بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Medical Laboratory Science

Estimation of Serum Calcium and Phosphorus levels in Sudanese Patients with Type 2 Diabetes Mellitus

تقدير مستويات الكالسيوم والفسفور في مصل الدم عند السودانيين المصابين بمرض السكري من النوع الثاني

A dissertation Submitted in Partial Fulfillment for B.Sc (honor) Degree in Medical Laboratory Science (Clinical Chemistry)

By:

Aziza Ali Mohammed Yousif

Shayma Osman Mohammed Ahmed

Supervisor:

Ust. Moawia Ali Yousif Abd Allah

M.Sc (Clinical Chemistry)

July 2014

قال تعالى:

بسم الله الرحمن الرحيم

(قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا)

صدق الله العظيم

سورة الكهف الآيه (109)

Dedication

We dedicate this work

To our lovely parents

To Our Family

To soul of Dr. Mohammed Abdul Rahim

To our perfect teachers

To our friends & to all people in the world love us

Aziza & Shayma

Acknowledgements

In the name of Allah, the Most Gracious and the Most Merciful

all praises to Allah for the strengths and His blessing in completing this work.

First and foremost, we have to thank our parents for their love and support throughout our life. Thank you for giving us strength to reach for the stars and chase our dreams.

We would like to sincerely thank our supervisor, Ust. ***Moawia Ali Yousif****, for his guidance and support throughout this study, and especially for his confidence in us. His guidance helped us in all the time of research and writing of this dissertation. We could not have imagined having a better advisor and mentor for our research.*

Last but not the least, our deepest gratitude go to our university, teachers, family and friends. To those who indirectly contributed in this research, your kindness means a lot to us. Thank you very much.

Abstract

An analytical hospital based case- control study conducted during the period, from February to July 2014, to estimate the serum levels of calcium and phosphorus of 40 Sudanese patients with type 2 diabetes mellitus as a test group compared to 40 healthy subjects as control group. Participants in this study were from Jabir Abu Al Eizz center in Khartoum state, Sudan. Males and females were matched in term of age (ranges 40-80 years). The serum levels of calcium and phosphorus were measured using colorimeter model JENWAY and commercial kits from Biosystem Company.

The results obtained and analyzed using software program (SPSS). The means and standards deviation of serum levels of calcium and phosphorus were detected and T-test was use for comparison (p. value of < 0.05 is considered to be significant), Linear regression analysis was used to assess correlation between the levels of calcium and phosphorus to the duration of diabetes mellitus and the age of diabetic patients.

The serum levels of calcium was not significantly differ between patients and controls (p=0.652).

The serum levels of phosphorus was significantly differ between patients and controls (p=0.000).

In the diabetic group, the serum levels of calcium showed insignificant very weak negative correlation with the duration and age, the serum levels of phosphorus showed insignificant very weak positive correlation with the duration and age, and both not affected by sex of diabetic patients.

From the results of this study, it conclude that: type 2 diabetes mellitus have no effect in calcium level but decrease serum level of phosphorus.

مستخلص الدراسة

اجريت هذة الدراسة التحليلية خلال الفترة من فبراير2014 وحتى يوليو2014 حيث تم مقارنة مستويات الكالسيوم والفسفور في مصل الدم عند 40 من المرضى السودانين المصابين بداء السكري من النوع الثاني كمجموعة اختبار مقارنة ب 40 الأصحاء كمجموعة ضابطه . كل المشاركين في هذه الدراسة كانوا من مركز جابر ابو العز بولاية الخرطوم. كان هنالك تطابق في العمرين الرجال والنساء (40-80 سنه)

.

تم قياس مستويات الكالسيوم والفوسفور في مصل الدم باستخدام نموذج مقياس الألوان والمحاليل المستخدمه كانت من شركه الانظمه الحيويه الاسبانيه.

النتائج المتحصل عليها تم تحليلها باستخدام برنامج الحزمه الاحصائيه للعلوم التطبيقيه. الوسط الحسابي والانحراف المعياري من مستويات الكالسيوم والفسفور تم الكشف عنها باستخدام اختبارT(القيمه الاحتماليه < 0.05 تعتبر ذات دلاله احصائيه), تم تحليل الانحدار الخطي لتقييم مدى الارتباط بين مستويات الكالسيوم والفسفور مع مده مرض السكري وعمر المرضى.

لم يكن هنالك فرق ذو دلالة إحصائية معنوية في مستوى الكالسيوم عند المصابين بمرض السكري من النوع الثاني مقارنة بالاصحاء (القيمة الإحتمالية=0.652).

كان هنالك إنخفاض ذو دلالة إحصائية معنوية في مستوى الفسفور عند المصابين بمرض السكري من النوع الثاني مقارنة بالاصحاء (القيمة الإحتمالية=0.000).

عند مقارنة مستويات كل من الكالسيوم و الفسفور في مجموعة الدراسة مع مدة الإصابة بمرض السكري اظهر الفسفور علاقة ضعيفة جدا و ليست ذات دلالة معنويه ,وأظهر الكالسيوم علاقة سالبه ضعيفه وليست ذات دلاله معنويه .

عند مقارنة مستويات كل من الكالسيوم و الفسفور في مجموعة الدراسة مع عمر المصابين بمرض السكري اظهر الفسفور علاقة ضعيفة جدا و ليست ذات دلالة معنوية, وأظهر الكالسيوم علاقة سالبه ضعيفه وليست ذات دلاله معنويه، ولا يتأثر كل منهما بجنس المريض.

من هذة الدراسة نخلص للاتي:

مرض السكري لا يؤثر في مستويات الكالسيوم ولكنه يخفض مستويات الفسفور.

Contents

Content	page
الاية	I
Dedication	II
Acknowledgments	III
Abstract	IV
مستخلص الدراسة	V
Table of contents	VI
List of tables	VIII
List of figures	IX
Abbreviations	X
Chapter One	
1 Introduction and Literature Review	1
1.1 Background	1
1.2 Literature Review	3
1.2.1 Diabetes mellitus	3
1.2.1.1 Glucose metabolism and regulation	3
1.2.1.2 Classification of diabetes mellitus	5
1.2.1.3 Causes of diabetes mellitus	6
1.2.1.4 Pathophysiology of diabetes mellitus	8
1.2.1.5 Signs and symptoms of diabetes mellitus	8
1.2.1.6 Complications of diabetes mellitus	8
1.2.2 Calcium	9
1.2.2.1 Calcium physiology	9
1.2.2.2 Regulation of calcium	10
1.2.2.3 Distribution of calcium	11
1.2.2.4 Clinical application of calcium	11
1.2.3 Phosphate	14
1.2.3.1 Phosphate physiology	14
1.2.3.2 Regulation of phosphate	15
1.2.3.3 Distribution of phosphate	16
1.2.3.4 Clinical application of phosphate	16
1.3 Rationale	18
1.4 Objectives	19

1.4.1 General objective	19
1.4.2 Specific objectives	19
Chapter Two	
2 Materials & methods	20
2.1 Study design	20
2.2 Study area	20
2.3 Study population	20
2.4 Inclusion criteria	20
2.5 Exclusion criteria	20
2.6 Samples	20
2.7 Ethical consideration	20
2.8 Equipments	21
2.9 Measurement of serum phosphorus	21
2.10 Measurement of serum calcium	21
Chapter Three	
3 Results	23
Chapter Four	
4 Discussion ,Conclusion and Recommendation	32
4.1 Discussion	32
4.2 Conclusion	33
4.3 Recommendations	34
References	35

Appendix (1): Questionnaire.
Appendix (2): The method used for measurements of serum level of phosphorus.
Appendix (3): The method used for measurements of serum level of calcium.

List of tables

Table (3-1): calcium level between test and control group.

Table (3-2): phosphorus level between test and control group.

List of figures

Figure (3-1): The means of the calcium level in reference to sex.

Figure (3-2): The means of the phosphorus level in reference to sex.

Figure (3-3): A correlation between calcium and duration.

Figure (3-4): Correlation between phosphorus levels and duration.

Figure (3-5): Correlation between calcium level and age.

