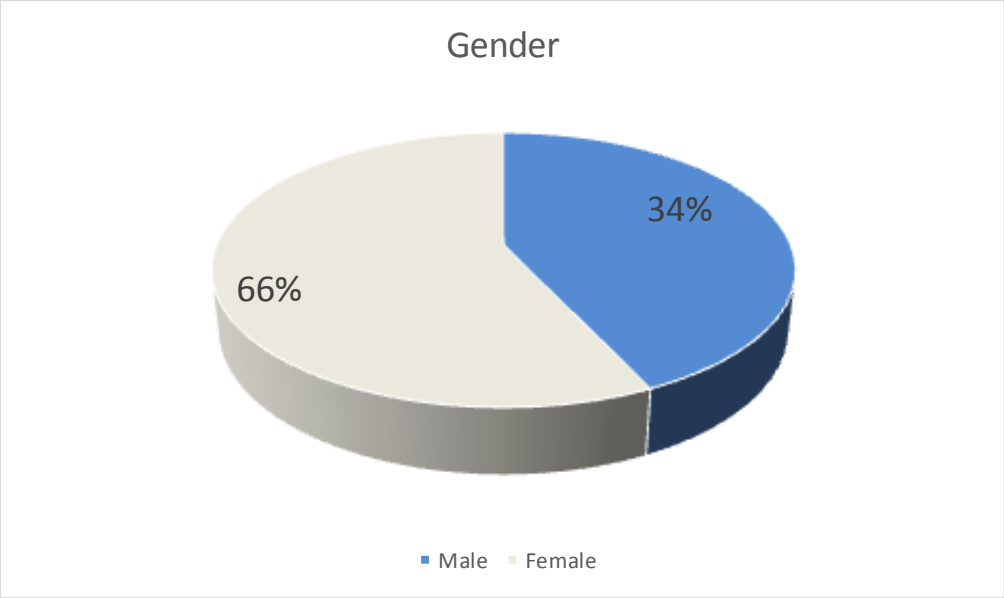

Figure (4.2): Gender distribution among metabolic syndrome patients.

Chapter five

Discussion, conclusion and recommendations

Chapter five

Discussion, Conclusion and Recommendations

5.1 Discussion:

Metabolic syndrome is a serious medical condition that increases the incidence of multiple cancers, and has effects on the quality of life. Albumin and total protein are considered to be two of the most important parameters in routine analysis and monitoring for disorders such as liver impairment and renal diseases. This study was primarily conducted to estimate albumin and total protein concentrations in Mets patients in an attempt to study the effects of metabolic syndrome on plasma albumin and total protein levels.

The findings of this study demonstrated that, Mets is most common among age group (56-70) years old. This finding agreed with another study carried by Chen *et al*, who reported that Mets is highly prevalent in the middle aged and elderly. (Chen *et al*., 2017). The findings obtained from especially designed questionnaire revealed that, Mets was more frequent in females than males. This finding agreed with another study carried by Maleki *et al* which showed that Mets patients most abundant in females than males. (Maleki *et al*., 2015).

This study showed, there were significant increases in mean values of plasma albumin, WC, BMI in Mets subjects when compared to healthy individuals (p- value 0.000). This results indicates those variables are closely related to Mets and considered as Mets components. This result agreed with study carried by (Bae *et al*., 2013); Cho *et al*., 2012), and disagree with (Augusthy *et al.*, 2016).

Bae et al explained that the increase of albumin levels in Mets subjects might be a consequence of increased albumin production in the liver under insulin resistant conditions. Insulin resistance goes hand – in - hand with increased insulin levels and insulin is known to stimulate albumin production in hepatocytes. (Peavy *et al*., 1985). Cho *et al* suggest that higher serum albumin levels are positively associated with Mets probably through increase abdominal obesity, high fasting blood glucose and triglycerides.

The study also demonstrated that, total protein concentrations was highly significant in Mets patients compared to control group (p- value 0.000).

Result of this study showed, there were moderate positive correlation between albumin and total protein level, (r= 0.456, P-value =0.0000).

