Sudan University of Science and Technology
College of Graduate Studies

Plasma Magnesium and Phosphorus Levels among Patients with Metabolic Syndrome in Khartoum State

مستوى الماغنسيوم و الفوسفات لدى المرضى المصابين بالمتلازمة الايضية في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirement of master degree in Medical Laboratory Science (Clinical Chemistry)

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الآيـــــــــــــة

قَالَ تَعَالَىٰ:

﴿ ءَامَنَ ٱلرَّسُولُ بِمَآ أُنزِلَ إِلَيۡهِ مِن رَّبِّهِۦ وَٱلۡمُؤۡمِنُونَۚ كُلٌّ ءَامَنَ بِٱللَّهِ وَمَلَٰٓئِكَتِهِۦ وَكُتُبِهِۦ وَرُسُلِهِۦ لَا نُفَرِّقُ بَيۡنَ أَحَدٖ مِّن رُّسُلِهِۦۚ وَقَالُواْ سَمِعۡنَا وَأَطَعۡنَاۖ غُفۡرَانَكَ رَبَّنَا وَإِلَيۡكَ ٱلۡمَصِيرُ ٢٨٥ ﴾

سورة البقرة الآية (285)

Dedication

To my Parents

My lovely sister Hiba

My Friends Toga , Saja and Shyma

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All and first thanks to the almighty ALLAH. Then I would like to express my gratitude and ever last appreciation to my supervisor **Dr. Nuha Eljaili Abubaker** for this guidance, helpful suggestions for solving problems, valuable supervision as well as precious advice, support continues assistance through the whole process of this research.

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Abstract

Metabolic Syndrome is a serious medical condition with increase risk of multiple cancers, has effects on the life quality. It is a problem found all over the world and Sudan. Also it is fatal unless controlled and treated properly.

A case control study conducted to compare the plasma level of magnesium and phosphorus of 50 randomly selected patients with metabolic syndrome as case group(age range 40-80 years) and 50 apparently healthy as control group (matched age and gender). Participants in this study were from Almotakamel center and Zinam Specialist Hospital in Khartoum state, Sudan. The plasma level of magnesium and phosphorus were measured using Mindray BS-200 (full automation instrument). The results were analyzed using statistical package for social science (SPSS version 16) computer program.

The study showed that, metabolic syndrome most common among age group (61-70) years (40%) and most abundant in females (62%) than males (38%).

The study showed that, there were significant decreased in mean of magnesium and phosphorus, while the mean of Waist Circumference and Body mass index (BMI) significantly increased in cases than control group (1.98±1.22 than 2.15±0.33mg/dl, P-value 0.000), (2.55±1.12 than 3.13±0.76mg/dl, P-value 0.003), (107.8±9.83 than 99.58±11.69cm, P-value 0.000) (30.02±5.05 than 27.42±4.59kg/m2, P-value 0.008) respectively.

There was insignificant difference in the mean of plasma magnesium and phosphorus level between males and females of metabolic syndrome

patients group (2.15±1.62 versus 1.66±0.62 *P*-value 0.165) (2.51±1.07 versus 2.62±1.42 *P*-value 0.730) respectively.

The study also showed, there was no correlation between plasma level of magnesium, BMI and age(r= 0.096, *P*-value= 0.508) (r= 0.058, *P*-value =0.687) respectively.

The study also showed that, there was no correlation between plasma level of phosphorus, BMI and age(r= -0.199, *P*-value=0.166) (r= -0.221, *P*-value=0.122) respectively.

The present study concluded that:

Patients with metabolic syndrome had lower concentration of magnesium and phosphorus, also metabolic syndrome is more common in females than males and more frequent in elderly.

المستخلص

المتلازمة الايضية حالة مرضية صحية خطيرة تهدد حياة الانسان ومساهمته في بناء المجتمع وتطويرية وهذة المتلازمة توجد في كل انحاء العالم والسودان بنسب كبيرة لذا توجب علينا دارستها لمعرفة كيفية تجنبها وتفادي مضعفاتها الخطيرة.

قارنت هذة الدراسة مستوى الماغنسيوم و الفوسفات في بلازما الدم عند ٥٠ من مجموعة المرضى السودانين المصابين الايضية (الفئة العمرية٤٠-٨٠) و٥٠ من مجموعة الاصحاء(كلا المجموعتين متطابقتين في العمر و النوع). كل المشاركين في هذة الدراسة كانو من مركز المتكامل و مستشفى زينام التخصصي في الخرطوم.

تم قياس مستوى الماغنسيوم والفوسفات باستخدام جهاز ميندري كامل الالية وتم تحليل البيانات بواسطة برنامج الحزم الإحصائية للعلوم الإجتماعية.

وجدت هذة الدراسة ان المتلازمة الايضية اكثر شيوعا في الفئة العمرية(٦١-٧٠)و اكثر شيوعا بين الاناث (٦٢٪) مقارنة بالذكور(٣٨٪)كان هنالك نقصان ذو دلالة احصائية في مستوى الماغنسيوم والفوسفات و ارتفاع في محيط البطن ومؤشر كتلة الجسم في مجموعة المرضى مقارنة بالاصحاء (١,٢٢ ± ١,٩٨مقابل ٢,١٥±٠,٣٣ مليغرام/ديسليتر ،الاحتمال الاحصائي للمقارنة ٠,٠٠٠) (٢,٥٥±١,١٢ مقابل ٣,١٣±٠,٧٦ مليغرام/ديسليتر ،الاحتمال الاحصائي للمقارنة٠,٠٠٣) (١٠٧±٩,٨٣ مقابل٩٩,٥٨±١١,٦٩سم،الاحتمال الاحصائي للمقارنة٠,٠٠٠) (٣٠,٠٢٥,٠٥± مقابل ٢٧,٤٢±٤,٥٩كيلوغرام/مترمربع،الاحتمال الاحصائي للمقارنة ٠,٠٠٨)على التوالي.

كما لا يوجد اختلاف في مستوى الماغنسيوم و الفوسفات بين الذكور و الاناث في مجموعة المرضى المصابين بالمتلازمة الايضية(٢,١٥±١,٦٢مقابل ١,٦٦±٠,٦٢ مليغرام/ديسليتر ،الاحتمال الاحصائي للمقارنة ٠,١٦٥) (٢,٥١±١,٠٧مقابل ٢,٦٢±١,٤٢ مليغرام/ديسليتر ،الاحتمال الاحصائي للمقارنة٠,٧٣٠) على التوالي.

ايضا لا يوجد ارتباط بين مستوى الماغنسيوم في بلازما الدم و محيط البطن و العمر في مجموعة المرضى المصابين بالمتلازمة الايضية (معامل بيرسون٠,٠٩٦ ومستوى المعنوية ٥٠٨,٠)(معامل بيرسون٠,٠٥٨ ومستوى المعنوية٠,٦٨٧).

ايضا لا يوجد ارتباط بين مستوى الفوسفات في بلازما الدم و محيط البطن و العمر في مجموعة المرضى المصابين بالمتلازمة الايضية(معامل بيرسون-٠,١٩٩ومستوى المعنوية٠,١٦٦)(معامل بيرسون -٠,٢٢١ ومستوى المعنوية٠,١٢٢).

وعليه خلصت الدراسة إلي أنه:

مرضى المتلازمة الايضية لديهم مستوى تركيز منخفض للماغنسيوم و الفوسفات كما ان هذة المتلازمة اكثر شيوعا بين الاناث مقارنة بالذكو ر واكثر شيوعا بين البالغين.

List of abbreviations

BMI	Body Mass Index
DM	Diabetes Mellitus
Cm	Centimeter
Mets	Metabolic syndrome
WC	Waist Circumference
ATP	Adenosine triphosphate
EGTA	Ethylene glycol tetraacetic acid

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Chapter one

Introduction

Chapter one

1-Introduction, Rationale and Objectives

1.1Introduction

Metabolic syndrome defined according to American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria as any three of the following: Waist circumference (102cm or greater in men, 88cm or greater in women), Triglycerides 150mg/dl or greater, HDL-cholesterol (< 40mg/dl in men and < 50mg/dl in women), BP 130/85mmHg or greater, Fasting glucose >110mg/dl (Shankar and Sundarka, 2003; Vyssoulis, *et al.* 2010; Kassi, *et al.* 2011; Razeena, *et al*. 2016).

Phosphorus is the most abundant anion in the body and comprises approximately1% of total body weight (Penido and Alon, 2012). It is a predominantly intracellular anion where its concentration is 100-fold greater than that in the plasma. Present in high concentration in many foods especially dairy products , meat and vegetables (Penido and Alon, 2012). The average intake of phosphate is about 50mmol/Day (Manghat, *et al.* 2014).

Magnesium is the second most abundant intracellular cation in the human body and is necessary as a cofactor for hundreds of enzymes, particularly for those cellular reactions involved in the transfer, storage, and utilization of energy. Also involved in the metabolism of proteins, carbohydrates and lipids, in stabilizing nucleic acids, and influencing the permeability of cell membranes. Rich in many unprocessed foods, such as whole grains and green leafy vegetables (Rotter, *et al.* 2015).

Patients with metabolic syndrome show significantly lower phosphate and magnesium concentrations compared with individuals who do not fulfill criteria for the diagnosis of this syndrome.

1.2 Rationale

Metabolic syndrome (Mets) represents a cluster of cardiovascular risk factors that recently has become a public health problem of epidemic proportions. Mets confers a 5-fold increase in the risk of type 2 diabetes mellitus and risk of developing cardiovascular disease (CVD) over the next 5 to 10 years. Further, patients with Mets are at increased risk of stroke, myocardial infarction (MI), and dying from such an event compared with those without the syndrome (Kaur, 2014). This problem found all over the world and Sudan and it is fatal unless controlled and treated properly. The worldwide prevalence of Mets in the adult population is estimated between 20%and 25% (Vidigal, *et al.* 2015)

Both phosphate and magnesium are vital to carbohydrate metabolism, it is possible that the reduced levels of these ions in patients with metabolic syndrome may decrease the peripheral utilization of glucose, thus leading to the development or exacerbation of insulin resistance (Kalaitzidis, *et al.* 2005). The prevalence of metabolic syndrome is increasing in epidemic proportions in both developed and developing countries. Not puplished yet in Sudan.