Figure (3-6): Correlation between phosphorus levels and age.

Abbreviations

1,25 DHCC: 1: 25-dihydroxycholecalciferol

2,3-BPG: 2,3 Bisphosphoglycerate

ATP: Adenine triphosphate

DNA: Deoxyribonucleic Acid

ECF: Extra Cellular Fluid

ECG: Electrocardiogragh

FHH: Familial hypocalciuric hypercalcaemia

GDM: Gestational Diabetes Mellitus

GH: Growth Hormone

HHM: Humoral Hypercalcaemia of Malignancy

HLA: Human Leukocyte Antigen

IDDM: Insulin Dependent Diabetes Mellitus

NIDDM: Non Insulin Dependent Diabetes Mellitus

PTH: Parathyroid Hormone

PTH-rP: Parathyroid Hormone related peptide

P-Value: Probability Value

RNA: Ribonucleic Acid

SPSS: Statistical Package for the Social Sciences

WHO: World Health Organization

CHAPTER ONE

1. Introduction and Literature Review

1.1 Background:

Diabetes mellitus is a metabolic disorder which affects many people in the world and they suffer from a number of disorders including bone disease (Hamad, *et al*. 2013).

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity (King, *et al.*2004). Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important to allow rational planning and allocation of resources (King, *et al.* 2004).

Diabetes is currently emerging as an important health problem with a significant global burden (Ahmed, *et al.* 2001). Assuming that age-specific prevalence remains constant, the number of people of diabetes in the world is expected to approximately double between 2000 and 2030, based solely upon demographic changes (Wild, *et al*. 2004). Accordingly the WHO has called the disease [the emerging epidemic] (Ahmed, *et al*. 2002).

The actual prevalence of the disease in Sudan is unknown although one small population based study of the prevalence in diabetes and impaired glucose tolerance in Sudan was conducted in 1996; the crude prevalence was 3.4% for diabetes and 2.9% for impaired glucose tolerance (Elbagir, *et al*. 2007).

The study aimed to evaluate the affect of hyperglycemia accompanying type 2diabetes mellitus in the serum levels of calcium and phosphorus.

Evidence for a disturbance of mineral metabolism in diabetes has accumulated since the eighties of the last decade. Calcium ion shown to play an important role in the biosynthesis, storage, release and activity of insulin, in addition, to glucose tolerance in human beings (Al-

selevany,2004). A significantly decreased level of calcium was reported in serum of diabetic patients .In another study, a significantly decrease in total ionized calcium was found in NIDDM patients (Al-selevany.2004).

Hypophosphatemia (low phosphorus levels) is clinically associated with diabetes mellitus (Bishop.2003). “Excessive amounts of phosphorus can also be lost in the urine of uncontrolled diabetics who have polyuria and acidosis” even if plasma phosphorus appears to be normal (Bishop, 2003).

1.2 Literature Review

1.2.1 Diabetes mellitus

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Bishop, 2003). It has been defined by the world health organization (WHO), on the basis of laboratory finding, as a fasting venous plasma glucose concentration greater than 7.8 mmol/L (140 mg/dl) or greater than 11.1 mmol/L (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion of the equivalent of 75 g of glucose, even if the fasting concentration is normal (Mayne, 1993)

1.2.1.1 Glucose metabolism and regulation:

Blood glucose is maintained within a narrow range imposed at the lower limit by the undesirable effects of hypoglycaemia, and at the upper limit by the potential for loss in the urine if the renal threshold is exceeded (Bechett, *et al*. 2010).

The liver, pancreas, and other endocrine glands are all involved in the controlling the blood glucose concentrations within a narrow range (Bishop, 2003) .The hormones mainly concerned with regulating glucose metabolism in the fed and fasting states are insulin , glucacon , GH, adrenaline and cortisol (Bechett, *et al*. 2010).

Insulin is the primary hormone responsible from the entry of glucose into the cell. It is synthesized by the β-cells of islets of langerhans in the pancreas. It decreases plasma glucose levels by increasing the transport entry of glucose in muscle and adipose tissue by way of nonspecific receptors. It also regulates glucose by increasing glycogenesis, lipogenesis, and glycolysis and inhibiting glycogenolysis. Insulin is the only hormone that decreases glucose levels and can be referred to as hypoglycemic agent (Bishop, 2003).

Glucagon is the primary hormone responsible for increasing glucose levels. It is synthesized by the α-cells of islets of langerhans in the pancreas. Glucagon acts by increasing plasma glucose levels by glycogenolysis in the liver and an increase in gluconeogenesis. It can be referred to as a hyperglycemic agent (Bishop, 2003).

Glucagon, GH, adrenaline and cortisol all tend, in general, to antagonise the actions of insulin (Bechett, *et al*. 2010).

I-Hyperglycemia:

Hyperglycemia is an increase in plasma glucose levels. In healthy patients, during a hyperglycemia state, insulin is secreted by the β cells of the pancreatic islets of langerhans. Insulin enhances membrane permeability to cell in the liver, muscle, and adipose tissue. It also alters the glucose metabolic pathway. Hyperglycemia, or increased plasma glucose levels, is caused by an imbalance of hormones (Bishop, 2003).

II-Hypoglycemia:

Hypoglycemia involves decreased plasma glucose levels and can have many causes some are transient and relatively insignificant; others can be life threatening (Bishop, 2003).

The plasma glucose concentration at which glucagon and other glycemic factors are released is between 65 and 70 mg/dl (3.6-3.9 mmol/L); at about 50-55 mg/dl (2.8-3.0 mmol/L), observable symptoms of hypoglycemic appear. The warning signs and symptoms of hypoglycemia are all related to the central nervous system (Bishop, 2003).

1.2.1.2 Classification of Diabetes Mellitus:

A) Primary diabetes mellitus:

Several classification of diabetes have been proposed (Marshall, 2004).In 1979, the National Diabetes Data Group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into tow broad categories; type 1, insulin dependent diabetes mellitus (IDDM) and type 2, non-insulin-dependent diabetes mellitus (NIDDM) (Bishop, 2005).

I-Type 1 Diabetes Mellitus:

Insulin dependent diabetes mellitus (IDDM) accounts for approximately 15% of all diabetics. It can occur at any age but is most common in the young. The absolute lack of insulin is a consequence of the autoimmune destruction of insulin-producing beta cells (Gaw, *et al.*1999).

Characteristics of type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency (Bishop, 2003).

II-Type 2 Diabetes Mellitus:

Non-insulin dependent diabetes mellitus (NIDDM) accounts for approximately 85% all diabetics and can occur at any age. It is most common between 40 and 80 years. In this condition there is resistance of peripheral tissues to the actions of insulin, so that the insulin level may be normal or even high (Gaw, *et al.*1999).

This type of diabetes is associated with a strong genetic predisposition with patients at an increased risk with an increase in age, obesity, lack of physical exercise, and sustained stress (Bishop. 2003, Mayne1998, Gaw, *et al.*1999).

Characteristics usually include an adult onset of the disease and milder symptoms than I type 1, with ketoacidois very seldom occurring. However, these patients are more likely to go into a hyperosmolar coma (Bishop, 2003).

B) Secondary Diabetes Mellitus:

Diabetes mellitus associated with other condition include:

-absolute insulin deficiency, due to pancreatic disease (chronic pancreatitis, haemochromatosis, cystic fibrosis).

-relative insulin deficiency, due to excessive growth hormone (acromegaly, glucocorticoid secretion (cushing,s syndrome), or increased plasma glucocorticoid concentration due to administration of steroids .

- drugs, such as thiazide diuretics (Mayne, 1998).

The characteristics and prognosis of this form of diabetes depends on the primary disorder (Bishop, 2003).

I-Gestational diabetes mellitus:

GDM is a temporary condition that occurs during pregnancy and is defined as any degree of glucose intolerance with onset or detection during pregnancy (Chatterjea and Chawla. 2010, Bishop.2003).

Causes of GDM include metabolic and hormonal changes (Bishop, 2003).If a woman had gestational diabetes during pregnancy, there is an increased risk of developing diabetes for both mother and the child. In most cases, gestational diabetes is managed by diet and exercise, and go away after the baby is born (Chatterjea and Chawla, 2010).

