بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Serum Calcium, Albumin and Phosphorus Levels among Female Patients with Polycystic Ovarian Syndrome in Sudan

تقييم مستوى الكالسيوم والألبيومين والفوسفات في مصل الدم لدى الإناث المصابات بمتلازمة التكيس المبيضي في السودان

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الآية

بسم الله الرحمن الرحيم

قال تعالي :

(وَلَوْ أَنَّمَا فِي الْأَرْضِ مِن شَجَرَةٍ أَقْلَامٌ وَالْبَحْرُ يَمُدُّهُ مِن بَعْدِهِ سَبْعَةُ أَبْحُرٍ مَّا نَفِدَتْ كَلِمَاتُ اللَّهِ ۗ إِنَّ اللَّهَ عَزِيزٌ حَكِيمٌ)

صدَقَ الله العَظِيم
سوره لقمان الايه (27)

DEDICATION

To all my Family Members.
To all my Teachers Elsewhere.
To my Father Taha Abubaker, God bless his soul.
To the great woman Haleema Tyrab Hassan .
To my wife Nabeela Abdelgabar.
To my Girlie Balsam and Raseel.

Mohamed

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Firstly, the great praise and thanks to God who gave me the ability to complete this work

I am gratefully acknowledging my supervisor DR. **Saifaldeen Ahmed Mohamed** for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this research.

My sincere thanks also goes to Dr. Nabeela Abdelgbar for enlightening the first glance of research.

Last but not the least; I would like to thank my parents and to my brothers and sisters for supporting me spiritually throughout writing this research.

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List of abbreviations:

PCOS	Polycystic ovarian syndrome
PTH	Parathyroid hormone
Ca	Calcium
BMI	Body Mass Index
SHBG	Six Hormone Binding Globulin
GNRH	Gonadotropin Releasing Hormone
CaSR	Calcium Sensor Receptor
ATP	Adenosine Tri phosphate

Abstract

Background: polycystic ovarian syndrome (PCOS) is a condition affects 5% ---10% of women of reproductive age worldwide. Characterized by increased ovarian and adrenal androgen secretion. Metabolism of calcium is regulated by vitaminD and Ca with vitamin D have direct effect on the ovarian and/or adrenal steroidgenesis pathway, Albumin is carrier of calcium in the blood and phosphorus metabolism also regulated by vitamin D

Objective: This study was carried out to assess serum level of calcium, albumin, and phosphorus in Sudanese female patients with polycystic ovarian syndrome

Material and Methods: hundred women are involved in this study divided to Fifty newly diagnosed PCOS subjects aged 19-40 years and fifty age-matched healthy women as control group. 3 ml venous blood collected into plain container from each volunteer under aseptic condition. All blood samples were allowed to clot at room temperature, and then there were centrifuged at 4000rpm to obtain serum, serum obtain was used to analysis calcium, phosphorus and albumin by using urit8021A fully auto analyzer. And the result obtain where analyzed by using statistic package for social science (SPSS) version20 on programmed computer.

Results: The mean level of serum calcium was significantly decreased in patients with polycystic ovarian syndrome in comparison with control group (8.714±1.1646)mg/dl versus (9.220±1.5260)mg/dl with p-value 0.031. In contrast the mean of serum phosphorus was insignificant different in patients with polycystic ovarian syndrome in comparison with control group (4.324±0.7925)mg/dl versus (4.076±0.8923)mg/dl with p-value0.133. On the other hand the mean level of albumin were insignificant different in patients with polycystic ovarian syndrome in comparison with control group (3.812±.6123)g/dl versus (4.050±.6594)g/dl with p-value0.068. Person's correlation showed, serum calcium inversely correlated with BMI of PCOS patient(r=-0.355,p-value0.011).

Conclusion: The study concluded that serum calcium is lower among PCOS patient while Albumin and phosphorus is insignificant changed.

المستخلص

خلفيه الدراسه:متلازمه التكيس المبيضي المتعدد،هي حاله تصيب 5%---10%من النساء في سن الانجاب حول العالم . تتميز بزياده افراز الاندروجين من المبيض والغده الكظريه. استقلاب الكالسيوم ينظم بواسطه فيتامين د والكالسيوم مع فيتامين د لهم تاثيرمباشر في مسار البويضه او استيرويد الغده الكظريه، الالبيومين حامل للكالسيوم في الدم والفسفور ايضا استقلابه يتم بواسطف فيتامين د,

الهدف:اجريت هذه الدراسه لتقييم مستوي مصل الكالسيوم ،الالبيومين والفوسفات لدي النساء السودانيات المصابات بمتلازمه التكيس المبيضي المتعدد

المواد والطرق:مائه امراءه شاركو في هذه الدراسه قسموالي خمسون من الذين تم تشخيصهم مؤخرأ بمتلازمه التكيس المبيضي المتعدد تتراوح اعمارهم بين 19و40 عاما والنساء الاصحاء المتطابقات معهم في العمر كمجموعه تحكم، 3مل من الدم الوريدي جمت من المتطوعين في وعاء عادي تحت ظروف تعقيم وتركت للتتجلط في درجه حراره الغرفه

ثم تم فصلها بواسطه جهاز طرد مركزي 4000 لفه في الدقيقه للحصول علي مصل الدم و المصل المتحصل عليه استخدم في تحليل الكالسيوم والفسفات والالبيومين بواسطه جهاز يوريت 8021ا الاوتوماتيكي.التحليل الاحصائي تم باستخدام برنامج الحزمه الاجتماعيه للعلوم الاحصائيه(اس بي اس اس).

النتائج : انخفض مستوي الكالسيوم في الدم بشكل ملحوظ في المرضي الذين يعانون من متلازمه التكيس المبيض المتعدد(8.714±1.1646)ملجرام لكل ديس لتر مقارنه مع مجموعه التحكم (9.220±1.5260) ملجرام لكل ديس لتر مع القيمه الاحتماليه0.031.في المقابل ليس هنالك اختلاف ذو دلاله احصائيه في مستوي الفوسفات في المرضي الذين يعانون من متلازمه التكيس المبيضي المتعدد (4.324±**0.**7925) ملجرام لكل ديس لتر مقارنه مع مجموعه التحكم(0.8923±4.076) مع القيمه الاحتماليه 0.133.من ناحيه اخري ليس هنالك اختلاف للالبيومين ذو دلاله احصائيه في المرضي الذين يعانون من متلازمه التكيس المبيضي المتعدد(3.812±**0.**6123) جرام لكل ديس لتر مقارنه مع مجموعه التحكم (4.050±6594) جرام لكل ديس لتر مع القيمه الاحتماليه0.068 كما بينت الدراسه وجود ارتباط عكسي للكالسيوم مع مؤشر كتله الجسم مع قيمه احتماليه0.011وارتبا ط 0.355

الخلاصه: خلصت الدراسه الي انه هنالك انخفاض واضح في مستوي تركيز الكالسيوم في الدم لدي النساء السودانيات المصابات بمتلازمه التكيس المبيضي المتعدد عند مقارنتهم بالاصحاء بينما لايوجد اختلاف في مستوي الفسوسفات والالبيومين.