1.3 Objectives

1.3.1 General Objective

To assess plasma phosphorus and magnesium levels among Sudanese patients with metabolic syndrome.

1.3.2 Specific objectives

1-To measure and compare the mean concentration of plasma phosphorus and magnesium levels in study groups.

2-To measure BMI by height and weight in study groups.

3-To assess relationship between plasma levels of phosphorus, magnesium and study variables (BMI, age and gender).

Chapter two

Literature review

Chapter two

2-Literature review

2.1 Metabolic syndrome

Metabolic syndrome represents a cluster of related metabolic abnormalities, including central obesity, hypertension, dyslipidemia, hyperglycemia, and insulin resistance, with central obesity in particular recognized as causative factors (Srikanthan, *et al.* 2016). These metabolic derangements present significant risk factors for both atherosclerotic cardiovascular disease (as the primary clinical outcome) and type2 diabetes mellitus (Kassi, *et al*. 2011; Abdalla, *et al*. 2014; Kaur, 2014). The commonly observed aggregation of metabolic risk Factors has gone by several different names: syndrome X, insulin resistance syndrome, pre-diabetes, metabolic syndrome, dysmetabolic syndrome, cardio metabolic syndrome, dyslipidemic hypertension, hypertriglyceridemic waist, and deadly quartet (Abdalla, *et al*. 2014).

2.1.1 Pathophsiology of metabolic syndrome

Abdominal obesity and insulin resistance are viewed as the core defects underlying the pathophysiology of metabolic syndrome. These two risk factors are highly interrelated; therefore, it is difficult to ascertain which one plays the predominant role in metabolic syndrome pathogenesis and progression. In addition, metabolic syndrome pathophysiology is complicated by contributing factors such as dysregulation of adipose tissue–derived cytokines, inflammation, genetics, race/ethnicity, physical inactivity, diet, hormone imbalances, drugs, and age. As such, it is unrealistic to assume that metabolic syndrome is caused by a single underlying defect. Instead, it is the combination of numerous risk factors, primarily driven by obesity and insulin resistance, which gives rise to the development of this clustering of metabolic health risk (Aganović and Dušek, 2005).

2.1.1.1 Obesity

Some experts view metabolic syndrome as the metabolic complications of obesity. Obesity is associated with numerous adverse health consequences such as cardiovascular disease, type 2 DM, hypertension, dyslipidemia, and insulin resistance. Adipose tissue is a metabolically active endocrine organ. Adipocytes release a variety of substances into the circulation including, but not limited to, free fatty acids, interleukin-6, tumor necrosis factor alpha, lipoprotein lipase, and angiotensinogen (Aganović and Dušek, 2005). Adipose tissue derived proteins cause alterations in glucose and lipid metabolism in muscle, liver, and fat. Furthermore, these substances promote local and systemic inflammatory and thrombotic states. Release of non-esterified fatty acids from adipose tissue is increased in obese states. Elevated circulating concentrations of free fatty acids result in diminished hepatic and muscle insulin sensitivity, increased hepatic cholesterol production, and altered endothelial function. The inflammatory cytokines interleukin-6 and tumor necrosis factor alpha impair insulin signaling and lead to insulin resistance. Furthermore, adipose tissue–derived interleukin-6 stimulates C-reactive protein production in the liver which is an acute-phase reactant and a major biomarker of the chronic low-grade inflammation present in obesity and metabolic syndrome (Aganović and Dušek, 2005).

Excess adipose tissue is recognized as a major contributor to metabolic syndrome; however, the location of this fat is also an important consideration. Visceral fat delivers free fatty acids and cytokines directly to the liver through the portal circulation; consequently, intra-abdominal fat is more closely tied to metabolic risk factors than subcutaneous fat. Taken together, adipose tissue (particularly fat in the visceral compartment) is an active endocrine organ that secretes a variety of substances that mediate the unfavorable metabolic, inflammatory, and thrombotic environment of metabolic syndrome (Aganović and Dušek, 2005).

2.1.1.2 Insulin resistance

a physiologic state in which the ability of target tissues (e.g., muscle, liver, fat) to respond to the normal actions of insulin is diminished. Consequently the ability of insulin to promote glucose uptake, inhibit hepatic glucose production, and suppress lipolysis in target tissues is decreased. Compensatory hyperinsulinemia is often present in insulin-resistant states as the body works to maintain glucose homeostasis. However, with time, the pancreas is often unable to secrete sufficient amounts of insulin to maintain glucose homeostasis. As a result, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or type 2 DM may ensue (Aganović and Dušek, 2005).

Excess free fatty acids are thought to be responsible for the development of insulin resistance in obesity and metabolic syndrome. In turn, a vicious cycle ensues whereby insulin resistance further promotes adipose tissue lipolysis, resulting in even greater release of free fatty acids into the circulation. Hepatic insulin resistance results in increased triglyceride and apolipoprotein B (apoB) production and in decreased high-density lipoprotein cholesterol (HDL-C), all of which are characteristic lipid abnormalities observed in metabolic syndrome. Insulin resistance also causes diminished glucose uptake in muscle and fat and causes increased glucose production in the liver. With time, this contributes to overt glucose abnormalities such as prediabetes or type 2 diabetes mellitus. Insulin resistance and hyperinsulinemia are associated with over activity of the sympathetic nervous system, increased sodium reabsorption in the kidney, and decreased vasodilation, all of which may contribute to the development of hypertension (Aganović and Dušek, 2005). In addition, through alterations in insulin signaling and the expression of cytokines, insulin resistance is thought to contribute to the inflammatory and thrombotic states observed in metabolic syndrome. The broad physiologic effects of insulin resistance on metabolic risk

factors have spurred some experts to view it as the underlying cause of metabolic syndrome (Aganović and Dušek, 2005).

2.1.2 Risk factors of metabolic syndrome

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

2.1.2.1 Stress

Prolonged stress can be an underlying cause of metabolic syndrome by upsetting the hormonal balance of the hypothalamic-pituitary-adrenal axis (HPA axis). Adysfunctional HPA axis causes high cortisol levels to circulate, which results in raising glucose and insulin levels, which in turn cause insulin mediated effects on adipose tissue, ultimately promoting visceral adiposity, insulin resistance, dyslipidemia and hypertension. This also may explain the reported risk indication of abdominal obesity to cardiovascular disease type2 diabetes and stroke (Abdalla, *et al*. 2014).

2.1.2.2 Obesity

Central obesity is a key feature of the syndrome, reflecting the fact that the syndrome's prevalence is driven by the strong relationship between waist circumference and increasing adiposity. However, despite the importance of obesity, patients who are of normal weight may also be insulin resistant and have the syndrome (Abdalla, *et al*. 2014).

2.1.2.3 Sedentary Life style

Many components of metabolic syndrome are associated with a sedentary life style, including increased adipose tissue (predominantly central), reduced HDL cholesterol, and a trend toward increased triglycerides, glucose in the genetically susceptible. Compared with individuals who watched television or videos or used their computers for less than one hour daily, those who carried out these behaviors for greater than four hours daily have a twofold increased risk of metabolic syndrome (Abdalla, *et al*. 2014).

2.1.2.4 Aging

Metabolic syndrome affects 44% of the U.S Population older than age of 50 years (Abdalla, *et al*. 2014).

2.1.2.5 Diabetes mellitus

It is estimated that majority of patients (~75%) with type2 diabetes or impaired glucose tolerance have the metabolic syndrome. The presence of metabolic syndrome in these populations is associated with a higher prevalence of CVD than found in patients with type 2 diabetes of impaired glucose tolerance without the syndrome (Abdalla, *et al*. 2014).

2.1.2.6 Coronary heart disease

The approximate prevalence of MS in patients with coronary heart disease is 50%, with a prevalence of 37% in patients with premature coronary artery disease, particularly in women. With appropriate cardiac rehabilitation and changes in lifestyle, the prevalence of the syndrome can be reduced (Abdalla, *et al*. 2014).

2.1.3 Signs and symptoms of metabolic syndrome

a. Metabolic syndrome has no symptoms, although a large waist circumference (central obesity) is a visible sign.

b. Blood sugar is very high, might have signs and symptoms of diabetes (including increased thirst and urination, fatigue, and blurred vision.)

c. Impaired fasting glucose, insulin resistance, or prediabetes.

d. High blood pressure.

e. Decreased fasting serum HDL cholesterol.

f. Elevated fasting serum triglyceride level (Knowler, 2002).

2.1.4 Diagnosis of metabolic syndrome

Several organizations have criteria for diagnosing metabolic syndrome. The 2001 National Cholesterol Education Program Adult Treatment Panel

guidelines (NCEP ATP III) diagnose metabolic syndrome if have three or more of these traits:

2.1.4.1 Large waist circumference

Waist circumference (102cm or greater in men, 88cm or greater in women)

2.1.4.2 High triglyceride level

Triglycerides 150 mg/dl or greater (Reis, *et al*. 2008).

2.1.4.3 Reduced high-density lipoprotein (HDL) cholesterol

Less than 40mg/dL, (1.04mmol/L) in men or less than 50 mg/dL in women (1.3 mmo1/L).

2.1.4.4 Increased blood pressure

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

2.1.4.5 Elevated fasting blood sugar

>110mg/dL (includes diabetes) (Reis, *et al*. 2008).

2.1.5 Treatment of metabolic syndrome

Pharmacological treatment should be considered for those whose risk factors are not adequately reduced with the preventive measures and lifestyle change. Most physicians treat each component of Mets separately. In fact, it is easier to prescribe a drug to lower blood pressure, blood glucose, or triglycerides rather than initiating a long-term strategy to change people's lifestyle(exercise more and eat better) in the hope that they will ultimately lose weight and tend to have a lower blood pressure, blood glucose, and triglycerides (Kaur, 2014).