1.2.1.3 Causes of diabetes mellitus

A) Causes of type 1 diabetes mellitus:

I- Genetics:

Susceptibility to type1 diabetes is inherited, but the mode of inheritance is complex and has not been completely defined. It's a multigenic trait, and the major locus is the major histocompatibility complex on chromosome 6 (Carl, *et al*. 2008). Subjects most at risk are those with HLA-types DR3 and DR4 of the major histocompatibility complex (Mayne, 1998).

II- Environmental factors:

Environmental factors are thought to be involved in initiating diabetes, for example, viruses, such as; rubella, mumps and coxsackievirus B, have been implicated others environmental factors that have been suggested include chemicals and cow's milk (Carl , *et al*. 2008).

B) Causes of type 2 diabetes mellitus:

I- Genetics:

Genetic factors contribute to the development of type 2 diabetes. For example, the concordance rate for type 2 diabetes in identical twins approaches 100%. In addition, type 2 diabetes it's 10 times more likely to occur in an obese individual with a parent who has diabetes than in an equally obese individual without a diabetic family history. The mode of inheritance however is unknown (Carl, *et al*. 2008).

A variety of approaches have identified several genes that are associated with type 2 diabetes. Therefore the gene or genes causing the common forms of type 2 diabetes remain unknown (Carl, *et al*. 2008).

II- Environmental factors:

Environmental factors such as diet and exercise are important determinants in the pathogenesis of type 2 diabetes. Although 60% to 80% of those with type 2 diabetes are obese, diabetes develops in fewer than 15% of obese individuals. In contrast, virtually all obese people even those with normal carbohydrate tolerance have hyperinsulinemia and are insulin resistant (Carl, *et al*. 2008).

Other factors such as:

-Family history of type 2 diabetes.

-The duration of obesity.

-The distribution of fat (Carl, *et al*. 2008).

1.2.1.4 Pathophysiology of Diabetes Mellitus

In both type 1 and type 2 diabetes, the individual will be hyperglycemic, which can be severe. Glucosuria can also occur after renal tubular transporter system for glucose becomes saturated. This happens when the glucose of plasma exceeds roughly `180mg/dL in an individual with normal renal function and urine output. As hepatic glucose over production continues, the plasma glucose concentration reaches a plateau around 300 mg/dL to 500 mg/dL (17mmol/l to 28mmol/l). Provided renal output is maintained, glucose excretion will match the over production causing the plateau (Bishop, 2003).

The individual with type 1 has a higher tendency to produce ketones, but instead have a greater tendency to develop hyperosmolar non-ketotic states.The difference in glucagon and insulin concentrations in this two group appear to be responsible for the generation of ketones through increased β- oxidation. In type 1, there is an absence of insulin with an excess glucagon. This permits gluconeogenesis and lipolysis to occur. In type 2, insulin is present as is (sometimes) hyperinsulinemia ; therefore , glucagon is attenuated. Fatty acid oxidation is inhibited in type 2. This causes fatty acids to be incorporated into triglycerides for release as very low- density lipoproteins (Bishop, 2003).

1.2.1.5 Signs and symptoms of diabetes:

Polydipsia (excessive thirst); Polyphagia (increase food intake); Polyuria (excessive urine production); Rapid weight loss; Hyperventilation; Mental confusion and possible loss of consciousness (due to increased glucose to brain) (Bishop, 2003).

1.2.1.6 Complications of diabetes:

I-Acute metabolic complications:

- Diabetic ketoacidosis .
- Hyperosmolal non-ketotic coma.

- Hypoglycaemia caused by excess insulin or sulphonylurea administration (Mayne, 1998).

II-Late complications:

Vascular disease is a common complication of diabetes mellitus (Mayne.1998).

Macrovascular disease, due to abnormalities of large vessels, my present as coronaryartery,cerebrovascular or peripheral vascular insufficiency. The condition is probably related to alterations in lipid metabolism.

Microvascular disease, due to abnormalities of small blood vessels, particularly affects the retina (diabetic retinopathy) and the kidney (leads ultimately to renal failure); the incidence of both may be related to inadequate glucose control (Mayne.1998. Gaw, *et al*.1999).

Neuropathy may become evident as diarrhoea, posturnal hypotension, impotence, neurogenic bladder and neuropathic food ulcer (Gaw, *et al*.1999).

1.2.2 Calcium

Calcium is the most abundant mineral in the body (Beckett, *et al*. 2010). Calcium is both absorbed from and secreted into the gut, but net absorption must equal urinary loss every day if balance is to be maintained (Gaw, *et al*.1999).

1.2.2.1 Calcium Physiology:

Unbound calcium is the biologically active fraction of the total calcium in plasma and maintenance of its concentration within tight limits is required for nerve function, membrane permeability, muscle contraction and glandular secretion, also calcium is essential for myocardial contraction (Gaw, *et al*.1999, Bishop.2003). Decreased ionized calcium concentrations in blood can cause neuromuscular irritability, which may become clinically apparent as irregular muscle spasms, called tetany (Bishop, 2003).

1.2.2.2 Regulation of calcium:

Three hormones are known to regulate serum calcium by altering their secretion rate in response to changes in ionized calcium. These hormones are parathyroid hormone PTH, vitamin D, and calcitonin (Bishop, 2003).

I-Parathyroid hormone (PTH):

PTH is the principal acute regulator of plasma calcium. Plasma PTH levels exhibit a diurnal rhythm. The active hormone is secreted in response to a fall in plasma calcium, and its actions are directed to increase plasma calcium. An increase in plasma calcium suppresses PTH secretion (Beckett, *et al*. 2010).

In bone, PTH stimulates bone resorption by osteoclasts, with a requirement for osteoblasts to mediate this effect. In the kidney, PTH increases the distal tubular reabsorption of calcium. It also reduces proximal tubular phosphate reabsorption and promotes activity of the 1α-hydroxylation of calcidiol (Beckett, *et al*. 2010).

II-1: 25-dihydroxycholecalciferol:

The principal action of 1:25-dihydroxycholecalciferol is to induce synthesis of calcium-binding protein in the intestinal epithelial cell necessary for the absorption of calcium from the small intestine (Beckett, *et al*. 2010).

III-Calcitonin:

The parafollicular cells of the thyroid produce calcitonin in response to high calcium levels, which decreases blood calcium levels, but its significance is much smaller than that of PTH (Beckett, *et al*. 2010).

Although calcitonin can decrease plasma [Ca^{2+}] by reducing osteoclast activity and decreasing renal reabsorption of calcium and phosphate ,its actions are transient, and chronic excess or deficiency is not associated with disordered calcium or bone metabolism(Beckett, *et al*. 2010) .

1.2.2.3 Distribution of calcium:

The average adult body contains in total approximately 1 kg. More than 99% of calcium in the body is part of bone, the remaining 1% is mostly in the blood and extracellular fluid (ECF) (Bishop.2003).The extracellular fluid (ECF) contains approximately 22.5 mmol, of which about 9 mmol is in the plasma. Approximately 500 mmol of calcium is exchanged between bone and the ECF over a period of twenty-four hours (Marshall and Bangert,1995).

Calcium in blood is distributed among several forms. About 45% circulates as free calcium ions, 40% is bound to protein, mostly albumin, and 15% is bound to anions such as bicarbonate, citrate, phosphate, and lactate (Bishop,2003).

1.2.2.4 Clinical applications of calcium

I-Hypercalcaemia:

Hypercalcaemia develops when the rate of entry of calcium into the extracellular fluid from the bone and gut exceeds the capacity of kidney to excrete it (Marshall and Bangert.1995).

A) Causes:

I- Primary hyperparathyroidism:

Primary hyperparathyroidism is the commonest cause of hypercalcaemia presenting outside hospital (Marshall and Bangert.1995).

Primary hyperparathyroidism may present with the symptoms of hypercalcaemia but frequently patients are symptom-free (Marshall and Bangert.1995).

Bone disease is apparent in fewer than 10% of subjects. Approximately 15% of patients have renal complications in the form of nephrolithiasis or nephrocalcinosis (Marshall and Bangert,1995).