CHAPTER ONE
INTORDUCTION – RATIONAL AND OBJECTIVES

CHAPTER ONE

INTRODUCTION-RATIONAL AND OBJECTIVES

1.1 Introduction:

Polycystic ovary syndrome which means that the ovaries become enlarged with multiple cyst and contain fluid filled sacs which surround the eggs, resulting in woman's ovaries or adrenal glands increased production of estrogen and androgen male hormone than normal, Which can stop eggs from ovulation leading to oligomenorrhea, hirsutism, infertility and obesity in young women (*Danish; 2010*). Polycystic ovaries syndrome is more common in woman who have obesity or mother or sister with PCOS (*Genazzani, ;2008*). According to Rotterdam criteria PCOS is defined by the existence of at least two of three criteria. Which are hyperandrogenism .chronic an ovulation, and polycystic ovaries on ultrasound findings (*Rotterdam Revised; 2003*)

The prevalence of PCOS is estimated to be approximately 48.5 million women aged 20–44 years (Rojas *et al.*,2014).The prevalence of polycystic ovary syndrome in the Qatari population between 16–20% (*Dargham et al.*;2017).The prevalence of PCOS in Australian women is estimated to be between 12-21% (*Jacqueline and Helena; 2012*). It has recently been demonstrated that elevated levels of phosphorus and parathyroid hormone (PTH) might be involved in the pathogenesis of the syndrome, possibly phosphorus was correlated negatively with insulin and insulin resistance and positively with 25-dihydroxyvitamin vitamin D3 (25OHD3)(*Diamanti et al ;2006*)Recently, it has been demonstrated that other parameters of bone

metabolism, namely lower osteocalcin and elevated serum levels of its carboxylated form, were associated with androgen levels, insulin resistance, and ovarian morphology in PCOS women ,These findings suggest a potential interaction between bone-derived markers and the metabolic/hormonal abnormalities observed in PCOS Some studies support the effect of vitamin D deficiency on pathophysiology of PCOS and even insulin resistance (*Mahmoudi et al ;2010*). Pal *et al* found that 3 months supplementation with vitamin D and calcium can reduce androgens. They believe that vitamin D and Ca have a direct effect on the ovarian and/or adrenal steroidgenesis pathway (*Pal et al* ;2012). Firouzabadi *et al* also found calcium and vitamin D supplementation can make a positive effect on weight loss, follicle maturation, menstrual regularity, and improvement of hyperandrogenism, in infertile women with PCOS (*Firouzabadi et al;* 2012). According to Thys-Jacobs *et al* calcium hemostasis disturbance can cause follicle growth disorders (*Thys-Jacobs et al ;1999*). In this review, 2 studies about calcium and vitamin D also showed a significant effect of these supplements on follicle growth and response to main treatment (*Mohammad et al; 2012*). Rashidi *et al* suggest combination of calcium-vitamin D therapy increases therapeutic effects of metformin treatment of menstrual disorders and maturation of follicles than metformin alone (*Rashidi et al; 2009*). the prevalence of obesity in PCOS patient is increased when compared to the general female population and, conversely, the prevalence of PCOS is increased in overweight and obese women when compared to their lean counterparts.(*martinez et al ;2007*)

1.2 Rationale:

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders that occurs in 4% -7% of women of reproductive age. It is the one

of the main cause of women infertility and hence potential risk to a woman's health over her lifecycle clearly remain a clinical investigational, genetic, and therapeutic challenge. PCOS is known to be associated with reproductive morbidity and increased risk for endometrial cancer, type 2diabetes mellitus, and hypertension, metabolic and cardiovascular diseases (*Galluzzo; 2008).*

Few Recent data showed that PCOS is related to abnormal minerals metabolism that have essential role in the regulation of ovarian follicle growth and ovulation rate. And suggest that may be associated with PCOS complication.

This study may help to support the Sudanese study suggested that abnormal calcium, albumin and phosphorus levels is associated with the development of PCOS in Sudan. Few study about the serum calcium and albumin in Sudanese women with PCOS, but now previous studies available about phosphorus in PCOS.

1.3 Objectives:

1.3.1General objective:

To assess serum level of calcium, albumin and phosphate among female patients with Polycystic Ovarian Syndrome in Sudan

1.3.2Specific objective:

1-To measure and compare serum levels of calcium, albumin and phosphorus in case (Women with polycystic ovary syndrome) and control group.

2- To calculate body mass index in both study groups.

3-To correlate between biochemical parameters and study variables (age – BMI) and correlate between calcium and albumin levels in case group.

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO

2. Literature review:

2.1 Polycystic ovary syndrome:

The polycystic ovary syndrome (PCOS) was first described by Stein and Leventhal in 1935(*Azzizet al; 2006).*Stein and Leventhal suggested that the ovarian change in bilateral cystic ovaries is most probably a result of some hormonal stimulation and that this stimulation was most likely the result of anterior pituitary secretions. PCOS is associated with several other diseases/morbidity-related factors such as obesity and other cardiovascular disease, which are becoming more prevalent among females today(*Schmidt, 2011).*

Poly cystic ovary syndrome has become one of the major public-health challenges It affects 5%–10% of women of reproductive age worldwide (*Walters et d; 2012*). . Woman with PCOS are higher risk of diabetes metabolic syndrome, heart disease, high blood pressure (*Dunaif et al ;2008*).

Published available online in march 2011 the result of study say women with polycystic ovaries syndrome have high serum level of phosphorus when compared with control, phosphorus may affect the pathogenesis of polycystic ovaries syndrome*(mohamed, 2011)* .

Article in April 2014 the study was conducted to assessed the a complex of calcium and phosphate incidence in patients' diagnosed with polycystic ovaries syndrome, total serum calcium level were low in polycystic ovaries syndrome patient but within normal range in women without PCOS , but phosphorus within the normal limit*(Mohamed ;2014).*

study published in march 2018 on Estradiol and calcium levels among

Female patients with Polycystic Ovarian Syndrome in Khartoum State, The mean level of plasma calcium was significantly decreased (p.value 0.000) whereas the mean level of AMH was significantly increased (p. value0.000) in patients with polycystic ovarian syndrome as compared to control group. On the other hand the mean level of estradiol and albumin were insignificantly decreased (p.value 0.086 and 0.283) respectively*(Elmugadam; 2018).*

2.1.1 A etiology of PCOS:

The etiology of PCOS remains unclear, and abnormal ovarian steroidogenesis, hyperinsulinemia, and neuroendocrine abnormalities have been proposed as a primary underlying abnormality (*Dunaif et al;* 2008). Abnormal steroidogenesis is suggested by studies showing that ovarian theca cells produce excessive androgens and show abnormal ovarian steroid responses to gonadotropin (*Rojas et al;* 2014). The etiology of PCOS is complex and seems to be influenced by multiple genetic and environmental factors. Among the genetic causes, mutation in genes involved in the synthesis, transport and regulation of androgens have been pointed out. Increasing evidence support relevant role of insulin resistance in blood promote the increased synthesis and secretion of androgens by theca cells in the ovaries through the insulin receptor and insulin growth factor-1 , also is note is that insulin decreases hepatic synthesis of sexual hormone – binding globulin (SHBG), which in turn increases the free fraction and the biological activity of androgen and estrogens(*Willia;2012*).