2.1.6 Management of the metabolic syndrome

Weight reduction: reduction in daily fat intake is essential. Saturated fat (mainly dairy and animal fat) worsens insulin resistance and increases LDL cholesterol level. Trans-unsaturated fatty acids (formed when vegetable oils are hydrogenated) behave similarly. Therefore, their daily intake should be

restricted to 7-10% of caloric intake. Dietary cholesterol should be restricted to less than 200 mg/day (Shankar and Sundarka, 2003).

Exercise: Regular exercise improves insulin sensitivity, decreases plasma triglyceride levels, and reduces cardiovascular morbidity and mortality. Accumulating at least 30 minutes of daily physical activity is enough to help reduce and maintain body weight. The activity should be in the form of aerobic exercise of moderate intensity like riding a bicycle, gardening, raking leaves, or even playing actively with kids(Shankar and Sundarka, 2003).

2.2 Plasma phosphorus

an essential element and plays an important role in multiple biological processes**.** The majority of phosphate is present in bone and teeth (85 %), with the remainder distributed between other tissues (14 %) and extracellular fluid (1%) (Manghat, *et al*. 2014).

In the skeleton, phosphate is primarily complexed with calcium in the form of hydroxyapatite crystals; the remaining phosphate appears as amorphous calcium phosphate. In soft tissue and cell membranes, phosphorus exists mainly as phosphate esters and to a lesser extent as phosphoproteins and free phosphate ions. In the extracellular fluid, about one-tenth of the phosphorus content is bound to proteins, one-third is complexed to sodium, calcium, and magnesium, and the remainder is present as inorganic phosphate. Serum phosphate concentration varies with age, with the highest concentration being in infants (normal range 4.5–8.3 mg/dl), who require more of the mineral for bone growth and soft tissue buildup, and concentrations declining towards adulthood (normal range2.5–4.5mg/dL (Manghat, *et al*. 2014).

Phosphorus containing compounds have important roles in cell structure (maintenance of cell membrane integrity and nucleic acids), cellular metabolism (generation of ATP), regulation of subcellular processes (cell signaling through protein phosphorylation of key enzymes), maintenance of acid–base

homeostasis (urinary buffering), and bone mineralization (Penido and Alon, 2012). Plasma phosphate concentration shows a marked diurnal rhythm. lowest in the morning and night Fasting can abolish this diurnal variation (Manghat, *et al*. 2014).

2.2.1 Phosphorus metabolism and regulation

The plasma phosphorus concentration is primarily regulated by the kidneys. Renal excretion of phosphorus is determined by the glomerular filtration rate and the maximum tubular reabsorption rate .Approximately 90% of plasma phosphorus is filtered by the glomerulus (Manghat, *et al*. 2014). Of this amount, 80% to 90% is reabsorbed in the renal tubules. Most renal phosphorus reabsorption occurs in the proximal convoluted tubule, with small amounts of phosphorus being reabsorbed in the distal nephron. Reabsorption is sodium dependent because phosphorus transport is performed by a brush border sodium-phosphate cotransporter (Manghat, *et al*. 2014). The sodium gradient is maintained by a sodium–phosphate adenosine triphosphatase and makes phosphorus reabsorption indirectly energy dependent. Maximal tubular reabsorption of phosphorus can normally be saturated, resulting in phosphaturia when excess phosphorus enters tubular fluid. The regulatory mechanism for renal phosphorus reabsorption can adapt to the body’s need for phosphorus through parathyroid hormone (PTH), the major hormonal regulator. PTH reduces reabsorption of phosphorus by decreasing the maximal tubular reabsorption rate in the proximal tubule while it enhances calcium reabsorption in the distal tubule. Thus PTH release results in an increased calcium concentration and phosphaturia. Low blood levels of PTH result in the opposite. The major stimulus for increased PTH synthesis and secretion is a reduced plasma calcium concentration. The major inhibitors of PTH synthesis and secretion are increased concentrations of plasma calcium and 1.25-dihydroxycholecalciferol, the active form of vitamin D. In the presence of

phosphorus depletion, the kidneys conserve phosphorus, and the renal response to the phosphaturic effect of PTH is blunted (Manghat, *et al*. 2014).

Although the kidneys are the major regulator of the plasma phosphorus concentration, ultimately, the concentration is the combined effect of intestinal absorption and excretion, bone resorption and accretion, and renal excretion and reabsorption. The skeleton functions as a reservoir from which phosphorus is mobilized during states of hypophosphatemia. Absorption of dietary phosphorus is approximately 80% and occurs by passive diffusion and by active transport using a sodium–phosphate cotransporter. Absorption occurs in the small intestine, primarily in the mid jejunum. Because of intestinal phosphorus excretion, a total of about 30% to 40% of ingested phosphorus is excreted in the feces .Decreased intestinal absorption of phosphorus occurs in vitamin D deficiency, in malabsorption syndromes (e.g., steatorrhea, pancreatitis, lymphangiectasia), and with a phosphorus-deficient diet. In addition, substances containing iron, aluminum (e.g., aluminum hydroxide), or unsaturated fatty acids interfere with intestinal phosphorus absorption (Hooft, *et al*. 2005). The active form of vitamin D, produced by epithelial cells of the proximal convoluted tubules in the kidneys, increases intestinal absorption of phosphorus. It also increases plasma phosphorus concentrations by stimulating bone resorption and possibly by some small contribution of increased renal tubular resorption (Hooft, *et al*. 2005).

Renal production of active vitamin D is increased by PTH and a low dietary content of phosphorus and/or hypophosphatemia. Renal synthesis is also increased by growth hormone, estrogen, and prolactin, which are important hormones during growth, pregnancy, and lactation, respectively. Renal production of active vitamin D is inhibited by hyperphosphatemia, hypercalcemia, and renal diseases characterized by loss of renal tubular mass (Hooft, *et al*. 2005).

2.2.2 Clinical significant of plasma phosphorus

Disorders of phosphate homeostasis occur in a wide range of clinical conditions. Both hyper and hypophosphatemia can be caused by cellular shifts of phosphate. The three primary conditions that lead to phosphate dysfunction are dietary intake, GI, and renal status (Manghat, *et al*. 2014).

2.2.2.1 Hyperphosphatemia

Serum phosphorus ≥ 4.4 mg/dl is associated with increased risk for chronic kidney disease and cardiovascular disease (Wojcicki, 2013). Occurs due to increased phosphate load due to endogenous or exogenous sources that exceeds the ability of renal excretory ability, or decreased urinary excretion of phosphate (Ghosh and Joshi, 2008).

Causes of hyperphosphatemia includes following:

First: Pseudohyperphosphatemia

Haemolysis, jaundice and lipaemia can interfere in some methods of phosphate measurement and cause pseudohyperphosphatemia. Also if blood samples are left to stand for prolonged periods (usually more than 4–6 h), phosphate will move out of blood cells and cause a false elevation (Manghat, *et al*. 2014).

Second: Increased phosphate intake

A very high intake of phosphate can lead to hyperphosphataemia especially if the renal function is poor. Also Vitamin D intoxication can cause hyperphosphataemia due to increased absorption of phosphate from the gastrointestinal tract and reduced renal phosphate excretion as a result of suppression of parathyroid caused by hypercalcaemia (Manghat, *et al*. 2014).

Third: Transcellular shift

Tumour lysis syndrome due to lysis of tumour cells by cytotoxic drugs can cause severe hyperphosphatemia. This syndrome is typically seen during

treatment of acute lymphoblastic leukaemia, probably due to the higher phosphate content in blast cells. A similar syndrome has been described after anti-infective treatment of leishmaniasis (Manghat, *et al*. 2014).

Fourth: Reduced renal excretion

Renal failure is the commonest cause of hyperphosphataemiain patients with CKD, the capacity to excrete phosphate decreases due to the reduction in the number of functioning nephrons. Also may be due to reduced PTH as in hypoparathyroidism which may be due to surgical removal of the parathyroid glands, autoimmune destruction of parathyroid glands or due to inherited disorders (Manghat, *et al*. 2014).

Fifth: Genetic causes of hyperphosphataemia (Manghat, *et al*. 2014).

2.2.2.2 Hypophosphatemia

Serum phosphate concentration less than 0.80mmol/L in adults defined as hypophosphataemia (Manghat, *et al*. 2014).

Causes of hypophosphataemia includes following:

First: Pseudohypophosphataemia

Spuriously low serum phosphate can arise due to analytical or pre-analytical factors. Gross elevation of leucocytes can cause hypophosphataemia in addition to hypokalaemia. Also high bilirubin concentration has been shown to interfere in the bichromatic analysis of phosphate but this was not seen in methods where a reducing agent was used (Manghat, *et al*. 2014)..

Second: Reduced absorption

It is unusual for reduced dietary intake alone to cause hypophosphataemia because there is usually renal and/or intestinal adaptation. Malabsorption or phosphate binding drugs can cause hypophosphataemia. It takes approximately three months for hypophosphataemia to develop in healthy subjects given a low

phosphate diet and antacid. Long-term use of antacids can lead to osteomalacia (Manghat, *et al*. 2014).

Third: Transcellular shift

Respiratory alkalosis and administration of carbohydrates, which are the commonest causes of hypophosphataemia, result from shift of phosphate into cells. Alkalosis, both respiratory and metabolic causes a decrease in serum phosphate; however, the effect is greater with respiratory alkalosis. Respiratory alkalosis causes intracellular CO2 to decrease, causing an intracellular alkalosis, which increases glycolysis, via stimulation of the key glycolytic enzyme phosphofructokinase. This leads to a reduction in intracellular phosphate and a consequent shift of phosphate into cells. In respiratory alkalosis serum phosphate can fall to <0.30 mmol/L. Serum phosphate falls within 20 min of hyperventilation and it persists for 90 min after the ventilation returns to normal. Carbohydrate administration has been shown to account for 40–43% of cases of hypophosphataemia (Manghat, *et al*. 2014).Even small amounts of intravenous glucose (4% or 5% dextrose) can cause significant hypophosphataemia. If there is accompanying hyperventilation, the fall in serum phosphate is greater. Infusion of carbohydrates increases insulin release, which causes a shift of phosphate into cells so the degree of hypophosphataemia is related to the amount of carbohydrates infused (Manghat, *et al*. 2014).