II- Familial hypocalciuric hypercalcaemia (FHH):

FHH is an autosomal dominant condition in which moderate hypercalcaemia with relative hypocalciuria is present throughout most of life (Marshall and Bangert,1995).

III- Hypercalcaemia of malignancy:

Malignancy can result in hypercalcaemia by two general mechanism:

-by secretion into the circulation of factors which increase bone resorption and/or decrease urine calcium loss (humoral hypercalcaemia of malignancy HHM) and

-as a result of metastases which lead to local bone destruction (Marshall and Bangert,1995).

PTH–related peptide (PTH-rP) appears to be an important mediator of the hypercalcaemia of malignancy. It is synthesized and secreted by number of tumors (e.g squamous cell carcinoma of bronchus, renal cell carcinoma, breast carcinoma) and produces biochemical changes similar to those of primary hyperparathyroidism (binds to normal PTH receptors and causes increased calcium levels), though the degree of hypercalcaemia is often more sever in HHM and plasma 1,25$(OH)_2$ D level tend to be suppressed rather than increased (Marshall and Bangert .1995,Bishop,2003).

IV- Granulomatous disease:

The macrophages in granulomata occurring in sarcoid tissue, pulmonary tuberculosis and berylliosis are capable of 1α-hydroxylating 25(OH) D, free from normal homeostatic regulation (Marshall and Bangert.1995).

V- Vitamin D toxicity:

Excessive intake of vitamin D or its analogues can produce hypercalcaemia (Marshall and Bangert,1995).

VI- Thiazide diuretics:

Increase calcium reabsorption, leadind to hypercalcemia.

VII- Prolonged immobilization:

May cause increased bone resorption. Hypercalcemia associated with immobilization is further compounded by renal insufficiency (Bishop.2003).

B) Clinical features:

- Neurological and psychiatric features such as lethargy, confusion, irritability and depression.
- Gastrointestinal problems such as anorexia, abdominal pain, nausea and vomiting, and constipation.
- Renal features such as thirst and polyuria, and renal calculi.
- Cardiac arrhythmias (Gaw, *et a*l.1999).

II-Hypocalcaemia:

A) Causes:

I- Hypoparathyrodism:

Idiopathic, after neck surgery, or occasionally due to magnesium deficiency (hypomagnesemia may cause hypocalcemia by three mechanisms: it inhibits the glandular secretion of PTH a cross the parathyroid gland membrane, it impairs PTH action at its receptor site on bone and it causes vitamin D resistance (Gaw, *et al*.1999, Bishop,2003).

II- Vitamin D deficiency:

This may be due to malabsorption, or an inadequate diet with little exposure to sunlight. It may lead to bone diseases, osteomalacia in adult and rickets in children.

III- Renal disease:

The diseased kidneys fail to synthesize 1,25 DHCC. Increased PTH secretion in response to the hypocalcaemia may lead to bone disease if untreated.

IV- **Pseudohypoparathyroidism**:

PTH is secreted but there is failure of target tissue receptors to respond to the hormone.

V- **Rarer causes** such as malignancy, acute rhabdomyolysis, acute pancreatitis, or bone marrow transplantation (Gaw, *et al*.1999).

B) Clinical features:

- Neurological features such as tingling, tetany and mental changes.
- Cardiovascular signs such as an abnormal ECG.
- Cataracts (Gaw, *et al*.1999).

1.2.3 Phosphate

Phosphate is an essential mineral that is required by every cell in the body for normal function (Higdon, 2007). The majority of the phosphorus in the body is found as phosphate (PO4). Approximately 85% of the body's phosphorus is found in bone (Higdon, 2007).

1.2.3.1 Phosphate Physiology:

Compounds of phosphate are everywhere in living cells and participate in many of the most important biochemical process (Bishop,2003). The genetic materials deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are complex phosphodiesters. Most coenzymes are esters of phosphoric or pyrophosphoric acid (Bishop,2003).

The most important reservoirs of biochemical energy are ATP, creatine phosphate, and phosphoenolpyruvate. Phosphate deficiency can lead to ATP depletion, which is ultimately responsible for many of the clinical symptoms observed in hypophosphatemia (Bishop,2003).

Alteration in the concentration of 2,3-bisphosphoglycerate (2,3-BPG) in the red blood cells affect the affinity of hemoglobin for oxygen, with an increase facilitating the release of oxygen in the tissues and a decrease making oxygen bound to hemoglobin less available. By affecting the formation of 2, 3-BPG, the concentration of inorganic phosphate indirectly affects the release of oxygen from hemoglobin.

Understanding the cause of an altered phosphate concentration in the blood is often difficult because transcellular shifts of phosphate are a major cause of hypophosphatemia in blood. That is, an increased shift of phosphate into cells can deplete phosphate in the blood. Once phosphate is taken up by the cell, it remains there to be used in the synthesis of phosphorylated compounds. As these phosphate compounds are metabolized, inorganic phosphate slowly leaks out of the cell into the blood, where it is regulated principally by the kidney (Bishop,2005).

1. **2.3.2 Regulation of phosphate:**

Phosphate in blood may be absorbed in the intestine from dietary sources, released from cells into blood, and lost from bone. In healthy individuals, all these process are relatively constant and easily regulated by renal excretion or reabsorption of phosphate (Bishop,2005).

Disturbances to any of these processes can alter phosphate concentration in the blood; however, the loss of regulation by the kidneys will have the most profound effect. Although other factors such as vitamin D, calcitonin, growth hormone, and acid-base status can affect renal regulation of phosphate, the most important factor is PTH, which overall lowers blood concentrations by increasing renal excretion (Bishop,2005).

Vitamin D acts to increase phosphate in the blood. Vitamin D increases both phosphate absorption in the intestine and phosphate reabsorption in the kidney (Bishop,2005).

Growth hormones, which helps regulated skeletal growth, can affect circulating concentration of phosphate. In cases of excessive secretion or administration of growth hormone, phosphate concentrations in the blood may increase because of decreased renal excretion of phosphate (Bishop,2005).

Because phosphorus is not as tightly regulated by the body as calcium, serum phosphate levels can rise slightly with a high phosphorus diet, especially after meals. High phosphate levels in the blood reduce the formation of the active form of vitamin D (calcitriol) in the kidneys, reduce blood calcium, and lead to increase PTH release by the parathyroid glands. However, high serum phosphorus levels also lead to decreased urinary calcium excretion (Calvo,1996, Calvo,2000).

1.2.3.3 Distribution of phosphate:

Although the concentration of all phosphate compounds in blood is about 12 mg/dL (3.9 mmol/L), most of that is organic phosphate and only about 3 mg/dL to 4mg/dL is inorganic phosphate. Phosphate is the predominant intracellular anion, with intracellular concentrations varying, depending on the type of cell. About 80% of the total body pool of phosphate is contained in bone (Bishop,2005).

1.2.3.4 Clinical applications of phosphate:

I-Hypophosphatemia:

Hypophosphatemia associated with disturbances of calcium metabolism is usually due to high circulating PTH concentration. In such conditions, and in renal tubular disorders of phosphate reabsorption, phosphate is lost from the body in urine. Hypophosphatemia may also be caused by severe and prolonged dietary deficiency; urinary phosphate excretion is then usually significantly reduced (Mayne,1998).

Phosphate enters cells from the extracellular fluid if the rate of glucose metabolism is increased. This may be associated with glucose infusion during, for example, the treatment of diabetic coma with insulin. The redistribution of phosphate is a common cause of hypophosphatemia in patients receiving parenteral nutrition with insulin and glucose. Long term parenteral feeding, without phosphate supplementation, may cause true phosphate depletion. Hypophosphatemia in such circumstances,

whether due to deficiency or redistribution, may cause neurological abnormalities such as convulsions (Mayne,1998).

II-Hyperphosphataemia:

Normal plasma phosphate concentration are higher in infants and children than in adults.The commonest cause of hyperphosphataemia is renal glomerular dysfunction, it is important not to correct hypocalcaemia until this abnormality has been corrected. Less common cause include hypoparathyroidism and acromegaly (Mayne,1998).