2.1.2 Pathophysiology of PCOS:

The pathophysiology of PCOS involves primary defects in the

hypothalamic–pituitary axis, insulin secretion and action, and ovarian function.(*Shannon;2012*) Although the cause of PCOS is unknown, PCOS has been linked to insulin resistance and obesity. The association with insulin function is expected; insulin helps to regulate ovarian function, and the ovaries respond to excess insulin by producing androgens, which can lead to anovulation.(*Diamenti et,al;2006*) Follicular maturation arrest is a hallmark sign that an ovarian abnormality exists. Clinical signs of PCOS include elevated luteinizing hormone (LH) and gonadotropin–releasing hormone (GnRH) levels, whereas follicular-stimulating hormone (FSH) levels are muted or unchanged. As a result of the increase in GnRH, stimulation of the ovarian thecal cells, in turn, produces more androgens.(*Urbanek;2007*.) Follicular arrest can be corrected by elevating endogenous FSH levels or by providing exogenous FSH. (*Shannon;2012*) some studies suggest that PCOS is a primary defect in young girls who are entering puberty and who have a family history of the disorder. Approximately 25% of patients with PCOS have elevated prolactin levels.(*Marx;2003*.)

2.1.3 Clinical presentation of PCOS:

PCOS is a hormonal disorder with a potential to lead to various diseases. It also continues to be a common cause of infertility among women (*NIH Pub ;2008*). Although signs and symptoms vary, the three most common factors associated with PCOS include ovulation irregularities, increased androgen levels, and cystic ovaries. Problems with ovulation and elevated androgen levels occur in the majority of women with PCOS. hirsutism, acne, and alopecia are directly associated with elevated androgen levels, and the prevalence of polycystic ovaries on pelvic ultrasound exceeds 70% in patients with PCOS (*Azziz et al;* 2006).

2.1.4Complication of PCOS:

(A) Infertility:

Infertility is defined as absence of pregnancy after tow years of regular intercourse, without using any contraception method**.** PCOS is characterized by anovulation du to Menstrual alteration by oligmenorrhea . in70%of patient's oligmenorrhea patterns with bleeding at intervals of more than 45 days or fewer that nine annual menstruation periods. alternated with the interval of secondary amenorrhea (absence of menstruation for at least 3consecutive months).this absence of ovulation or dys ovulation implies and alteration in fertility present in 30% to 70% of the patients, infertility are related to the hyper secretion of LH(70)present in women with hyper androgenism an ovulatory women, which entails a longer period of time to achieve pregnancy . (*Izzo ;2013*).infertility has been considered by world health organization(WHO)as public health problem.one of the central goals of the UN conference program on action on population and Development in2015was to guarantee, for all individuals, access to quality reproductive health services(*piazza; 2009*).Treatment in these women with fertility problems is focused on inducting ovulation. The objective is to increase levels of endogenous FSH that stimulate follicular development. This can be done in one of two ways; by increasing endogenous production with the use of anti-estrogens drugs or aromatase inhibitors. Through exogenous administration of the hormone (*legro et al ;2013*).

(B)Hirsutism:

Hirsutism defined as an excessive growth of terminal hair in androgen dependent areas of women. Is one of the most widely used clinical criteria for the diagnosis of androgen excess and is observed in 50% - 80% of patients with hyperandrogenism(*yildiz; 2006)*

(C)Acne:

acne is a disorder of the pilosebaceous unit,with lesions on the face,neck,back and chest area. The importance of androgens in the acne pathogenesis is well - know and authenticated. As vulgaris acne, the androgen levels are usually normal. It is believed that the local conversion has been increased for a greater receptors sensibility for androgens in patients with acne in relations to normal population. Perhaps, it represents the most important cause in the disease activation(*Fraser et al ;2004*).

(D)Androgenicalopecia:

the androgenic alopecia in women is characterized by hair loss in the central region of the scalp, with important psychosocial repercussions. In the presence of androgens with increased level of 5-alpha-reductase,the higher concentration of androgen receptors, and lower levels of cytochrome p450enzyme,is shortened anagen phase and terminal follicles suffer miniaturization, becoming vellus hairs(lee *et al;2007*). Most patients with androgenetic alopecia have the normal endocrine function. the anamnesis and physical examination are important to search of other signs of hyper androgenism(*yildiz; 2006*).

(E)Acantosenigricans:

the acanthosisnigricansis characterized by the presence of a brown and velvety plate with accentuation in the furrows of skin. the dermatopatology is the most commonly observed in the neck and intertriginous areas such as armpits. groin and inframammary region and it is reported in 5% of patient pcos(*Araujo et al; 2002*).Although to be Acnto associated with obesity, PCOS and diabetes, my be present in genetic disease, drug reaction(nicotinic acid),and malignancies. The presence of Acanto indicates the glucose tolerance test. When severe, extensive and progressive, may be associated

with malignancy, especially when the mucus is also involved(*Araujo et al; 2002*).

2.2. Calcium:

Calcium is most important mineral in the body. Most of which (98%) is present in the skeleton. One half of the remaining calcium is found in extracellular fluid and the rest in tissues. Calcium has a crucial role in bone mineralization, follicle growth and is also vital for basic physiological processes such as blood coagulation, neuromuscular conduction. Found in many foods, the body need calcium to maintain strong bones and to carry out many important functions (sizer *et al; 2007*). Calcium is storage in bones and teeth. Body also need calcium for muscle for move and for nerves to carry messages between the brain and every body part, also calcium is used to help blood vessels move blood throughout the body and to help release hormone and enzyme that affect almost every function in the human body, also have important role in weight loss or prevent weight gain by its claimed to burn fat and decrease the fat absorption (*NIH ;2010).* Calcium and Vitamin D Supplementation Calcium Supplementation Dietary reference intakes, developed in 1997, recommend calcium intakes of 1000 to 1500 mg/d in healthy individuals, depending on age *(Matkovic;2008).*

2.2.1. Calcium metabolism: Calcium is absorbed and circulated in multiple form,50%from serum calcium is freely ionized witch 45%is bound to protein .while 5%exist in poorly defined complexes(this is not filtrated by the kidney), three hormones' that regulate the serum calcium(1) parathyroid (PTH) from the parathyroid glands; (2) calcitonin (CT) from the C cells of thyroid and ultimobranchial bodies, and (3) dihydroxycholecalciferol "calciferol", formed from vitamin D in liver and kidney by alternating the

secretion rate in response to change in ionized calcium, which increases calcium absorption from the gut.(*paul; 2011*). PTH stimulates calcium release from bone and calcium reabsorption in the kidney, moreover it stimulates 1α-hydroxylation of 25-hydroxy vitamin D"in active" leading to the production of active 1,25-dihydroxyvitamin D (calcitriol) which modulates gastrointestinal calcium absorption (*Holick;* 2007).