Also the findings of this study showed, there were no correlation between values of albumin and study variables (age, BMI, WC, duration of disease) among Mets group. (r=-0.023, P-value =0.874), (r = -0.177, P-value = 0.218), (r = -0.191, P-value = 0.185), (r = -0.137, P-value = 0.343). Also there were no correlations between values of total protein and study variables (age, BMI, WC, duration of disease) among Mets group, (r = -0.01, P-value = 0.945), (r = -0.168, P-value = 0.243), (r = 0.025, P-value = 0.861), (r=-0.188, P-value =0.190), respectively.

5.2 Conclusion:

According to the results of this study it is concluded that:

The level of plasma total protein and albumin are increased in metabolic syndrome patients. The Mets is most abundant in females than males, and most common in age group (56 - 70) years old.

5.3 Recommendations:

1.Life style modification program such as exercise, healthy diets, low calories intake, and physical activities should be implemented in whole community specially females to reduce the susceptibility to metabolic syndrome.

2. Another parameters such amino acids, electrolytes, trace elements should be measured in Mets patients.

3. The other researches should take into account other data such as exercise, diet, and medication use for previously diagnosed hypertension and/or hyperlipidemia into account and observe its effect on the prevalence and pathogenesis of metabolic syndrome.

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Appendices (I)

بسم الله الرحمن الرحيم

Sudan University of science and technology

Collage of graduate studies

Estimation of plasma total proteins and albumin levels in metabolic syndrome patients

Questionnaire No ( )

A. **General information:**

Name: …….....……..

Gender: Male ☐ Female ☐

Weight: …… (Kg). Height: …….. (Cm).

WC…….. (Cm).

B. Clinical information:

Family history of disease: ……. ……...

HT ☐ **D.M** ☐

Duration of diseases……Month……...Years.

Life style……..

Smoker ☐ Alcoholism ☐ Tobaco ☐

Laboratory investigation:

Parameters:

Albumin……..g/dl

Total protein……..g/dl

Date: ……../……./2018 **Signature:**

Appendices (II)

COD 11547 2 x 250 mL	COD 11573 1 x 250 mL
STORE AT 2-8ºC	
Reagents for measurement of albumin concentration Only for in vitro use in the clinical laboratory	

ALBUMIN

CE

BioSystems

ALBUMIN
BROMOCRESOL GREEN

PRINCIPLE OF THE METHOD

Albumin in the sample reacts with bromocresol green in acid medium forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS

	COD 11547	COD 11573
A. Reagent S. Standard	2 x 250 mL 1 x 5 mL	1 x 250 mL 1 x 5 mL

COMPOSITION

A. Reagent: Acetate buffer 100 mmol/L, bromocresol green 0.27 mmol/L, detergent, pH 4.1.

S. Albumin Standard: Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology, USA).

STORAGE

Reagent (A): Store at 2-8ºC.

Albumin Standard (S): Store at 2-8ºC, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 630 nm (1 cm cuvette).

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 630 nm (610 - 670 nm).

SAMPLES

Serum or plasma (EDTA, citrate or heparine) collected by standard procedures.

Albumin in serum is stable for 3 days at 2-8ºC.

PROCEDURE

1. Pipette into labelled test tubes: (Notes 1, 2)

	Blank	Standard	Sample
Albumin Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 1 minute at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 630 nm against the Blank. The colour is stable for 30 minutes.

CALCULATIONS

The albumin concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

REFERENCE VALUES

Serum[2]:

Newborn, 3 to 4 days	28-44 g/L
4 days to 14 years	38-54 g/L
Adult	35-50 g/L
> 60 years	34-48 g/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own Internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.1 g/L.

– Linearity limit: 70 g/L.

– Repeatibility (within run):

Mean Concentration	CV	n
26.3 g/L	1.4 %	20
42.1 g/L	1.0 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
26.3 g/L	1.8 %	20
42.1 g/L	1.8 %	20

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.

– Interferences: Bilirubin (>10 mg/dL), lipemia (triglycerides >7.5 g/L) and hemoglobin (>2.5 g/L) may affect the results. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Albumin is the most abundant protein in human plasma. It has three main functions: it contributes towards maintaining the colloid oncotic pressure of plasma, it acts as non-specific transport vehicle for many nonpolar compounds and it is a source of endogenous amino acids.