1.3 Rationale:

Diabetes mellitus is a medical condition that can be life-threatening if not treated properly. The incidence and prevalence of type 2 diabetes mellitus is seemed to be increasing in Sudan. Many studies reported abnormal results for serum level of calcium and phosphorus in associated with type 2 diabetes mellitus, in different countries. This study aims to answer the question “Are there any significant change in serum levels of calcium and phosphorus in Sudanese patients with type 2 diabetes mellitus?

1.4 Objectives:

1.4.1 General objective:

To estimate the levels of serum calcium and phosphorus in type 2 diabetic patients.

1.4.2 Specific objectives:

1) To estimate the levels of serum calcium and phosphorus in case and control groups.

2) To compare between the means of calcium and phosphorus in case and control groups.

3) To correlate between calcium and phosphorus levels and the duration of diabetes.

4) To correlate between the mean of calcium and phosphorus levels and sex of diabetic patients.

5) To assess the correlation between calcium and phosphorus levels, and the age of diabetic patients.

CHAPTER TWO

2. Materials and Methods

2.1 Study design:

This is analytical hospital based case-control study.

2.2 Study area:

The study was conducted in type 2 diabetic patients in Khartoum state.

2.3 Study population:

This study included 40 blood samples collected randomly from type 2 diabetic patients in Jabir Abu Elazz diabetes center as test group and 40 blood sample from healthy volunteers as control group to determine level of calcium and phosphorus. The clinical data were obtained from history and record in a questionnaire sheet.

2.4 Inclusion Criteria:

Patients with type 2 diabetes mellitus.

2.5 Exclusion criteria:

Patients with type 1 diabetes mellitus and have already congestive heart failure, hemorrhage, rhabomyolysis and tumor were excluded.

2.6 Samples:

About 2.5ml of venous blood were collected by venipuncture technique from each patient. The samples collected under aseptic conditions then allowed to clot, centrifuged at 3000 rpm for 5 minutes to obtain serum to determine levels of calcium and phosphorus.

2.7 Ethical consideration:

Patients who voluntarily accepted to participate in the study were included.

2.8 Equipments:

- Colorimeter, model JENWAY.
- Centrifuge
- Sterile plane containers
- Disposable syringes
- 70% alcohol
- Tourniquets
- Cotton
- Micropipettes (automatic pipettes)
- Graduated pipettes

2.9 Determination of phosphate by phosphomolybdate colorimetric method:

I-Principle:

Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum color. The intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample.

II-Reagents: see appendix no.2

III-Procedure: see appendix no.2

IV-Reference range: see appendix no.2

V- Quality control: see appendix no.2

2.10 Determination of calcium by O-cresolphatalein colorimetric method:

I-Principle:

The measurement of calcium in the sample is based on formation of color complex between calcium and O-cresolphatalein in alkaline

medium. The intensity of the color formed is proportional to the calcium concentration in the sample.

II- Reagent: see appendix no.3

III- Procedure: see appendix no.3

IV- Reference range: see appendix no.3

V- Quality control: see appendix no.3

CHAPTER THREE

3. Results

Forty Sudanese patients with type 2 diabetes mellitus as test group and forty healthy volunteers as control group were enrolled in this study. Males and females were matched for (age range 40-80 years).

Table (3.1) Shows insignificant difference between the means of calcium level of test group compared to control group. (Mean ±SD): (8.468 ± 0.616) versus (8.530 ± 0.620) respectively, P= 0.652

Table (3.2) Shows significant difference between the means of phosphorus level of test group compared to control group. (Mean ±SD): (3.8125+0.5958) versus (4.9275±1.3105) respectively, P=0.000. The mean of the test group is significantly decrease.

Figure (3.1) Shows no difference between the mean of calcium level of males compared to females.

Figure (3.2) Shows no difference between the mean of phosphorus level of males compared to females.

Figure (3.3) A scatter plot shows insignificant very weak negative correlation between the calcium level (mg/dL) and the duration (year) of diabetes (r= -0.088 P= 0.587)

Figure (3.4) A scatter plot shows insignificant very weak positive correlation between the phosphorus level (mg/dL) and the duration (year) of diabetes (r=0.025 P= 0.877)

Figure (3.5) A scatter plot shows insignificant very weak negative correlation between the calcium level (mg/dl) and the age (year) of diabetic patients (r= -0.018 p= 0.912)

Figure (3.6) A scatter plot shows insignificant very weak positive correlation between the phosphorus level (mg/dl) and the age (year) of diabetic patients (r= 0.125 p=0.442)

Table (3-1): calcium level between test and control group

	Group	N	Mean	Std. Deviation	P-value
Calcium	Test	40	8.468	.6162	.652
	Control	40	8.530	.6203	

Table (3-2): phosphorus level between test and control group

	NO	N	Mean	Std. Deviation	P-value
Phosphorus	Test	40	3.8125	.59579	.000
	Control	40	4.9275	1.31051	