2.2.2Hypocalcaemia:

Hypocalcaemia can affect many organ systems and causes arrhythmias, prolongation of QTc interval, hypotension, coarse hair, dry skin, muscle twitching and tingling, paresthesias, tetany, and seizures. Trousseau's and Chvostek's signs are used to examine for increased neuromuscular activity. Chvostek sign is noted by contraction of facial muscles by tapping on the facial nerve near the temporomandibular joint and Trousseau's sign is elicited by inflating the blood pressure (BP) cuff above the patient's systolic BP for 3 minutes which results in spasm of the involved hand. The inherited causes of hypocalcemia can be broadly classified in to disorders of vitamin D metabolism, CaSR and parathyroid gland. (*Egbuna et al ;2008*).

2.2.3Hypercalcemia:

The symptoms of hypercalcemia are dependent not only on the severity of hypercalcemia but the rate of rise in serum calcium levels. The spectrum of symptoms includes three mains organ systems; renal, GI, neurological and cardiac. Patients can present with constipation, loss of appetite, weight loss, abdominal pain, acute pancreatitis, vomiting, irritability, poor concentrating capacity, memory loss, muscle weakness, lethargy, hypertension, shortened QTc interval and cardiac arrhythmias. Renal symptoms include polyuria, polydipsia, volume depletion, nephrocalcinosis and renal failure. The

inherited disorders of hypercalcemia can be broadly classified in to disorders of parathyroid gland (*Lietman et al ;2010*).

2.2.4Calcium and polycystic ovarian syndrome:

Calcium is involved in egg activity oocyte maturation , progression of follicular development and regulation of cell division in mammalian oocytes, calcium deficiency could be related to risk of obesity because the insulin signaling pathway is calcium dependent therefore it is considered that abnormalities of calcium concentration could be associated with insulin resistant and promoting polycystic ovarian syndrome pathologies, biochemical studies have showing that decreased calcium levels are observed in obese women with polycystic ovarian syndrome when compared with health women. calcium homeostasis is depend on vitamin D receptor (VDR), parathyroid hormone (PTH)and calcium – sensing receptor (CaSR) in addition adiponectin concentration is strongly associated with calcium and Vit D level . to determine the role of the polymorphisms of calcium homeostasis –linked factors in initiating PCOS , VDR, PTH, CaSR,insulin receptor and adiponectin genes were analyzed and compared with PCOS – associated biochemical parameters .consequently , polymorphisms of VDR are related to increased LH and reduced SHBG levels and the gene variant of CaSR is linked to higher homeostatic model assessment – IR (HOMA – IR) and IR, combined supplementation of vitamin D 100,000IU/month ca 1000mg/day, and metformin 1500mg/day , for 6months in100 infertile patients with PCOS resulted in significantly reduced body mass index (BMI).in addition menstrual cyclicity , follicular maturation ,and pregnancy rates were affected positively , but the alterations were not statistically significant(*Gray; 2003)*.

2.3 Phosphorus:

phosphorus is a mineral that makes up 1% of persons total body weight.it is the second most abundant mineral in the body.it is present in every cell of the body. Most of the phosphorus is found in the bones and teeth. The main function of phosphorus is in the formation of bones and teeth also play important role in how the body uses carbohydrate and fats and also need for the body to make protein for the growth, maintenance and repair of cells and tissues. Phosphate also help body to make ATP, a molecule the body uses to store energy,(*knochel; 2006*) high serum phosphorus concentrations have been associated with increased rates of cardiovascular disease and mortality in subjects with or without kidney disease. Abnormal deposition of calcium phosphate in soft tissue may predispose individuals to vascular dysfunction and cardiovascular disease, inadequate phosphorus intake rarely results in renal reabsorption of phosphorus increase to compensate for decreased intake (martin *et al;2012).*

2.3.1 Phosphorus metabolism:

phosphorus is either inorganic 30%or organic 70% from 30% of inorganic 10% is ionic ally bound to protein(this is not filtrated by the kidney) 90%is ionic and freely filtrated by the kidney , three hormones' that regulate the serum phosphate ,(parathyroid hormone (PTH), vitamin D, calcitonin) by alternating the secretion rate in response to change in ionized calcium , PTH in bone stimulates osteoclastic activity which release calcium and phosphorus , in kidney PTH promote absorption of calcium and execration of phosphorus , vitamin D is circulating as inactive form 25-OH which can activated to 1-25(OH)2 , vit D act in intestine to increase absorption of calcium and phosphorus and act in kidney to promote reabsorption of calcium and phosphorus (*Bringhures; 2006)*

2.3.2hypophosphatemia:

Phosphorus absorption is rarely limited. Dietary phosphorus, which parallels dietary protein, is present in abundance in most foods. Dietary phosphorus is absorbed almost twice as efficiently as dietary calcium, phosphorus absorption, unlike calcium, is rarely a nutritional problem, also maintained by intestinal absorption, renal excretion, and bone accretion. Bone is the major store for both phosphorus and calcium. There are much larger stores of phosphorus than calcium in soft tissue,hypophosphatemia is also really duo to of phosphorus is absorbed passively and not by the 1,25(OH)2D-dependent active transport system. Hypophosphatemia occurs in diseases with increased PTH secretion, including primary and secondary hyperparathyroidism. The hypophosphatemia is usually mild and asymptomatic. In diseases with increased serum FGF-23, including oncogenic osteomalacia and various forms of hereditary osteomalacia, hypophosphatemia is symptomatic and causes mineralization failure in bone. The acute symptoms of hypophosphatemia include myopathy, fatigue, bone pain, increased risk for rhabdomyolysis and hemolysis. Respiratory and cardiac failure can occur with hypophosphatemia. Chronic hypophosphatemia results in skeletal abnormalities including rickets and osteomalacia*(Bergwitz et al;2010).*

2.3.3 Hyper phosphatemia:

Bioavailability of phosphorus can be reduced by excessive use of compounds that bind dietary phosphate, such as aluminum hydroxide , Renal phosphate excretion is regulated by tubular reabsorption and filtered phosphate load. Similar to calcium**,** hyperphosphatemia occurs in diseases with decreased PTH secretion, including various forms of hypoparathyroidism, and is usually asymptomatic. In contrast, in hereditary

diseases in which the FGF-23 receptor/Klotho receptor complex is disrupted. *(Lightwood; 1953),* hyperphosphatemia is marked and leads to ectopic soft tissue calcification.. GFR hyperphosphatemia occurs in CKD because of the inability of the kidney to excrete the dietary phosphorus load independent of the tubular phosphate reabsorption rate and occurs in the face of increased serum concentrations of both PTH and FGF-23. The acute symptoms of hyperphosphatemia are primarily due to the resulting hypocalcemia and its symptoms (*Shiber;2002*)An acute phosphate load especially after phosphate enemas has been shown to cause phosphate nephropathy and acute kidney injury (*Markowitz et al;2005*). Chronic hyperphosphatemia has been associated with vascular calcifications especially in patients with chronic kidney disease and end stage renal disease *(Neven et al;2011*).