Fourth: Increased loss of phosphate

Is seen in more than 90% of patients after renal transplantation. The main mechanism of hypophosphataemiain this case is suggested to be increased renal loss as shown by an increased fractional excretion of phosphate. Primary, secondary and tertiary hyperparathyroidism cause an increased urinary loss of phosphate leading to hypophosphataemia (Manghat, *et al*. 2014).

Hypophosphataemia in vitamin D deficiency is usually more severe than that in primary hyperparathyroidism because of the relatively higher PTH concentration in the former. Decreased reabsorption of phosphate by proximal tubules is seen in renal tubular disorders such as Fanconi syndrome (Manghat,*et al*. 2014).

Fifth: Hypophosphataemia after hepatic surgery

Is observed almost invariably after hepatic surgery. Serum phosphate starts to decrease on the first or second post-operative day and returns to normal by the ninth day. The mechanism for hypophosphataemia post-hepatic surgery is not known, but it has been suggested that uptake of phosphate by rapidly regenerating liver cells may be a possible cause (Manghat,*et al*. 2014).

Sixth: Genetic disorders causing hypophosphataemia (Manghat,*et al*. 2014).

2.3 Plasma magnesium

Is the fourth most abundant cation in the body and second most abundant intracellular ion. Normal total plasma magnesium concentration varies in a narrow range (1.7–2.4mg/dL) (Khairi, *et al.* 2014).

About 99% of total body magnesium is located in bone, muscles and non-muscular soft tissue. The magnesium content of bone decreases with age, and magnesium stored in this way is not completely bioavailable during magnesium deprivation (Sarrafzadegan, *et al.* 2016).

Intracellular magnesium concentrations range from 5 to 20mmol/L; 1–5% is ionized, the remainder is bound to proteins, negatively charged molecules and adenosine tri-phosphate (ATP). Extracellular magnesium accounts for ~1% of total body magnesium which is primarily found in serum and red blood cells (RBCs). Serum magnesium can -just like calcium-be categorized into three fractions. It is either free/ionized, bound to protein or complexed with anions such as phosphate, bicarbonate and citrate or sulphate. Of the three fractions in

plasma, however, ionized magnesium has the greatest biological activity (Sarrafzadegan, *et al.* 2016).

Magnesium is primarily found within the cell where it acts as a counter ion for the energy-rich ATP and nuclear acids. It is an essential cofactor of more than 300 enzymatic reactions so it critically stabilizes enzymes, including many ATP-generating reactions. Thus, one should keep in mind that ATP metabolism, muscle contraction and relaxation, normal neurological function and release of neurotransmitters are all magnesium dependent. It is also important to note that magnesium contributes to the regulation of vascular tone, heart rhythm, platelet-activated thrombosis and bone formation (Sarrafzadegan, *et al.* 2016).

2.3.1 Magnesium metabolism and regulation

Many studies have shown intestinal Mg absorption is balanced against renal Mg excretion. In times of a temporary Mg deficit, the body depends on the availability of Mg in bone to maintain constant serum levels. Therefore, Mg homeostasis depends on three organs: the intestine, facilitating Mg uptake; bone, the Mg storage system of the body and the kidneys, which are responsible for Mg excretion (Gommers, *et al*. 2016).

a. Intestinal magnesium uptake

In healthy people, Mg plasma concentrations range between 0.65 and 1.05mmol/L. In order to maintain these levels, a daily Mg intake of 320 mg for men and 420 mg for women is recommended by the US Food and Nutrition Board. Approximately 30–50% of dietary Mg is absorbed by the intestine. However, when Mg intake is low, the absorption percentage can rise to~80%. Mg absorption takes place mainly in the distal small intestine and in the colon (Gommers, *et al*. 2016).

b. Magnesium storage

While Mg can be stored in muscle fibers, where it plays an important role in the regulation of muscle contraction by antagonizing the action of Ca, bone tissue is the largest Mg store in the human body, where it also contributes to the density and strength of the skeleton. Depletion of Mg is, therefore, a risk factor for osteoporosis. A model of Mg induced bone loss has been proposed in which low blood plasma concentrations lead to activation of bone resorption by osteoclasts and decreased osteoblast bone formation. Moreover, bone surface Mg concentrations (of~30%) are closely related to serum Mg concentrations, indicating a continuous exchange of Mg between bone and blood (Gommers, *et al*. 2016)

c. Magnesium reabsorption in the kidney

The kidney plays a major role in magnesium homeostasis and the maintenance of plasma magnesium concentration. Urinary magnesium excretion normally matches net intestinal absorption and is ~4mmol/d (100 mg/day). Regulation of serum magnesium concentration is achieved mainly by control of renal magnesium reabsorption. Under normal circumstances, when 80% of the total plasma magnesium is ultra-filtrable, 84mmol of magnesium is filtered daily and 95% of this reabsorbed, leaving about 3-5mmol to appear in the urine. Under normal circumstances, approximately only 20% of filtered magnesium is reabsorbed in the proximal tubule, whereas 60% is reclaimed in the cortical thick ascending limb (TAL) of loop of Henle and the remaining 5-10% in the distal convoluted tubule (DCT) (Seo and Park, 2008).

2.3.2Clinical significant of plasma magnesium

2.3.2.1 Hypermagnesaemia

Defined as a serum magnesium level more than 2.6mg/dl**.** At higher concentrations, magnesium might lead to neuromuscular dysfunction, ranging from drowsiness to respiratory depression, hypotonia, areflexia and coma in severe cases (Seo and Park, 2008).

Causes of Hypermagnesaemia includes following:

First: Excessive intake

Hypermagnesemia has often been described with the use of magnesium containing cathartics for treatment of drug overdose, antacids for therapeutic purposes and following rectal administration of magnesium, even in the presence of normal renal function (Seo and Park, 2008).

Second: Renal failure

Hypermagnesemia is common in patients with end stage renal disease, in those undergoing dialysis and in acute renal failure. In chronic renal failure, serum magnesium concentration is usually maintained until the glomerular filtration rate falls below 30mL/min. However, severe hypermagnesemia may result, especially if magnesium- containing medications are used. In patients undergoing regular dialysis, the serum magnesium concentration is directly related to the dialysate magnesium concentration (Seo and Park, 2008).

Third: Miscellaneous causes

Lithium therapy causes mild hypermagnesemia as well as hypercalcaemia. Mild hypermagnesemia has also been seen in hypothyroidism and Addison's disease (Seo and Park, 2008).

2.3.2.2 Hypomagnesaemia

Is generally defined as serum magnesium less than 1.8 mg/dl (Chaudhary, *et al*. 2018). As magnesium deficiency is usually secondary to other disease processes or drugs, the features of the primary disease process may complicate or mask magnesium deficiency. Signs and symptoms of magnesium deficiency are usually not seen until serum magnesium decreases to 0.5mmol/L or lower (Seo and Park, 2008).

Causes of Hypomagnesaemia includes following:

First: Hypomagnesaemia due to redistribution

The shift of magnesium from extracellular fluid into cells or bone is seen in refeeding of starved patients (refeeding syndrome),during treatment of metabolic acidosis, and in hungry bone syndrome which is seen after Parathyroidectomy or in patients with diffuse osteoblastic metastases (Seo and Park, 2008).

Second: Gastrointestinal causes

Magnesium deficiency entirely due to reduced dietary intake in otherwise healthy subjects is very uncommon. Hypomagnesemia may be seen in patients who are maintained on magnesium free intravenous fluids or total parenteral nutrition, especially in those patients who have a marginal or reduced serum magnesium to start off with. An inherited disorder of isolated magnesium malabsorption associated with hypocalcemia, tetany, and seizures has been described in infants as well as in older individuals (Seo and Park, 2008).

Third: Renal causes

Proximal tubular magnesium reabsorption is proportional to sodium reabsorption, and a reduction in sodium reabsorption during long-term intravenous fluid therapy may result in magnesium deficiency. Also hypomagnesemia is occasionally observed in chronic renal failure due to an obligatory renal magnesium loss. It is also seen during the diuretic phase of acute renal failure, in post-obstructive diuresis and after renal transplantation (Seo and Park, 2008).

2.4 Relationship between phosphorus, magnesium and Metabolic Syndrome

Patients with metabolic syndrome show significantly lower phosphate and magnesium concentrations compared with individuals who do not fulfill criteria for the diagnosis of this syndrome. This reduction may represent the consequence of increased transfer of phosphate from the extracellular to the

intracellular compartment. Increased insulin levels in patients with metabolic syndrome could be a major determinant of this process. In addition, the activation of the sympathetic nervous system observed in patients with metabolic syndrome and the resulting increment in serum catecholamine levels. Both insulin and catecholamine stimulate glycolysis, thus increasing the intracellular formation of phosphorylated carbohydrate compounds in the liver and skeletal muscles. The source of this phosphate is the inorganic phosphate of the extracellular fluid, and, as a result, serum phosphate concentrations may decrease rapidly. Lower magnesium concentrations in patients with metabolic syndrome compared with the control population can be attributed to the same mechanisms as lower serum phosphate levels. Additionally, the hyperinsulinemia induced renal magnesium wasting also may have a contributory role (Kalaitzidis, *et al.* 2005).

Chapter three

Materials and methods

Chapter three

3-Materials and methods

3.1 Materials

3.1.1 Study approach

A quantitative method was used to measure plasma levels of phosphorus and magnesium in metabolic syndrome Sudanese male and female patients during the period from July to September 2018.

3.1.2 Study design

This was analytical case control study.

3.1.3 Study population

The study included 50 randomly selected patients with metabolic syndrome as case and 50 apparently healthy individual as control.

3.1.4 Inclusion criteria

Sudanese patients with metabolic syndrome and healthy individuals serve as control were included (matched age and gender).

3.1.5 Exclusion area

Patients with Renal, hepatic, thyroid disease, cardiovascular disease and smokers were excluded.