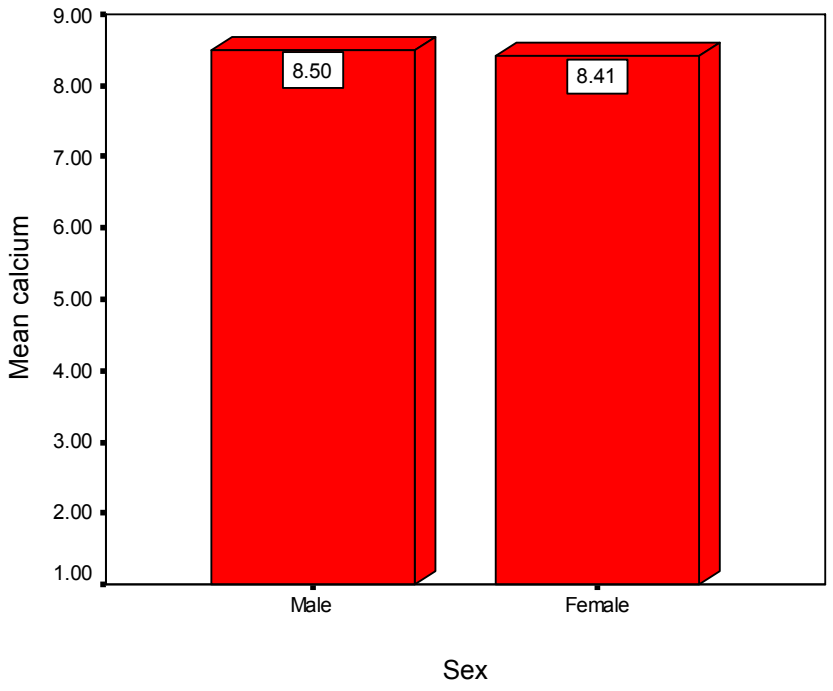

Figure (3-1): The means of the calcium level in reference to sex

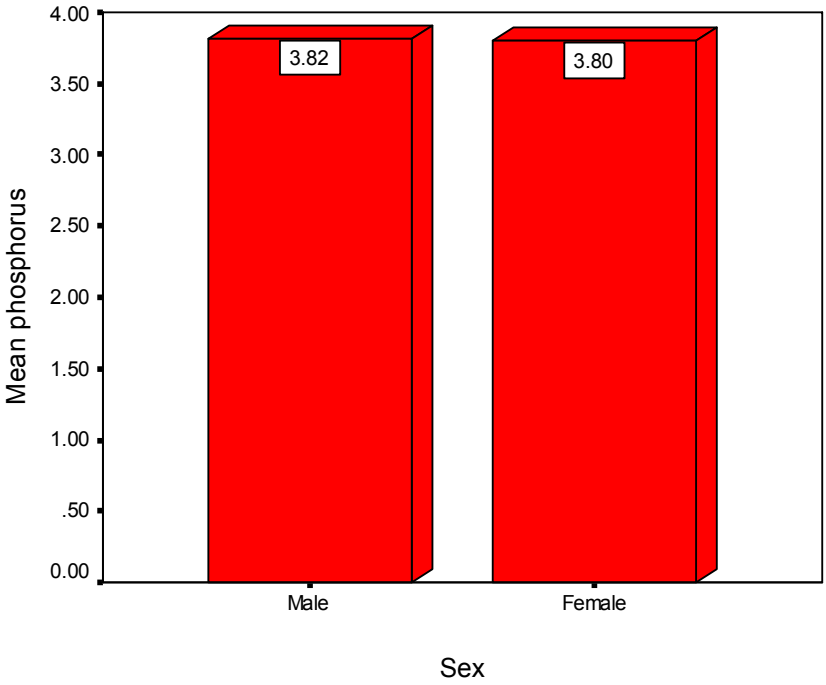

Figure (3-2): The means of the phosphorus level in reference to sex

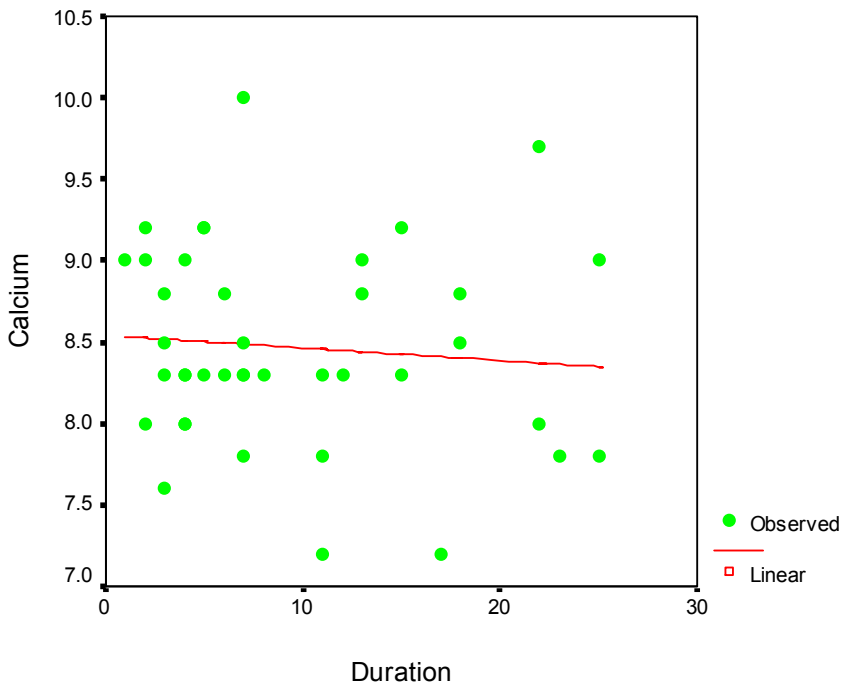

Figure (3-3): A correlation between calcium and duration (r= -0.088 p= 0.587)

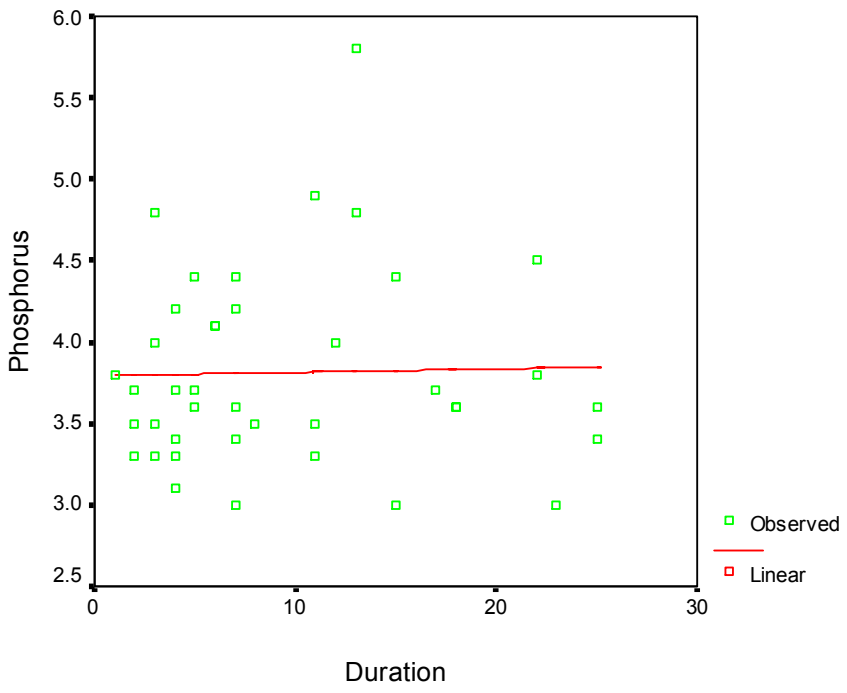

Figure (3-4): Correlation between phosphorus levels and duration (r= 0.025 p=0.877)

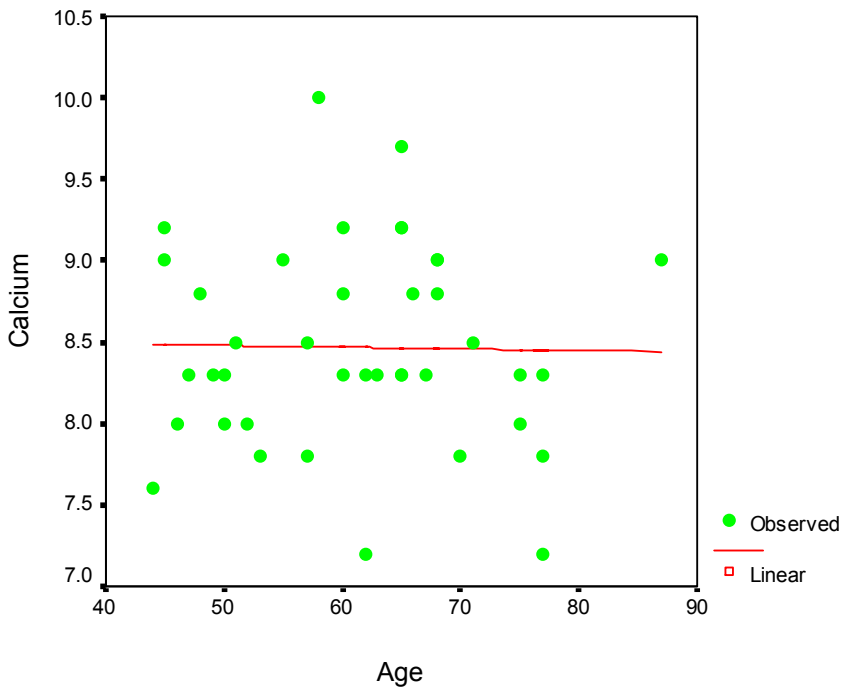

Figure (3-5): Correlation between calcium level and age

(r= -0.018 p=0.912)

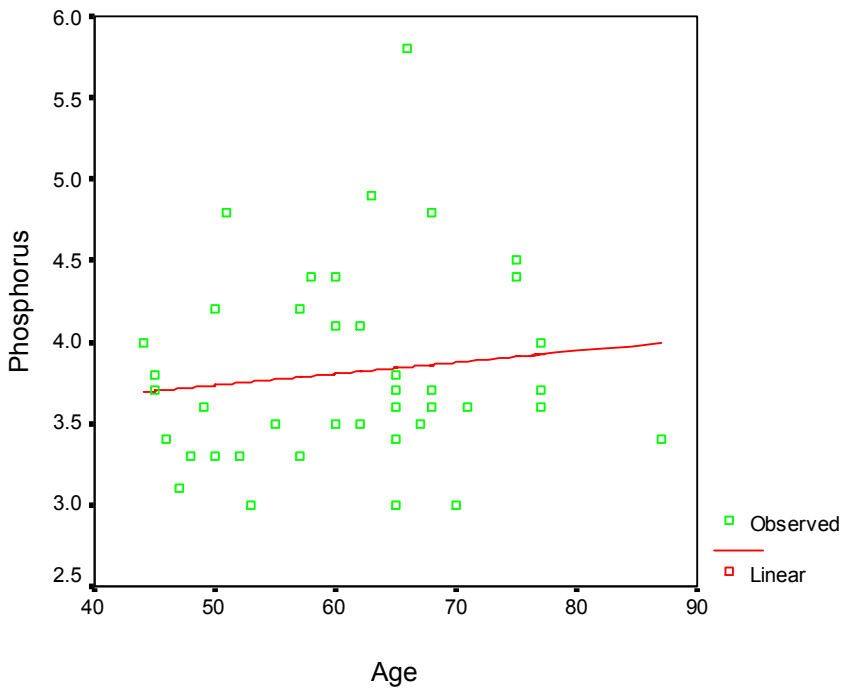

Figure (3-6): Correlation between phosphorus levels and age (r= 0.125 p=0.442)

CHAPTER FOUR

4. Discussion, Conclusions and Recommendations

4.1 Discussion:

It is well established that inadequate management and control hyperglycemia predispose diabetic patients to number of complications. The aim of this study is to compare the level of serum calcium and phosphorus between type 2 diabetic patients and non diabetic patients. In the present study out of 40 type2 diabetic patients were involved as test group and 40 healthy subjects as control group.