Hyperalbuminemia is of little diagnostic significance except in dehydration[2].

Hypoalbuminemia is found as a result of several factors: reduced synthesis caused by liver diseases; reduced absorption of amino acids due to malabsorption syndromes or malnutrition; increased catabolism as a result of inflammation or tissue damage; altered distribution between intravascular and extravascular space due to increased capillary permeability, overhydration or ascites; abnormal losses caused by renal disease (nephrotic syndrome, diabetes mellitus, chronic glomerulonephritis, systemic lupus erythematosus), gastrointestinal tract disease (ulcerative colitis, Crohn's disease) or skin damage (exfoliative dermatitis, extensive burns); congenital absence of albumin or analbuminemia[2,4].

Albumin plasma concentrations, although important for management and follow-up, have very little value in diagnosis[2].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automated analysers. Instructions for many of them are available on request.
2. Albumin reaction with bromocresol green is immediate. It is not recommended to delay readings, since other proteins react slowly.
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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M11547-20

BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain)
Quality System certified according to
EN ISO 13485 and EN ISO 9001 standards

12/2008

Appendices (III)

COD 11600 1 x 50 mL	COD 11500 2 x 250 mL	COD 11572 1 x 250 mL	COD 11553 1 x 1 L
STORE AT 2-30ºC			
Reagents for measurement of protein concentration Only for in vitro use in the clinical laboratory			

PROTEIN (TOTAL)

CE

BioSystems

PROTEIN (TOTAL)
BIURET

PRINCIPLE OF THE METHOD

Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS

	COD 11600	COD 11500	COD 11572	COD 11553
A. Reagent S. Standard	1 x 50 mL 1 x 5 mL	2 x 250 mL 1 x 5 mL	1 x 250 mL 1 x 5 mL	1 x 1L 1 x 5 mL

COMPOSITION

A. Reagent: Copper (II) acetate 6 mmol/L, potassium iodide 12 mmol/L, sodium hydroxide 1.15 mol/L, detergent.

DANGER: H314: Causes severe skin burns and eye damage. P260: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

S. Protein Standard: Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology, USA).

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Reagent (A): Store at 2-30ºC.

Protein Standard (S): Store at 2-8ºC, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 545 nm.

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 545 ± 10 nm.

SAMPLES

Serum or heparinized plasma collected by standard procedures. Stable for 4 weeks at 4-8ºC[2].

Anticoagulants other than heparin should not be used.

PROCEDURE

1. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Distilled water	20 µL	—	—
Protein Standard (S)	—	20 µL	—
Sample	—	—	20 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 10 minutes at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 545 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

The protein concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

REFERENCE VALUES

Serum, adults[3]:

Ambulatory	64-83 g/L
Recumbent	60-78 g/L

Concentrations are lower in child. Plasma total protein concentration is 2 to 4 g/L higher due to the presence of fibrinogen as well as some other trace proteins[4].

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18008 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own Internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 4.6 g/L.

– Linearity limit: 150 g/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
44 g/L	1,1 %	20
57 g/L	0,9 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
44 g/L	1,8 %	25
57 g/L	1,9 %	25

– Sensitivity: 5 mA·L/g

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.

– Interferences: Hemoglobin (2.5 g/L) and lipemia interfere. Bilirubin (20 mg/dL) does not affect the results. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Most of the plasma proteins are synthesized by the liver. The major exception to this is the immunoglobulins which are produced by plasma cells found in the spleen, lymph nodes and bone marrow.

The two general causes of alterations of serum total protein are a change in the volume of plasma water and a change in the concentration of one or more of the serum proteins.

Hyperproteinemia can be caused by dehydration (inadequate water intake, severe vomiting, diarrhea, Addison's disease, diabetic acidosis) or as a result of an increase in the concentration of specific proteins (immunoglobulins in chronic infections, multiple myeloma)[4].

Hypoproteinemia may be caused by hemodilution (salt retention syndromes, massive intravenous infusions), by an impaired synthesis (severe malnutrition, chronic liver disease, intestinal malabsorptive disease), or by an excessive protein loss due to a chronic kidney disease or severe burns[4].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automated analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analysers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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MT13001-23

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09/2018