2.4 Albumin:

Albumin is the most abundant plasma protein with a concentration ranging from 35 to 50 g/L.*(Cabrerizo et al; 2015).* Albumin represents 50% of the total protein content of plasma, with globulins making up most of the rest. It is a single peptide chain of 585 amino acids in a globular structure. The molecular weight of albumin is approximately 66 kDa, and it has a half-life of 21 days. Albumin is exclusively synthesized by the liver, initially a pre-proalbumin and then proalbumin, which in the Golgi apparatus is converted to albumin, which is the final form secreted by the hepatocyte, very little albumin is stored in the liver. The synthetic rate is about 10 to 15 grams per day and then secreted into the circulation of which around 40% remains in circulation with a fraction moving from the intravascular to the interstitial space(*Brock et al; 2016*).Factors that stimulate albumin synthesis include the

action of hormones such as insulin and growth hormone. Albumin production may be inhibited by pro-inflammatory mediators such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor*(Ballmer ;2001*).Albumin has several physiological roles. One of the most important is to maintain the oncotic pressure within the vascular compartments preventing leaking of fluids into the extravascular spaces.albumin functions as a low-affinity, high-capacity carrier of several different endogenous and exogenous compounds. . Also, albumin binds at least 40% of the circulating calcium, fatty acid, bilirubin and many drugs, and is a transporter of hormones such as thyroxin, cortisol, testosterone, fatty acid, bilirubin and many drugsAlbumin is also involved with maintaining acid-base balance as it acts as a plasma buffer. (*Brock et al; 2016*).. Renal and gut loss of albumin may account for around 6% and 10% respectively of albumin loss in healthy individuals. A decrease in serum albumin levels below the reference interval hypoalbuminemia(William;*2012*).

2.4.1 Albumin and calcium relationship:

Albumin is carrier of various hydrophobic substance in the blood such as minerals, calcium is one from minerals carried by albumin and calcium level in blood is lowered in hypoalbuminemia ,thus even though total calcium level in blood is lowered ,ionized calcium level may be normal, ionized calcium binds to negatively charge site on protein molecules, competing with hydrogen ions for the some binding sites on albumin and other calcium-binding proteins. This binding is pH defendant and alters the level of ionized calcium in blood. An increase in PH ,alkalosis, promote increased protein binding witch decreases free calcium levels .acidosis, on the other hand, decreased albumin binding, resulting in increased free calcium

levels(*anderson;2005*).

2.5 Body Mass Index (BMI):

Body mass index is a measure of body fat based on height and weight that applies to adult men and women. Universally express in units of kg/m2,resulting from mass in kilogram and height in meters, BMI is used to broadly categorize apearson as underweight, normal weight ,overweight or obese based on tissue mass(muscle, fat and bone)and height(*malcom;2015).*commonly accepted BMI ranges are under weight:18.5kg/m2 , normal weight 18.5 to 24.9 , over weight or pre obesity 25 to 29.9 kg/m2 , obese over 30 kg/m2 . obesity also classified to obesity class I(30 – 34.9 kg/m2) . Class II (35 – 39.9 kg/m2) . class III above 40kg/m2(*WHO;2019).*

2.5.1 Polycystic ovary syndrome and obesity:

Obesity has been recognized as a common feature of the polycystic ovary syndrome (PCOS). In the United States, some studies report that the prevalence of overweight and obesity in women with PCOS is as high as 80%. The prevalence and severity of obesity are lower in women with PCOS outside the U.S. This observation suggests that environmental factors, such as lifestyle, contribute to development of obesity in PCOS(*susan; 2007*). Obesity is a common finding in women with PCOS and between 40–80% of women with this condition is reported to be overweight or obese. Familial aggregation of PCOS strongly supports a genetic susceptibility to this disorder *(Sam et al; 2003)* the metabolic abnormalities associated with PCOS, such as β-cell dysfunction and type 2 diabetes, have heritable components in families of women with PCOS. , the genes responsible for PCOS have not been clearly identified. Considering the close association between PCOS and obesity, it is likely that similar or interrelated genes may

also predispose to obesity in affected women. Environmental factors (high-caloric diets and reduced exercise) also play a major role in the high prevalence of obesity in women with PCOS . Insulin resistance is a common finding in PCOS that is independent of obesity. Insulin-mediated glucose disposal, reflecting mainly insulin action on skeletal muscle is decreased by 35–40% in women with PCOS compared to weight comparable reproductively normal women (*Dunaif ;1999*) Lower fasting levels of the peptide hormone, ghrelin, have been reported in women with PCOS compared to weight-matched control women *(Moran et al; 2004*).

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1Study design:

This study is analytical hospital based-case control study

3.1.2 Study Area:

The study was conducted in Saad Aboalella Hospital and Alser Abulhassan fertility Center among polycystic ovary syndrome patients.

3.1.3 Study population:

The study will be consisted of 50 Patients diagnosis with polycystic ovary syndrome (50) controls (healthy volunteer) will be involved in the study, age will be matched.

3.1.4 Inclusion criteria:

Polycystic ovary syndrome women who are clinically diagnosed according to Rotterdam criteria and healthy subject will be enrolled in this study.

3.1.5 Exclusion criteria:

Postmenopausal status and Smokers

Taking the oral contraceptive pill "OCP" or have taking in the last month

Have any medical condition that maybe responsible for the symptoms of PCOS such as congenital hyperplasia. Androgen secreting tumor. will be excluded.

3.1.6 Ethical Consideration:

The Study was approved from research committee in college of medical laboratory sciences in Sudan University of Sciences and Technology, all patients and controls were informed about the aim of the study and accepted

. Their participation in this study was fully voluntary and a verbal consent was taken from all participant's included in the study.

3.1.7 Data collection tools:

3.1.7.1 Questionnaire:

The data will be collected from all participants through closed ended questionnaire; patient's records will be reviewed to confirm diagnosis and information.

3.1.7.2 Sample collection:

After informed consent a sample were collected by using dry, plastic syringes, tourniquet was not used ,venous blood (3 mL) will be collected into plain container from each volunteer under aseptic condition. All blood samples were allowed to clot at room temperature, and then there were centrifuged at 4000rpm for 10 mints to obtain serum for calcium, phosphorus and albumin. The sample will be stored at -20°C until analysis .The sample with interference substances like lipemic, hemolysis and icteric samples will be excluded.

3.2 Methods

3.2.1 Sample estimation:

3.2.1.1 Principle of Urit 8021A photo electric full auto analyzer :

Operate in the visible portion of the electromagnetic spectrum whereas measures the absorbance of specific colors and give the concentration by Beer's law. **3.2.2 Biochemical parameters**:

3.2.2.1 Estimation of Calcium:

principle: At a neutral PH,the calcium form with Arsenazolll acomplx, the color intensity of which is directly proportional to the concentration of calcium in sample that will be measured by photoelectric colorimeter.