In this study, level of serum calcium between patients and control remained unaltered without significant effect of hyperglycemia accompanying diabetes. Relative availability of dietary calcium sources and the partially controlled patients hyperglycemia; may all contribute to these results.

Our finding is in agreement with results that obtained in study done by Hamad,*et al.* which reported no change in levels of calcium between diabetic and non diabetics patients (p value = 0.124).

Levels of serum phosphorus were lower in diabetic in comparison to healthy subjects; this may indicate a possible negative effect of hyperglycemia on serum phosphorus. Also low phosphate levels were known to affect those who have type 2 diabetes (legan,1994); this could justify our finding. In study done in Alneelain Medical research center-Sudan in 2013, which reported levels of phosphorus were decreased among diabetics in comparison to control group (p value = 0.001).

In this study there was an insignificant correlation between the age of diabetic patients and serum levels of calcium and phosphorus.

Also there was insignificant correlation between sex of diabetic patients and serum levels of calcium and phosphorus.

Duration of diabetes is known to be related to the development of complications, however in this study no association was found.

4.2 Conclusions:

From the results of this study it conclude that:

-Hyperglycemia in type 2 diabetic patients associated with lower levels of serum phosphorus.

-Unaltered levels of serum calcium.

-Duration of disease, age and sex of patients don't affect the levels of serum calcium and phosphorus.

4.3 Recommendations:

In type 2 diabetic patients, determination of phosphate should be one of the basic parts of monitoring, because of its importance that mentioned before and to prevent or minimize the complications that resulted from hypophosphatemia.

Patients with type 2 diabetes must be check regularly for serum calcium level and should be supplement with calcium to prevent complications of hypocalcaemia.

References

References

Ahmed A.M.A: (2002) Brief history of Diabetes Mellitus, Saudi Med J; 23: in press.

Ahmed AM, Ahmed NH: (2001) Diabetes mellitus in Sudan, practical diabetes. 18(9); 324-327.

Al-Selevany BK: (2005) Serum Calcium level in patients with type II Diabetes Mellitus; Iraqi Journal of Medical Sciences, 4 [1]; 84.

Beckett G, Walker S, Rae P: (2010) Clinical Biochemistry. Eighth edition; by wiley- Blackwell; 71-93.

Bishop ML, Edward P: (2005) Clinical Chemistry Principles, Procedures, Correlation. Fifth edition; By William and Wilkins Lipincott; 267.

Bishop ML: (2003) Clinical Chemistry Principles, Procedures, Correlation. Fourth edition; By William and Wilkins Lipincott; 219-316.

Botolin S, Mscabe LR: (2006) Chronic hyperglycemia modulates osteoblast gene expression through osmotic and non osmotic pathway. 1;99 [2]: 411-24.

Calvo Ms, park yk: (1996) Changing Phosphorus content of the U.S. diet: potential for adverse effects on bone. J nutrition; 126.

Calvo Ms: (2000) Dietary considerations to prevent loss of bone and renal function. Nutrition; 16.

Carl A, Edward R, David E: (2008) Fundamentals of Clinical Chemistry. Sixth edition; 381-382.

Chaterjea MN, Chwla R: (2010) Clinical Chemistry (Organ Function Test, Laboratory investigations and inborn metabolic diseases). Second edition; by jaypee; 88.

Elbagir MN, Eitam MA, Elmahdi EMA: (2007) A population Based Study of prevalence of Diabetes in Adults in North Sudan, Diabetes care: 19; 1126-1128.

Grow A, Cowan RA, Stewart MJ, Shepherd J: (1999) Clinical Biochemistry. Second edition; by Churchill Livingstone; 56-67.

Hamad NA, Eltayeb LB, Brair SL, Bakheit KH, Hamdan ZK, Omer WH: (2013), a clinical study of serum calcium, phosphorus, and Alkaline Phosphatase level in type II Diabetes mellitus among Sudanese population in Khartoum state; Al Neelain in Medical Journal , 3[10]; 42-50.

Higdon J: (2007) Micronutrient Research for Optimum Health, Ph.D. Linus Pauling Institute.

King H, Aubert RE, Herman WH: (2004) Global prevalence of Diabetes Estimates for the year 2000 and projections for 2030, Diabetes care, 27[25]; 1047-1053.

Legan M: (1994) the Influence of Insulin Growth 1, Growth Hormone and Sex Steroids on Bone Mineral density; Medicinski Razgeldi; 33: 519-526.

Marshall WJ and Bangert SK: (1995) Clinical Biochemistry Metabolic and Clinical Aspects; 87-268.

Marshall WJ and Bangert SK: (2004) Clinical Chemistry. Fifth edition; 194-232.

Mayne PD: (1998) Clinical Chemistry in diagnosis and treatment. Sixth edition; 172-209.

Wild S, Roglic G, Green A, Sicree R, King H: (2004) Global Prevalence of diabetes, diabetes care; 27[5]; 1047-1053.

Appendices

Sudan University of Science and Technology

College of Medical Laboratory Science

Estimation of Serum Calcium and Phosphorus levels in Sudanese Patients with Type 2 Diabetes Mellitus

Appendix (1) Questionnaire

(a) General Information:

Name: ……

Age:……

Sex: Male ☐ Female ☐

(b) Clinical Information

Duration of the disease:……../ year.

Type of treatment:

Insulin ☐ Tablet ☐ Diet ☐

Other disease:

Yes ☐ no ☐

If yes specify …….

(c) Laboratory investigation:

Test	Result	
Calcium	mg/dl	mmol/L
Phosphorus	mg/dl	mmol/L

Date:…… Signature:…… …

SPINREACT

Appendix (2) A.

CE PHOSPHORUS -C

Phosphorus

Phosphomolybdate. Colorimetric

Quantitative determination of phosphorus
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum colour.
The intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample[1,2].

CLINICAL SIGNIFICANCE

Phosphorus is an essential mineral for tissue bone formation and is required by every cell in the body for normal function. Approximately 85% of the body phosphorus is found in bone and in teeth.
Low levels of phosphorus, can be caused by hypervitaminosis D, primary hyperparathyroidism, renal tubular disorders, antacids or malabsortion.
High levels of phosphorus can be caused by diet, bone metastases, liver disease, alcohol ingestion, diarrhea and vomiting[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Molybdic	Molybdate-Borate Sulphuric acid (H_2SO_4)	1.21 mmol/L 100 mmol/L
R 2 Catalyzer	1,2 Phenylenediamine	2.59 mmol/L
PHOSPHORUS CAL	Phosphorus aqueous primary standard 5 mg/dL	

PRECAUTIONS

R1/RT: Corrosive (C): R35: Causes severe burns.
S24: Avoid contact with the skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S30: Never add water to this product. S45: In case of accident or if you feel unwell, seek medical advice immediately.

PREPARATION

Working reagent (WR):
Mix equal volumes of R 1 (Molybdic) and R 2 (Catalyzer)
Stability: 10 h at 2-8ºC, protected from light.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 710 nm ≥ 0.40.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 710 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment[(Note 1)].