3.1.6 Ethical consideration

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

Data were collected using structural interviewing questionnaire, which was designed to collect and maintain all valuable information concerning each case examined.

3.2 Methods

3.2.1 Sample collection and processing

About 3ml of venous blood were collected by under aseptic condition in heparin container. Plasma was obtained after centrifugation at least 15 minutes at 2500RPM.

3.2.2 Estimation of phosphorus level

3.2.2.1 Principle of phosphorus level

Direct phosphomolybdate reaction without deproteinization. Phosphate ions form with molybdate ions in acid solution proportional amounts unreduced phosphomolybdate complex. The concentration of the complex formed is determined by measuring its absorbance.

3.2.2.2 Procedure of phosphorus level: Appendix II.

3.2.3 Estimation of magnesium level

3.2.3.1 Principle of magnesium level

Magnesium forms a purple coloured complex in alkaline solution. In the presence of EGTA, the reaction is specific. The intensity of the purple colour is proportional to the magnesium concentration.

3.2.3.2 Procedure of magnesium level: Appendix III

3.3 Quality control

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 test and control samples.

3.4 Statistical analysis

Data obtained from this study was analyzed using statistical package for the social science (SPSS version 16). Independent *t* test was used for comparison and Persons correlation was used for correlation.

Chapter Four

Results

Chapter four

4-Results

The results of the biochemical determination of plasma Magnesium and phosphorus levels in metabolic syndrome patients (case group) and control group are given in figures and tables:

Figure (4-1): Age distribution among metabolic syndrome patients group, the result showed that, (14%) of patients between (40-50), (26%) between (51-60), (40%) between (61-70) and (20%) between (71-80) years.

Figure (4-2): Show gender distribution among metabolic syndrome patients group, the result showed that (62%) were females while (38%) were males.

Table (4-1): Represent the comparison mean± SD of plasma magnesium, phosphorus, WC and BMI in case versus control group, the result showed there were significant decrease in mean concentration of magnesium and phosphorus (1.98±1.22 versus 2.15±0.33mg/dl, P-value 0.000), (2.55±1.12 versus 3.13±0.76mg/dl, P-value 0.003), and there were significant increase in WC and BMI (107.8±9.83 versus 99.58±11.69cm, P-value 0.000), (30.02±5.05 versus 27.42±4.59kg/m2, P-value 0.008) respectively.

Table (4-2): Represent the comparison mean ±SD of plasma magnesium and phosphorus in male versus female in metabolic syndrome patients group, the result showed there was insignificant difference between two groups.

Figure (4-3): Show Correlation between plasma magnesium level and BMI in metabolic syndrome patients group: (r= 0.096, P-value= 0.508). (There was no correlation).

Figure (4-4): show Correlation between plasma magnesium level and age of metabolic syndrome patients group: (r= 0.058, P-value =0.687). (There was no correlation).

Figure (4-5): Show Correlation between plasma phosphorus level and BMI of metabolic syndrome patients group: (r= -0.199, P-value=0.166). (There was no correlation).

Figure (4-6): Show Correlation between plasma phosphorus level and age of metabolic syndrome patients group: (r= -0.221, P-value=0.122). (There was no correlation).

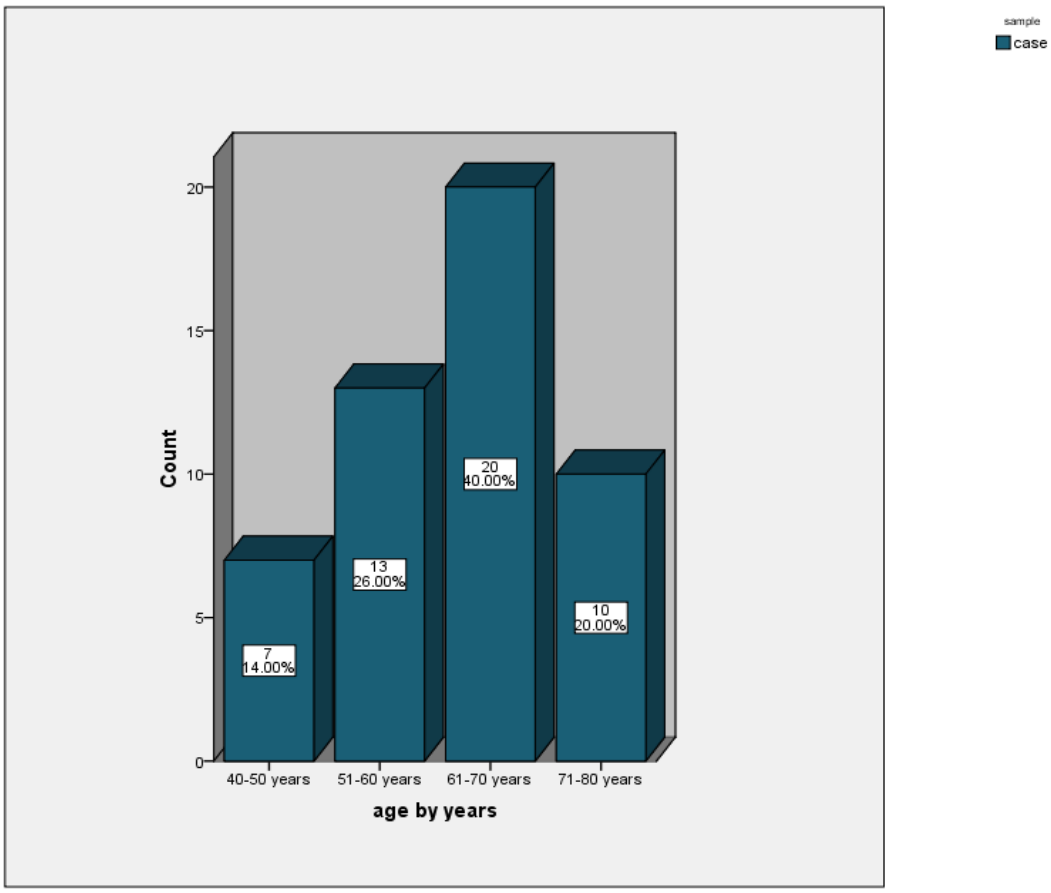

Figure (4-1): Age groups distribution among metabolic syndrome patients group

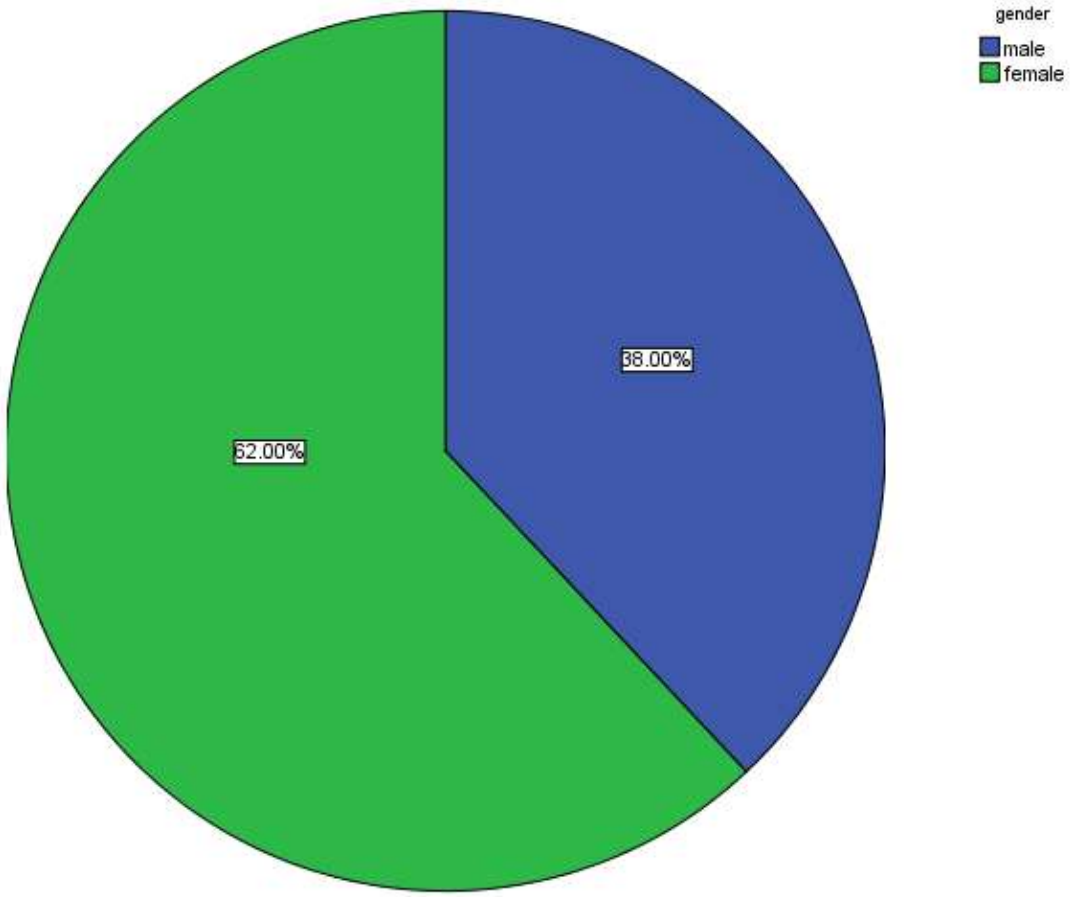

Figure (4-2): Gender distribution among metabolic syndrome patients group

Table (4-1): Mean concentrations and values of magnesium and phosphorus, WC and BMI in case and control groups

Variable	Case Mean ±SD N=50	Control Mean ±SD N=50	P-value
Magnesium mg/dl	1.98±1.22	2.15±0.33	0.000
Phosphorus mg/dl	2.55±1.12	3.13±0.76	0.003
WC cm	107.8±9.83	99.58±11.69	0.000
BMI kg/m2	30.02±5.05	27.42±4.59	0.008

Result given in mean ±SD, P.value≤0.05 considered significant.