3.2.2.1.1Reagent , procedure , calculation appendix II

3.2.2.2Estimation phosphorus:

Principle: Inorganic phosphate in the sample react with molybdate in acid medium(molybodic acid) forming aphosphomolybdate complex(phosphomolybodic acid)color less that can be measured by photoelectric colorimeter.

inorganic phosphate + ammonium molibdate + sulphuric acid <————colour complex.

3.2.2.2.1Reagent, Procedure, calculation appendix III

3.2.2.3 Estimation of albumin:

Principle:-Albumin reacts with BCG at acidic PH 4.2 to give blue - green color complex that can be measured by photoelectric colorimeter.

3.2.2.3.1Reagent, prouder, calculation appendix IV

3.2.3 Quality control:

The precision and accuracy of all method used in this study will be checked by commercially prepared control sample before its application for the measurement of test and control samples.

3.2.4 Statistical analysis:

Statistical procedure will be followed using statistic package for social science (SPSS) version 20 on programmed computer. T-test will be applied to compare biochemical quantitative data between case and the control groups and person correlation test for correlation and the level of significance will be expressed as $P < 0.05$.

CHAPTER FOUR
RESULTS

CHAPTER FOUR

RESULTS

This study included 50 female with PCOS and 50 without PCOS age matched.

The mean of body mass index was significantly increased among PCOS patient (27.448±4.5665) in comparison with (25.258±4.6717)control group with p- value 0.015 presented in table 4.1.

The mean Concentration of calcium was significantly decreased among PCOS patient(8.714±1.1646)in comparison with(9.220±1.5260)non PCOS with p value 0.031 which presented in table 4.1 In contrast the mean of phosphorus level showed insignificant differnt in PCOS patient (4.324±.7925) versus non PCOS female (4.076±.8923) with p-value 0.133 which presented in table 4.2 Also our results revealed insignificant differnt in mean concentration of albumin in PCOS (3.812±.6123) in Comparison with non PCOS (4.050±.6594) with(p value=0.068) is presented in table 4.1.

Person's correlation showed no correlation between serum calcium and age of PCOS patient(r 0.241 , p value 0.092)presented in figure 4.1 Also no correlation observed between serum albumin and phosphorus with age of PCOS patient (r - 0.110 p-value 0.449) (r – 0.027 , p-value 0.851)respectively presented in figure 4.2 and figure 4.3 . , serum calcium level inversely correlated with body mass index of PCOS patient (r=-0.355, p-value 0.011)presented in figuer4.4 , while no correlation observed between serum albumin and BMI of PCOS patient(r – 0.262 p- value 0.067) presented in figure4.5,Also no correlation observed between phosphorus and BMI of PCOS patient(r - 0.109 , p- value 0.451) presented in figure 4.6 serum calcium not correlated with serum albumin of PCOS patient(r 0.214 p value 0.136)presented in figure 4.7.

Table 4:1

Comparison between the mean of phosphorus, calcium and albumin in patient with PCOS(case)and (control)

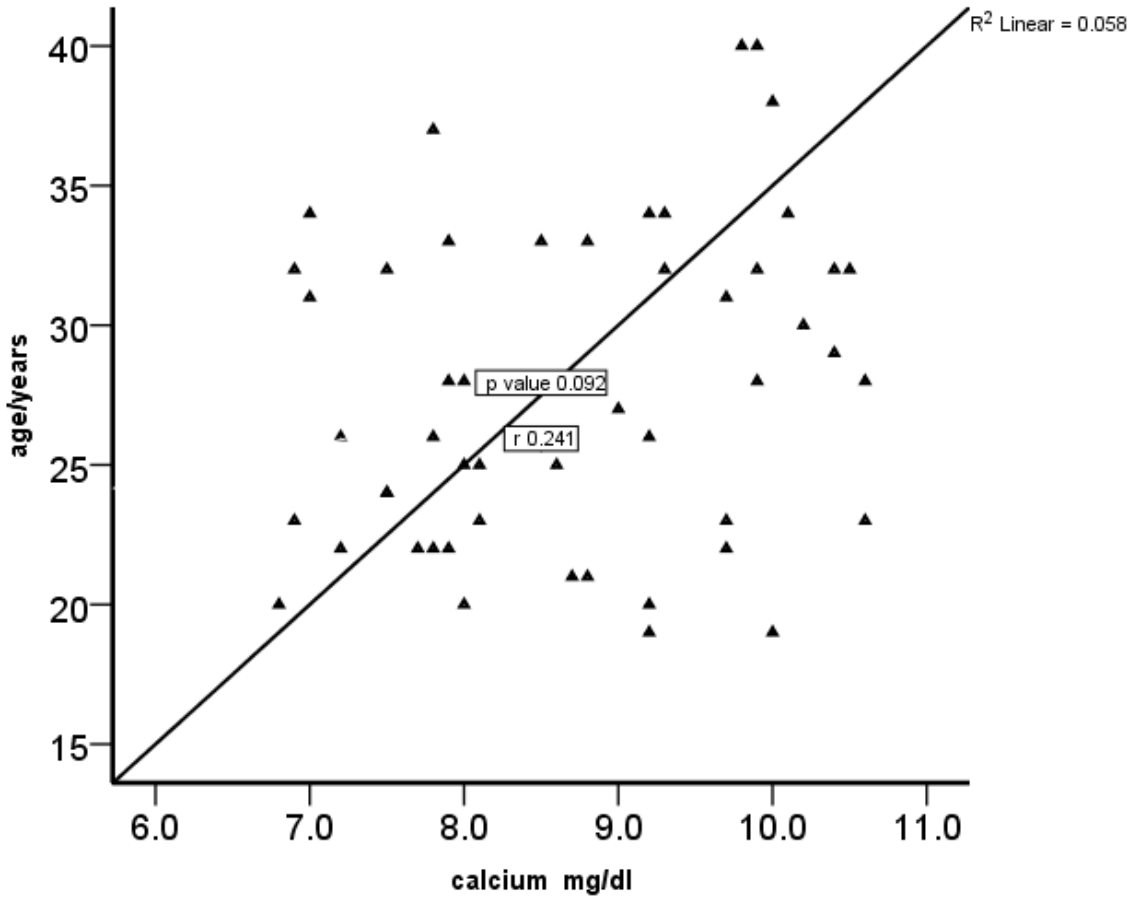
Variable	**Case (n=50) mean**±SD	**Control (n=50) mean**±SD	**P value**
Calcium mg/dl	**8.714**±1.1646	**9.220**±1.5260	**.031**
Phosphorus mg/dl	**4.324**±.7925	**4.076**±.8923	**.133**
Albumin g/dl	**3.812**±.6123	**4.050**±.6594	**.068**
BMI	**27.448**±4.5665	**25.258**±4.6717	**.015**