SAMPLES

- Serum[1]:
  Free of hemolysis. Serum should be removed from the clot as quickly as possible to avoid elevation of serum phosphorus from hydrolysis or leakage of phosphate present in erythrocytes.
  Stability: 7 days at 2-8ºC.
- Urine[1,2] (24 h):
  Collect the specimen into a bottle containing 10 mL of 10% v/v hydrochloric acid (HCl) to avoid phosphate precipitations. Adjust to pH 2. Dilute the sample 1/10 with distilled water. Mix. Multiply the result by 10 (dilution factor). Stability: 10 days at 2-8ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: ..... 710 nm (620-750)
   Cuvette: ..... 1 cm. light path
   Temperature ..... 37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.5	1.5	1.5
Standard[(Note 2-3)] (µL)	--	50	--
Sample (µL)	--	--	50

4. Mix and incubate for 10 min at 37ºC or 30 min at room temperature (15-30ºC).
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

Serum

$$\frac{(A)\text{Sample}}{(A)\text{Calibrator}} \times 5 \text{ (Calibrator conc.)} = \text{mg/dL of phosphorus in the sample}$$

Urine 24 h

$$\frac{(A)\text{Sample}}{(A)\text{Calibrator}} \times 5 \times \text{vol. (dL) urine 24 h} = \text{mg/24 h of phosphorus}$$

Conversion factor: mg/dL x 0.323 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum:

Children	:	4.0 - 7.0 mg/dL	(1.3 – 2.2 mmol/L)
Adults	:	2.5 - 5.0 mg/dL	(0.8 – 1.8 mmol/L)

Urine: 300 - 1000 mg/24 horas (10 - 33 mmol/24h)

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,008 mg/dL to *linearity limit* of 15 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	3.64	6.25	3.72	6.17
SD	0.05	0.04	0.22	0.03
CV (%)	1.28	0.69	5.99	0.56

Sensitivity: 1 mg/dL = 0.104 A.
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.9928
Regression equation: y=99782x + 0.0329
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed with bilirubin up to 20 mg/dL, hemoglobin up to 150 mg/dL and ascorbic acid up to 30 mg/dL[1].
A list of drugs and other interfering substances with phosphorus determination has been reported by Young et. al[3,4].

NOTES

1. PHOSPHORUS CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. Most of the detergents and water softening products used in the laboratories contain chelating agents and phosphates. It is recommended to rinse glassware in diluted nitric acid and water before using.
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.
5. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

1. Farrell E C. Phosphorus. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1072-1074 and 418.
2. Daly J A. et al. Clin Chem 1972; 18 (3): 263-265.
3. Young DS. Effects of drugs on Clinical Lab. Tests. 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests. 4th ed. AACC 2001.
5. Burtis A. et al. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed. AACC 1995.

PACKAGING

Ref: 1001150 | Cont. | R1:1 x 150 mL, R2: 1 x 150 mL, CAL: 1 x 5 mL

SPINREACT,S.A./S.A.U. Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
Tel. +34 972 69 08 00 Fax +34 972 69 00 99. e-mail: spinreact@spinreact.com

SPINREACT

Appendix (3)

CE

CALCIUM-oC

Calcium

o-Cresolphtalein. Colorimetric

Quantitative determination of calcium
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD
The measurement of calcium in the sample is based on formation of color complex between calcium and *o*-cresolphtalein in alkaline medium:

$$Ca^{++} + o\text{-Cresolphtalein} \xrightarrow{OH^{-}} \text{Colored complex}$$

The intensity of the colour formed is proportional to the calcium concentration in the sample[1,2,3].

CLINICAL SIGNIFICANCE
Calcium is the most abundant and one of the most important minerals in the human body. Approximately 99% of body calcium is found in bones.
A decrease in albumin level causes a decrease in serum calcium. Low levels of calcium are found in hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsortion.
Among causes of hypercalcemia are cancers, large intake of vitamin D, enhaced renal retention, osteoporosis, sarcosidosis, thyrotoxicosis, hyperparathyroidism[1,6,7].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	Ethanolamine	500 mmol/L
R 2 Chromogen	*o*-Cresolphtalein 8-Hidroxyquinolein	0.62 mmol/L 69 mmol/L
CALCIUM CAL	Calcium aqueous primary standard 10 mg/dL	

PRECAUTIONS
R2: Corrosive (C):R35:Causes severe burns.

PREPARATION
All the reagents are ready to use.
To prepare monoreagent, mix according to this proportion: 50 vol. of R1 and 1 vol. of R2.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations are prevented during their use.
Do not use reagents over the expiration date.
The monoreagent stability is 5 days at 2-8ºC.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 570 nm ≥ 0.2.

ADDITIONAL EQUIPMENT
- Spectrophotometer or colorimeter measuring at 570 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment[(Note 1,2)].

SAMPLES
- Serum or plasma[1]: Separated from cells as rapidly as possible. Blood anticoagulants with oxalate or EDTA are not acceptable since these chemicals will strongly chelate calcium.
- Urine[1]: Collect 24 hour urine specimen in calcium free containers. The collecting bottles should contain 10 ml of diluted Nitric acid (50% v/v). Record the volume.
  Dilute a sample 1/2 in distilled water. Mix. Multiply results by 2 (dilution factor).

Stability of the samples: Calcium is stable 10 days at 2-8ºC.

PROCEDURE
1. Assay conditions:
   Wavelength: . . . . . 570 nm (550-590)
   Cuvette: . . . . . 1 cm. light path
   Temperature . . . . . 37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R 1 (mL)	2.0	2.0	2.0
R 2 (drop)	1	1	1
Standard[(Note 3,4)] (µL)	--	20	--
Sample (µL)	--	--	20

4. Mix and incubate for 5 min at 37ºC / 15-25ºC.
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The color is stable for at least 40 minutes.

CALCULATIONS
Serum and plasma $\frac{(A)\text{Sample}}{(A)\text{Standard}}$ x 10 (Standard conc.) = mg/dL calcium in

Urine 24 h $\frac{(A)\text{Sample}}{(A)\text{Standard}}$ x 10 x vol. (dL) urine/24 h =mg/24 h calcium

Conversion factor: mg/dL x 0.25= mmol/L.

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]
Serum or plasma:

Adults	8.5-10.5 mg /dL	≅	2.1-2.6 mmol/L
Children	10 -12 mg/dL	≅	2.5 - 3 mmol/L
Newborns	8 -13 mg/dL	≅	2 - 3.25 mmol/L
Urine:			
Adults	50 - 300 mg/24h	≅	1.25 - 7.5 mmol/24h
Children	80 -160 mg/24h	≅	2 - 4 mmol/24h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: From detection limit of 0,17 mg/dL to linearity limit of 15 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	8.62	14.9	8.02	14.9
SD	0.06	0.12	0.14	0.28
CV (%)	0.68	0.81	1.76	1.89

Sensitivity: 1 mg/dL = 0,043 A.
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.99.
Regression equation: y= 0.97x + 0.26.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
No interferences were observed with triglycerides up to 1.25 g/L[1,2,3].
A list of drugs and other interfering substances with calcium determination has been reported by Young et. al[4,5].

NOTES
1. CALCIUM CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with diluted 1/1 HNO_3 in water and then thoroughly rinsed it with distilled water.
3. Most of the detergents and water softening products used in the laboratories contains chelating agents. A defective rinsing will invalidate the procedure.
4. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
5. Use clean disposable pipette tips for its dispensation.
6. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

1. Farell E C. Calcium. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1051-1255 and 418.
2. Kessler G. et al. Clin Chem 1964; 10 (8); 686-706.
3. Connerty H. V. et al. Am J Clin Path 1996; 45 (3); 200-296.
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed. AACC 2001.
6. Burtis A. et al. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed. AACC 1995.

PACKAGING

Ref:1001060 | Cont. | R1:2 x 150 mL, R2: 1 x 10 mL, CAL: 1 x 5 mL

SPINREACT, S.A. / S.A.U. Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
Tel. +34 972 69 08 00 Fax +34 972 69 00 99. e-mail: spinreact@spinreact.com