Independent sample T test was used for comparison.

Table (4-2): Mean concentrations and values of magnesium and phosphorus in male versus female in metabolic syndrome patients group

Variable	Male group N=19	Female group N=31	P-value
Magnesium level mg/dl	2.15±1.62	1.66±0.62	0.165
Phosphorus level mg/dl	2.51±1.07	2.62±1.42	0.730

Result given in mean ±SD, P.value≤0.05 considered significant.

Independent sample T test was used for comparison.

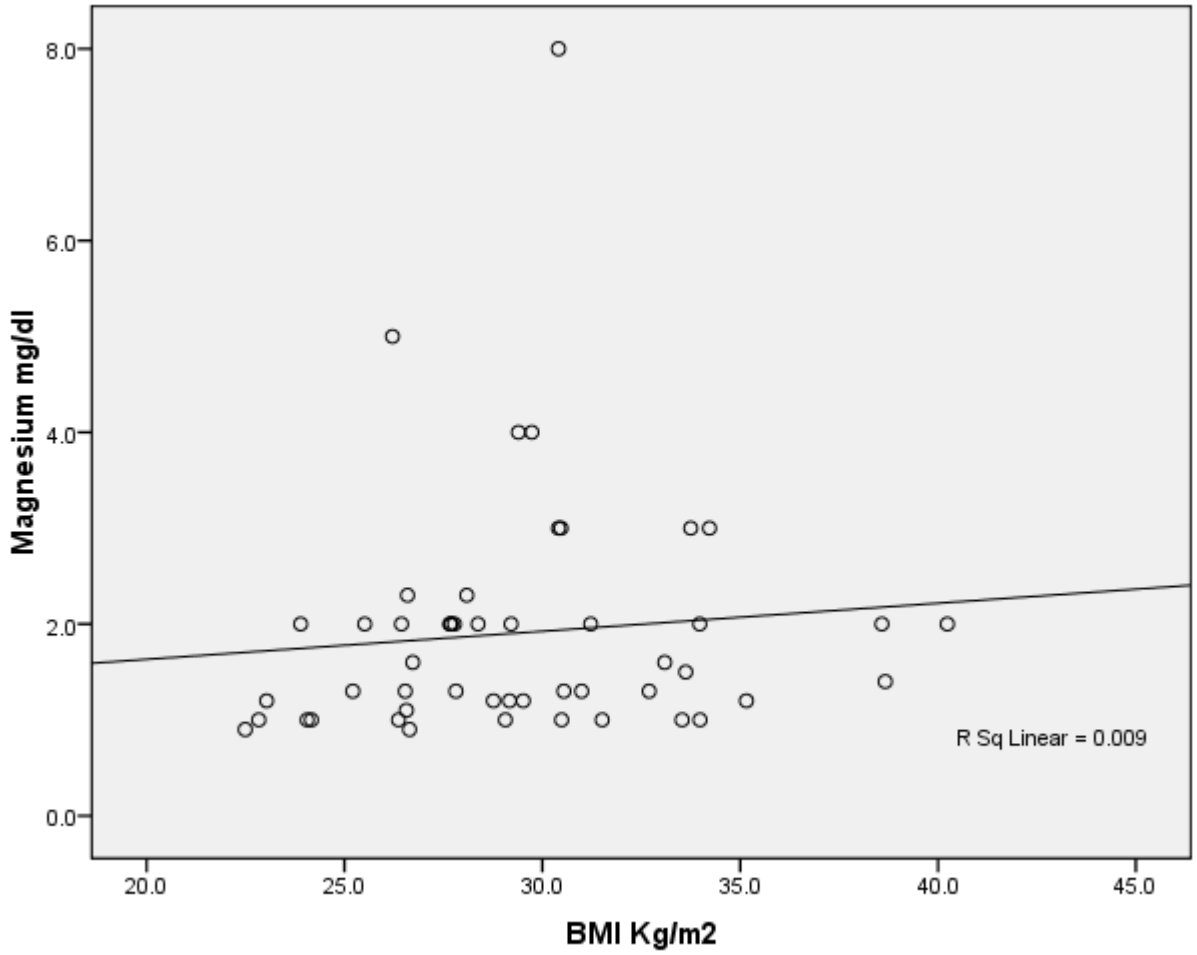

Figure (4-3): Correlation between plasma magnesium level and BMI in metabolic syndrome patients group. (r= 0.096, P-value = 0.508).

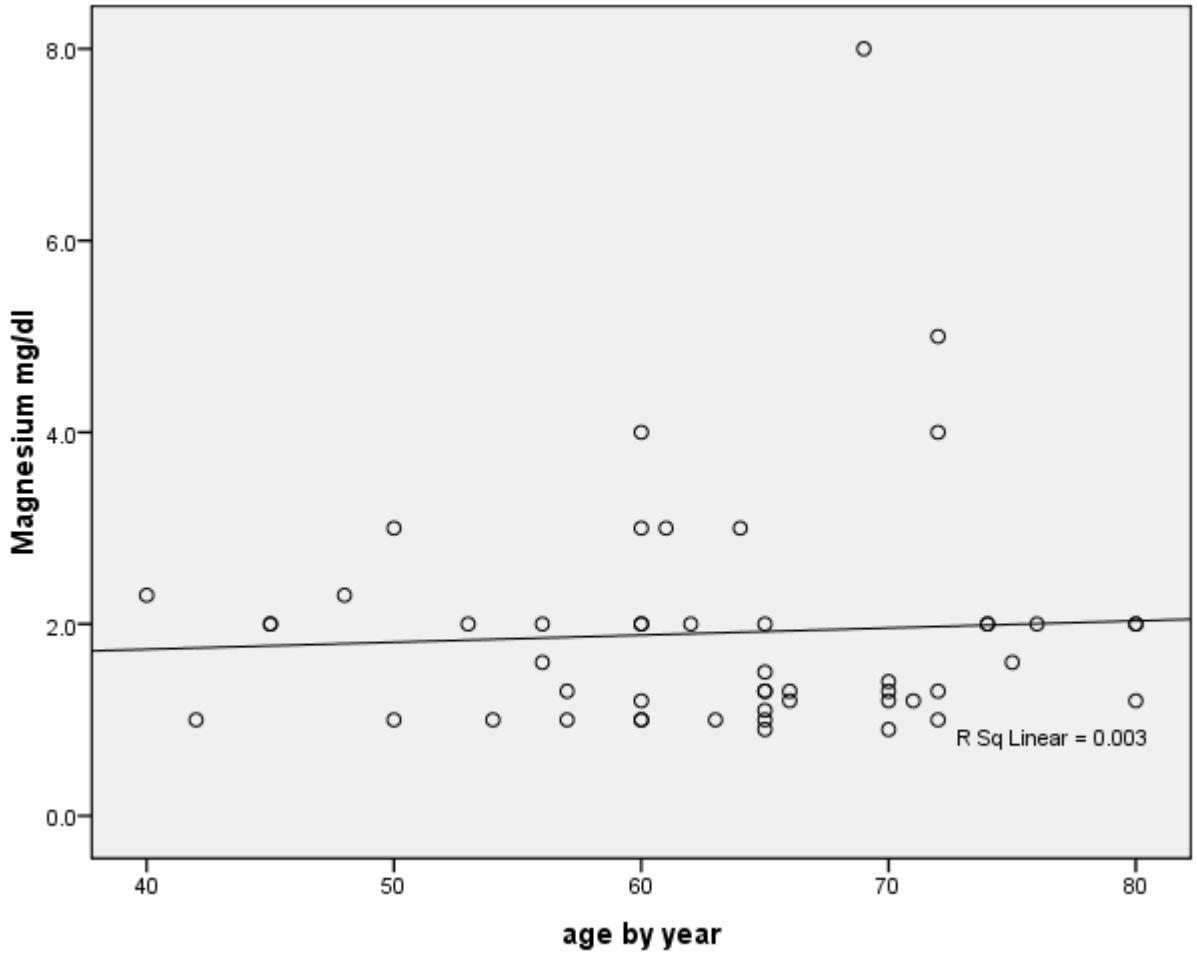

Figure (4-4): Correlation between plasma magnesium level and age of metabolic syndrome patients group: (r= 0.058, P-value = 0.687).