Table show the mean ± standard deviation and probability value (p)

T test was used for comparison

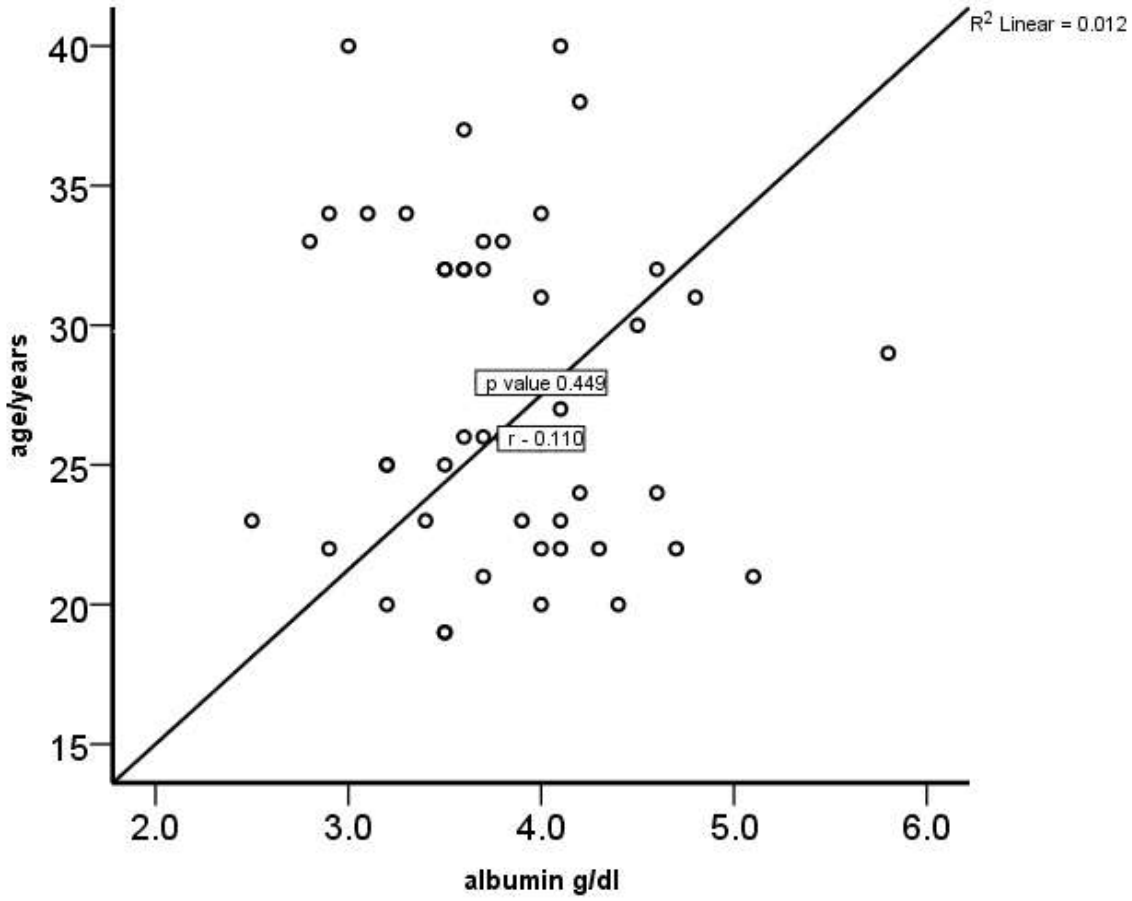
P value < 0.05 considered significant

Figure 4.1



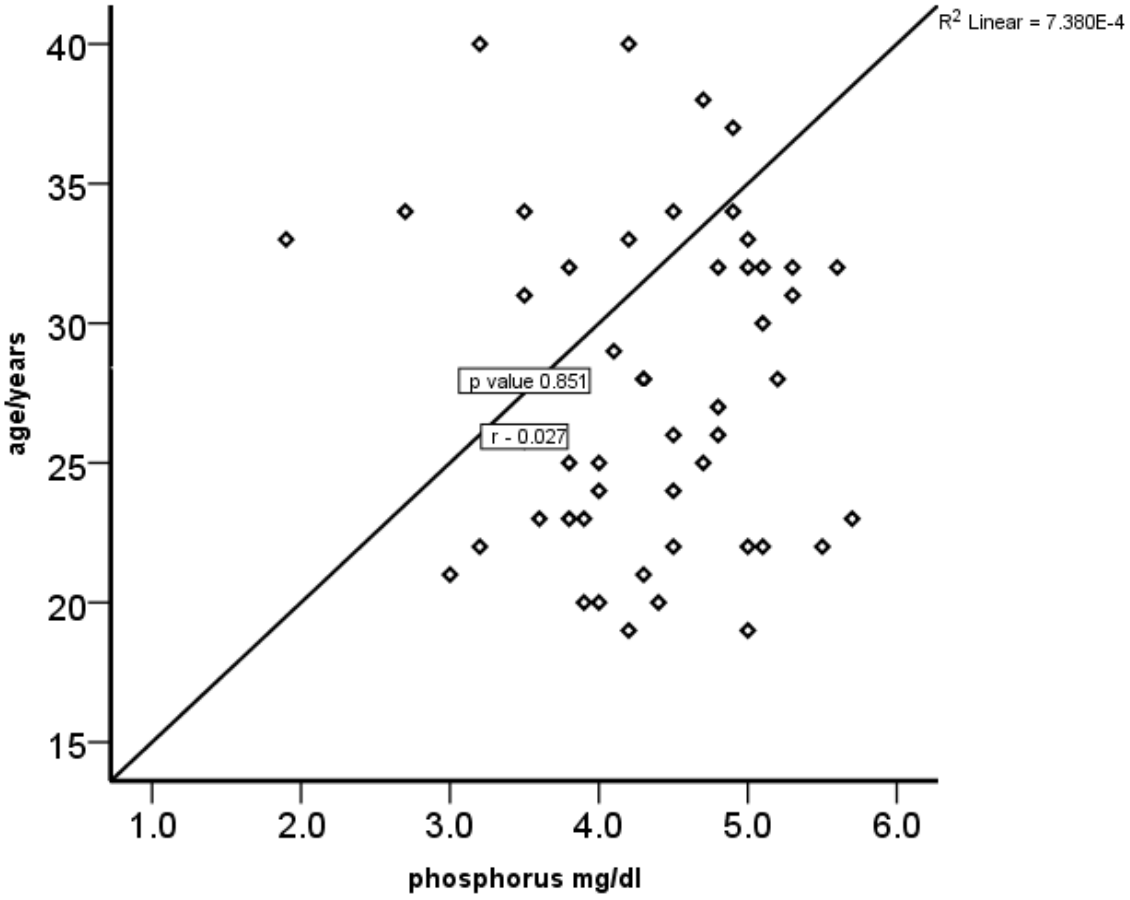
Correlation between calcium and age(r 0.241, value 0.092)