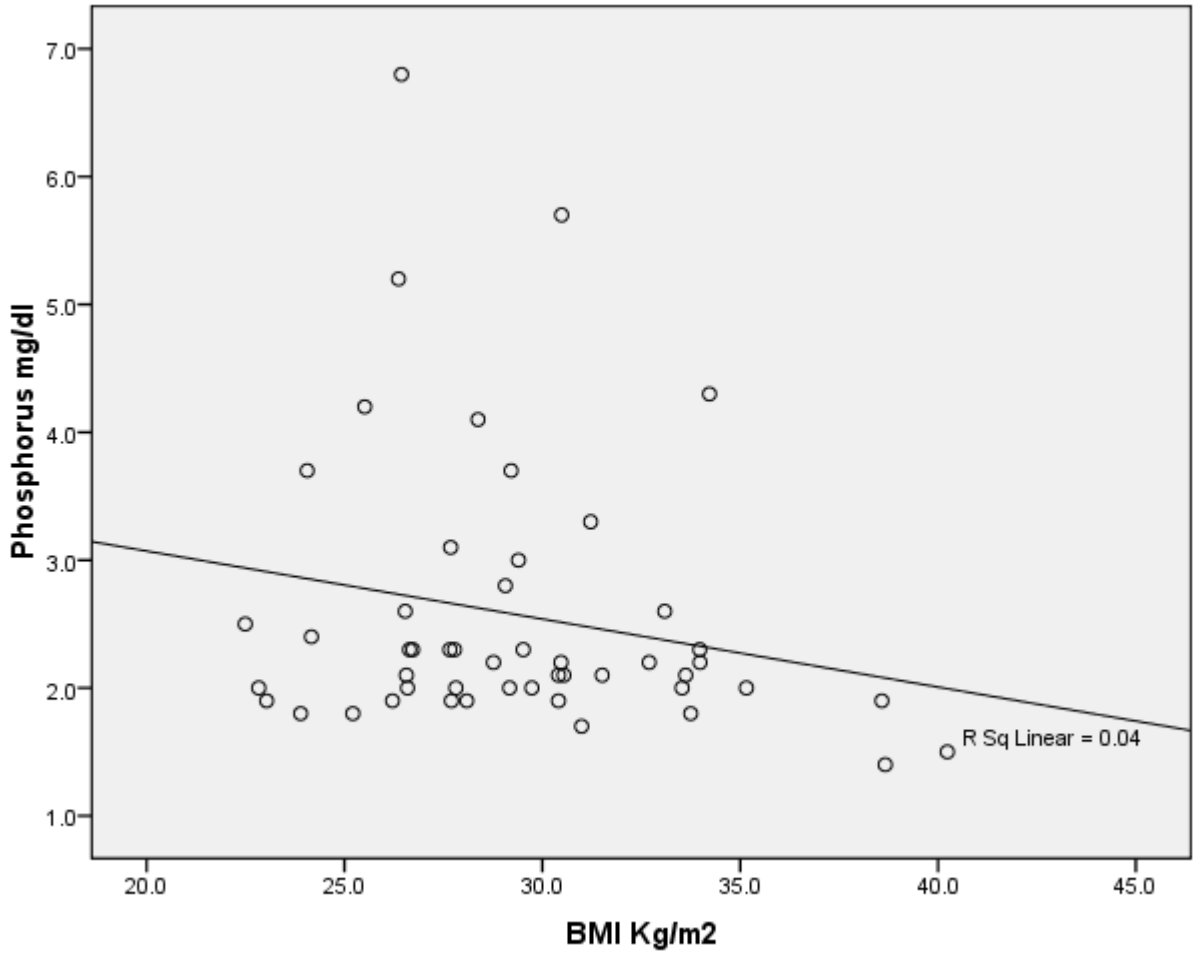

Figure (4-5): Correlation between plasma phosphorus level and BMI of metabolic syndrome patients group: (r= -0.199, P-value= 0.166).

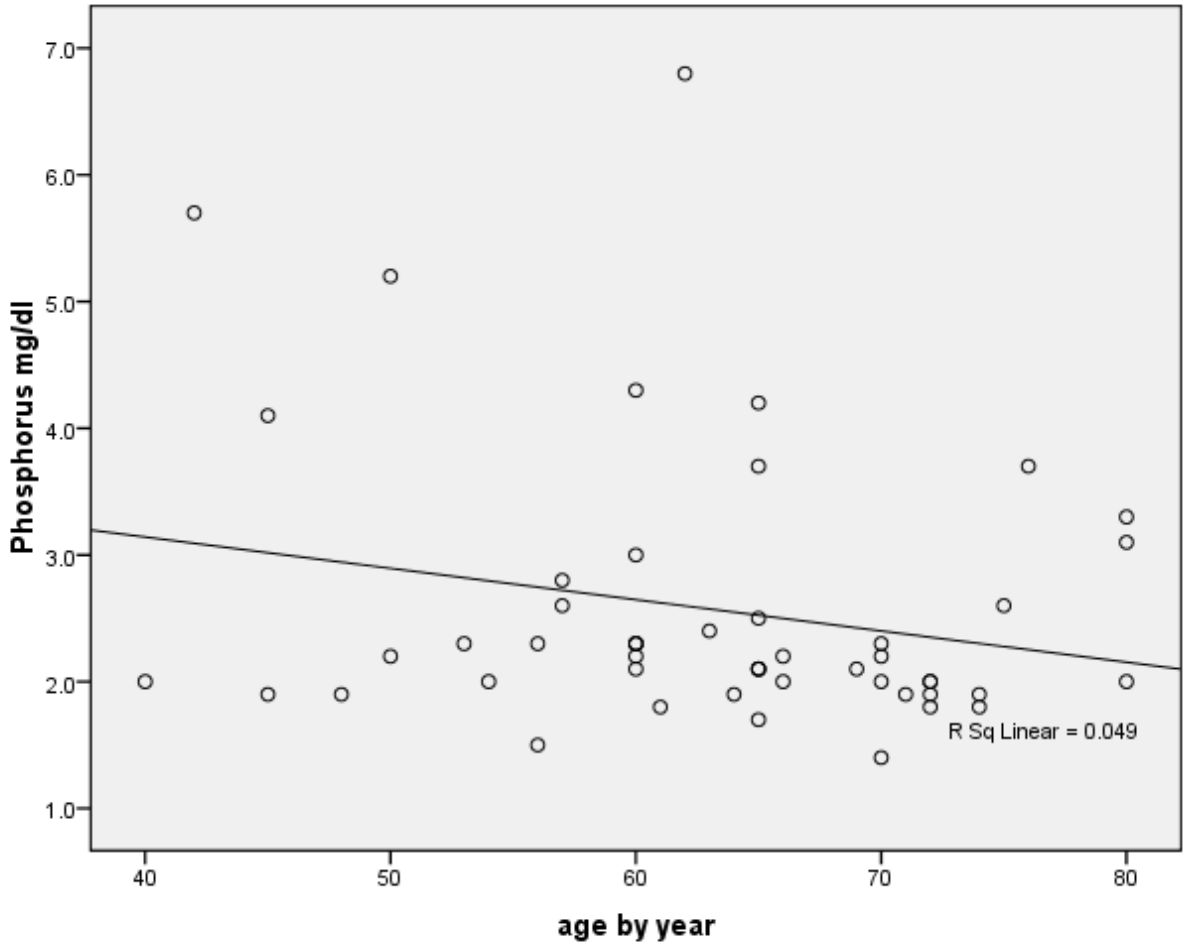

Figure (4-6): Correlation between plasma phosphorus level and age of metabolic syndrome patients group. (r = -0.221, P-v= 0.122).

Chapter Five

Discussion, Conclusion and recommendations

Chapter five

5-Discussion, Conclusion and Recommendations

5.1 Discussion

Metabolic Syndrome is a serious medical condition with increase incidence of multiple cancers risk, has effects on the life quality. It is a problem found all over the world and Sudan. Also it is fatal unless controlled and treated properly.

This study conducted to assess plasma level of magnesium and phosphorus among Sudanese patients with metabolic syndrome in Khartoum state.

The findings of this study showed that Metabolic syndrome most common among in elderly (40%). This finding agreed with another study carried by (Kraja, *et al*. 2006).

The findings obtained from especially designed questionnaire revealed that, (62%) of patients were females and (38%) were males. This finding agreed with another study carried by (Beigh and Jain, 2012).

Also the findings of this study showed that, there were decrease in mean values of plasma phosphorus and magnesium, and increase in mean of WC and BMI in metabolic syndrome patients compared to control group this result agreed with another studies carried by (Kalaitzidis, *et al*. 2005; Guerrero-Romero, *et al*. 2011; Stoian, *et al*. 2013). That indicates those variables are closely related to metabolic syndrome and also showed that the reason of decreased phosphorus and magnesium levels in Mets may result from increased insulin levels in patients with metabolic syndrome (Kalaitzidis, *et al*. 2005).

Also the findings of this study showed that, there was insignificant difference in mean plasma phosphorus level in males compared to

females in metabolic syndrome group. This result disagree with another studies carried by (Kalaitzidis, *et al*. 2005; Stoian, *et al*. 2013).

Also the findings of this study showed that, there was insignificant difference in mean plasma magnesium level in males compared to females in metabolic syndrome group. This result agree with another studies carried by (Kalaitzidis, *et al*. 2005; Guerrero-Romero, *et al*. 2011).

Also the result showed that, there was no correlation between plasma phosphorus level and study variables (BMI and age) in metabolic syndrome patients. This result disagree with another study carried by (Haglin, *et al*. 2001).

Also the result showed that, there was no correlation between plasma magnesium level and study variables (BMI and age) in metabolic syndrome patients. This result disagree with another result obtained by (Rotter, *et al.* 2015).

5.2 Conclusion

According to the results of this study it is conclude that patients with metabolic syndrome had lower level of phosphorus and magnesium.

5.3 Recommendations

From the findings of this study it is recommended that:

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

2-Plasma levels of phosphorus and magnesium should be done as monitoring tests to minimize the complications of metabolic syndrome that result from insulin resistance.

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Appendices

Sudan University for Sciences and Technology
College of Graduate Studies

Appendix (I)

Questionnaire
No. ( )

Research title: Plasma Magnesium and Phosphorus Level among Sudanese Patients with Metabolic Syndrome in Khartoum State

Name: ..... **Age:** .....

Gender:
Male ☐ **Female** ☐

Height: ☐ cm **Weight:** ☐ Kg **waist circumference** ☐ cm

Results:
1- Plasma magnesium:.....mg/dl

2-Plasma phosphorus:.....mg/dl

Date: / /2018

Cat. No 7-243

Appendix II

II GENERATION

DIAGNOSTIC KIT FOR DETERMINATION OF INORGANIC PHOSPHORUS CONCENTRATION

ACCENT-200 PHOSPHORUS

INTRODUCTION

Phosphorus is present in all body cells as a component of nucleic acids, phospholipids and phosphoproteins. Phosphorus is essential for intracellular storage and conversion of energy (ATP, creatine phosphate) and participates in carbohydrates metabolism. In the blood phosphorus is present as a mixture of inorganic phosphates HPO_4^{-2} and $H_2PO_4^-$. Besides phosphorus and calcium constitute mineral portion of bone. Continuous flux of phosphorus in organism is controlled by parathyroid hormone (PTH), vitamin D and calcitonin. Phosphorus serum level abnormalities are caused usually by disorders of vitamin D metabolism or parathyroid and kidney diseases.

METHOD PRINCIPLE

Direct phosphomolybdate reaction without deproteinization. Phosphate ions form with molybdate ions in acid solution proportional amounts of unreduced phosphomolybdate complex. The concentration of the complex formed is determined by measuring its absorbance.

REAGENTS

Package

1-Reagent	1 x 32 ml

The reagent, stored at 2-8°C is stable up to expiry date printed on the package The reagent stored on board of the analyser at 2-10°C is stable for 12 weeks. Protect from light and avoid contamination!

Concentrations in the test

ammonium molybdate	0.4 mmol/l
sulphuric acid	150 mmol/l
hydrochloric acid	100 mmol/l
detergents	

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for the intended purpose, by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- Contaminated glassware is the greatest source of error. Disposable plastic ware is recommended for the test.
- 1-REAGENT meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients:

1- REAGENT contains sulfuric acid (VI) and hydrochloric acid.

Danger

H314 Causes severe skin burns and eye damage.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing.

P305 +P351 +P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 - Immediately call a POISON CENTER or doctor.

SPECIMEN

Serum, heparinized plasma (recommended: heparine lithium, sodium or ammonium salt) free from hemolysis, 24-hours urine.

Serum is the preferred specimen. Level of inorganic phosphate in heparinized plasma is about 0.2 to 0.3 mg/dl (0.06-0.10 mmol/l) lower than in serum.

Serum should be separated from red blood cells as soon as possible after blood collection, because erythrocytes contain several times higher phosphate concentration than normal serum.

Urine preparation: To prevent phosphate precipitation in urine, specimens should be collected in HCl, 20-30 ml of 6 mol/L for 24-h specimen. Then dilute 1 part of acidified urine with 10 parts of distilled water. Multiply the result by the dilution factor.

Serum and plasma can be stored up to 7 days at 2 – 8°C. For longer storage samples should be frozen at -20°C.

24-hours urine samples can be stored up to 7 days at 2-8°C. Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

This reagent may be used in automatic analysers: ACCENT-200, ACCENT-200 II GEN, ACCENT-220S and BS-120 / BS-130.

1-Reagent is ready to use.

Deionized water is recommended as a reagent blank.

Actions required:

When performing assays with kits ACCENT-200 there is a probability of **cross-contamination** affecting the tests results: CALCIUM ARSENAZO – PHOSPHORUS II GEN. To avoid this effect follow the recommendations contained in the advisory note **"Carry-over – Preventive Actions".**

REFERENCE VALUES [7]

serum / plasma	mg/dl	mmol/l
age: 0 – 10 d	4.5 – 9.0	1.45 – 2.91
10 d – 24 mo	4.5 – 6.7	1.45 – 2.16
24 mo – 12 y	4.5 – 5.5	1.45 – 1.78
12 – 60 y	2.7 – 4.5	0.87 – 1.45
> 60 y males	2.3 – 3.7	0.74 – 1.20
> 60 y females	2.8 – 4.1	0.90 – 1.32
24–hours urine	g/24h	mmol/24h
	0.4 – 1.3	12.9 – 42.0

It is recommended for each laboratory to establish its own reference ranges for local population.

Phosphorus concentration in 24-hours urine – calculation

$$\text{phosphorus concentration in 24-hours urine [g/24h]} = \text{phosphorus concentration in sample of 24-hours urine [mg/dl]} \times \frac{\text{urine volume of 24-hours urine [dl/24h]}}{100}$$

QUALITY CONTROL

For internal quality control it is recommended to use the following controls with each batch of samples:

CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) for determination in serum;

CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) and LEVEL 2 (Cat. No 5-162) for determination in urine.

For the calibration of automatic analysers: ACCENT-200, ACCENT-200 II GEN, ACCENT-220S, BS-120 / BS-130, the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) are recommended. Deionised water should be used as a calibrator 0.
The calibration curve should be prepared every 12 weeks with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

The following results have been obtained using the automatic analysers: ACCENT-200 and/or ACCENT-200 II GEN and/or ACCENT-220S and/or BS-120 / BS-130. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity**

0.22 mg/dl (0.07 mmol/l) - ACCENT-200
0.23 mg/dl (0.07 mmol/l) - ACCENT-200 II GEN
0.05 mg/dl (0.02 mmol/l) - ACCENT-220S

- **Linearity**

up to 18.0 mg/dl (5.8 mmol/l) - ACCENT-200.
up to 15.0 mg/dl (4.9 mmol/l) - ACCENT-200 II GEN
up to 17.5 mg/dl (5.7 mmol/l) - ACCENT-220S

For higher concentration dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

- **Specificity / Interferences**

Haemoglobin up to 0.16 g/dl, ascorbate up to 62 mg/l, bilirubin up to 15 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n=20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	3.70	0.04	1.02
level 2	6.39	0.05	0.71
Reproducibility (day to day) n=	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	4.34	0.11	2.47
level 2	7.02	0.08	1.16

- **Method comparison**

A comparison between phosphorus values determined at **ACCENT-200** (y) and at **ADVIA 1650** (x) using 23 samples gave following results:
$y = 0.9593\ x + 0.1595$ mg/dl;
$R = 0.986$ (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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Appendix III

DIAGNOSTIC KIT FOR DETERMINATION OF MAGNESIUM CONCENTRATION

ACCENT-200 MG

INTRODUCTION
Magnesium in human organism occurs mainly in bone (about 50%) but is present also intracellularly in other tissues. Magnesium serves as a cofactor for multiple enzymatic reactions involved in nucleic acids synthesis, transport and production of energy. Magnesium is important in neuromuscular conduction and activation. Reduced magnesium level generates: concentration disturbances, fatigue, muscle tremor, anxiety state.

METHOD PRINCIPLE
Magnesium forms a purple coloured complex in alkaline solution. In the presence of EGTA, the reaction is specific. The intensity of the purple colour is proportional to the magnesium concentration.

REAGENTS
Package

1-Reagent	1 x 31 ml

The reagent is stable up to the kit expiry date printed on the package when stored at 2-8°C. After first opening the reagent's stability on board of the analyser at 2-10°C is 3 weeks. The reagent is air sensitive, to extend reagents stability it is recommended to keep reagent's bottles recapped on the board of analyser. Protect from light and avoid contamination!

Concentrations in the test

xylidyl blue	0.15 mmol/l
EGTA	0.1 mmol/l
buffer (pH 11.5)	
detergent	

Warnings and notes
- Product for in vitro diagnostic use only.
- The reagents must be used only for the intended purpose, by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- The reagent contains sodium azide (< 0.1%) as a preservative. Avoid contact with skin and mucous membranes.
- It is recommended to use disposable plastic materials. If it is not possible, the glassware should be washed with 1% HCl solution and rinsed with plenty of distilled water.
- It is recommended to precede the MG test by wash cycle with ACCENT-200 ACID WASHING SOLUTION (Cat. no 3-109) using APPLICATION for WASHING in order to avoid interference with other tests.
- 1-Reagent meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients:
1-Reagent contains potassium hydroxide.
Danger

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P305 +P351 +P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor.

SPECIMEN
Serum, heparinized plasma free from hemolysis, 24-hours urine.
Recommended anticoagulants: heparine lithium, sodium or ammonium salt.
Serum should be separated from red blood cells as soon as possible after blood collection, because erythrocytes contain approximately 3 times the magnesium concentration found in normal serum.
Urine preparation: Acidify urine with some drops of concentrated hydrochloride acid to pH 1.0. Then dilute 1 part of acidified urine with 4 parts of distilled water. Multiply the result by 5. Mix well samples before analysis.
Serum and plasma can be stored up to 7 days at 2-8°C. For longer storage samples should be frozen at -20°C.
24-hours urine samples can be stored up to 7 days at 2-8°C.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE
This reagent may be used in automatic analysers: ACCENT-200, ACCENT-200 II GEN, ACCENT-220S and BS-120 / BS-130.
1-Reagent is ready to use. Avoid foaming.
Actions required:
Use the reagent in conjunction with APPLICATION for WASHING (see page 3,4 of this instruction for use). A bottle of cleaning solution ACCENT-200 ACID WASHING SOLUTION (Cat. No 3-109) must be also put in reagent tray.
Deionised water is recommended as a reagent blank.

REFERENCE VALUES [6]

serum / plasma	mg/dl	mmol/l
newborn 2 – 4 d	1.5 – 2.2	0.62 – 0.91
children 5 mo – 6 y	1.7 – 2.3	0.70 – 0.95
6 – 12 y	1.7 – 2.1	0.70 – 0.86
12 – 20 y	1.7 – 2.2	0.70 – 0.91
adults	1.6 – 2.6	0.66 – 1.07
24-hours urine:	mg/24h	mmol/24h
	72.9 – 145.8	3 – 5

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL
For internal quality control it is recommended to use the following controls for each batch of samples:
CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) - for determination in serum:
CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) and LEVEL 2 (Cat. No 5-162) - for determination in urine.
For the calibration of automatic analysers: ACCENT-200, ACCENT-200 II GEN, ACCENT-220S, BS-120 / BS-130, the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) and deionised water is recommended.
The calibration curve should be prepared every week, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS
The following results have been obtained using automatic analysers ACCENT-200 and/or ACCENT-200 II GEN, and/or ACCENT-220S. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity**

0.12 mg/dl (0.05 mmol/l) - ACCENT 200
0.12 mg/dl (0.05 mmol/l) - ACCENT 200 II GEN
0.12 mg/dl (0.05 mmol/l) - ACCENT 220S

- **Linearity**

up to 5.0 mg/dl (2.05 mmol/l) - ACCENT 200
up to 5.0 mg/dl (2.05 mmol/l) - ACCENT 200 II GEN
up to 5.0 mg/dl (2.05 mmol/l) - ACCENT 220S

- **Specificity / Interferences**

Haemoglobin up to 0.313 g/dl, ascorbate up to 62 mg/l, bilirubin up to 15 mg/dl, triglycerides up to 1000 mg/dl and calcium up to 20 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n=10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	1.97	0.02	1.22
level 2	4.26	0.03	0.63
Reproducibility (day to day) n=10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	2.06	0.05	2.31
level 2	4.21	0.09	2.04

- **Method comparison**

A comparison between magnesium values determined at **ACCENT-200** (y) and at **ADVIA 1650** (x) using 25 samples gave following results:
y = 0.9218 x + 0.0901 mg/dl;
R = 0.964 (R – correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

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