Figure 4.2



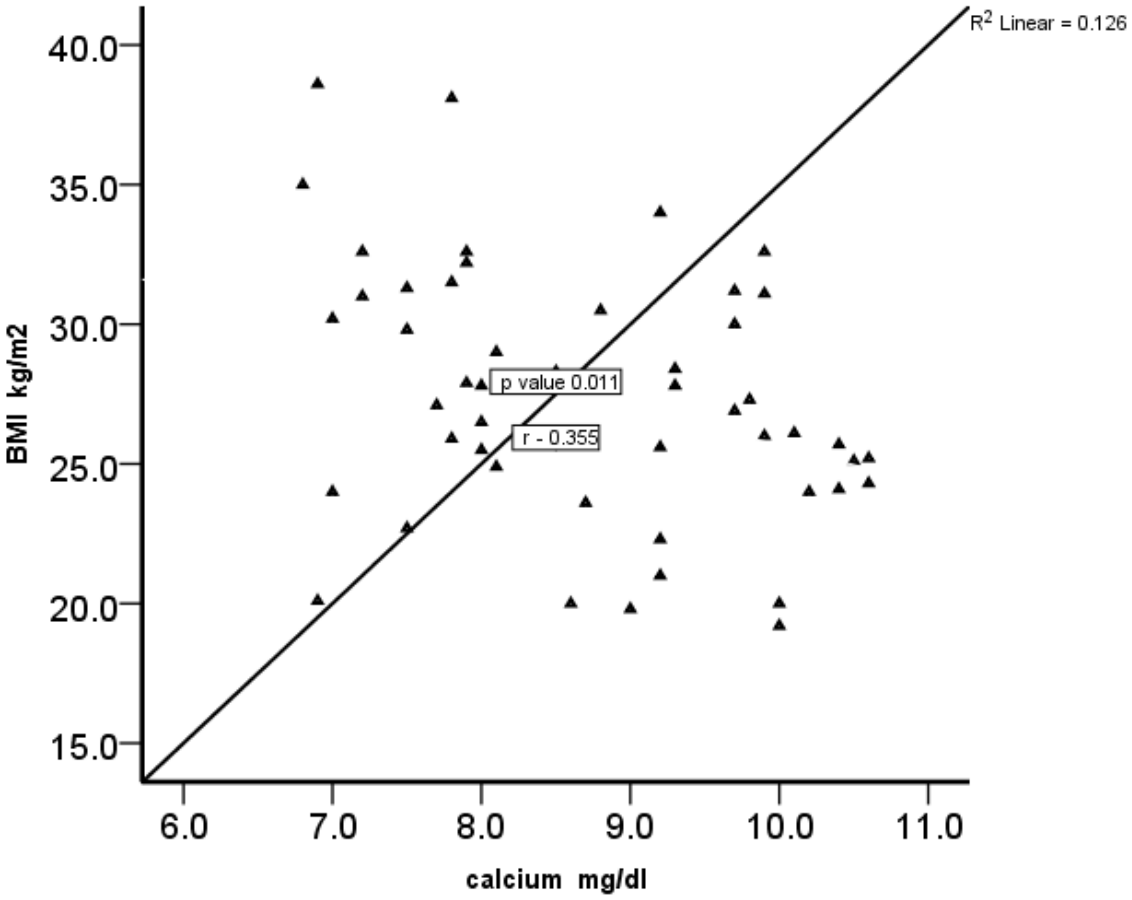
Correlation between Albumin and age(r-0.110,pvalue 0.449)

Figure 4.3



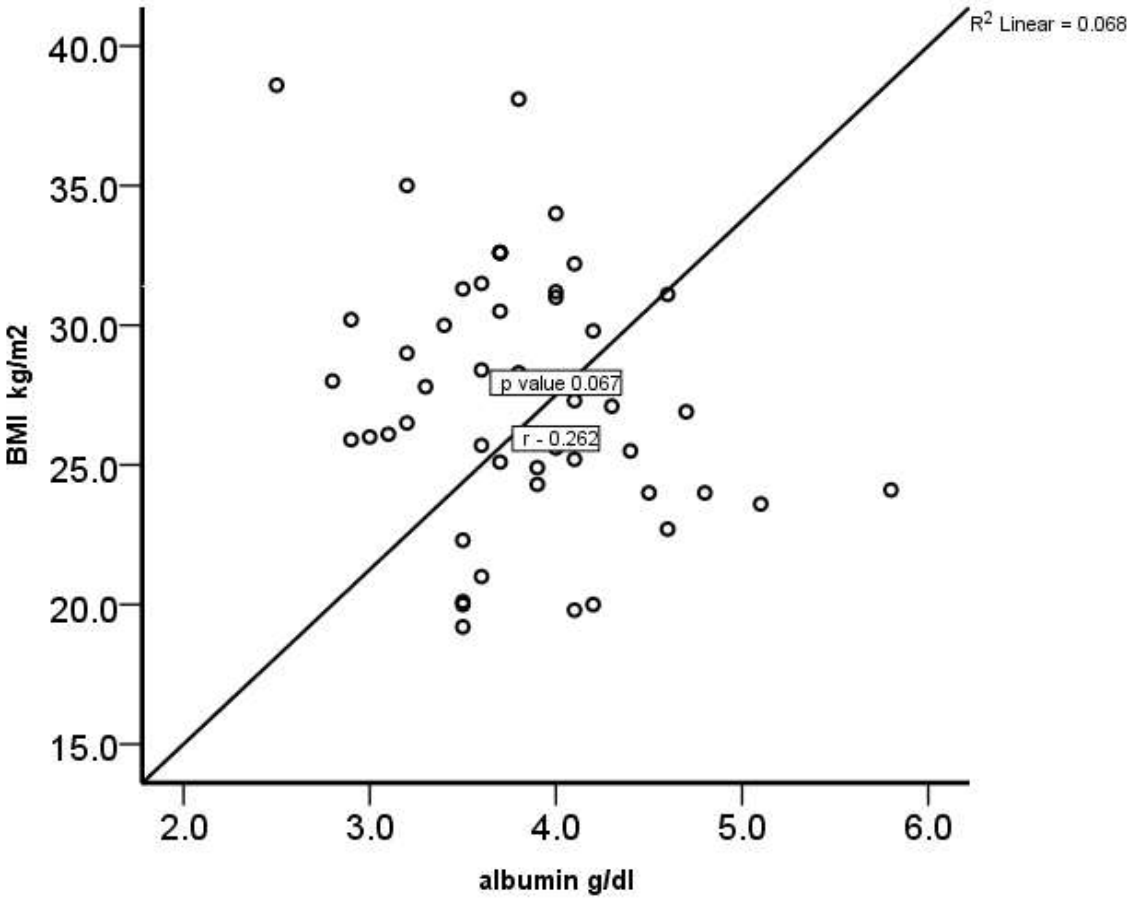
Correlation between phosphorus and age (r -0.027,pvalue 0.851)

Figure 4:4



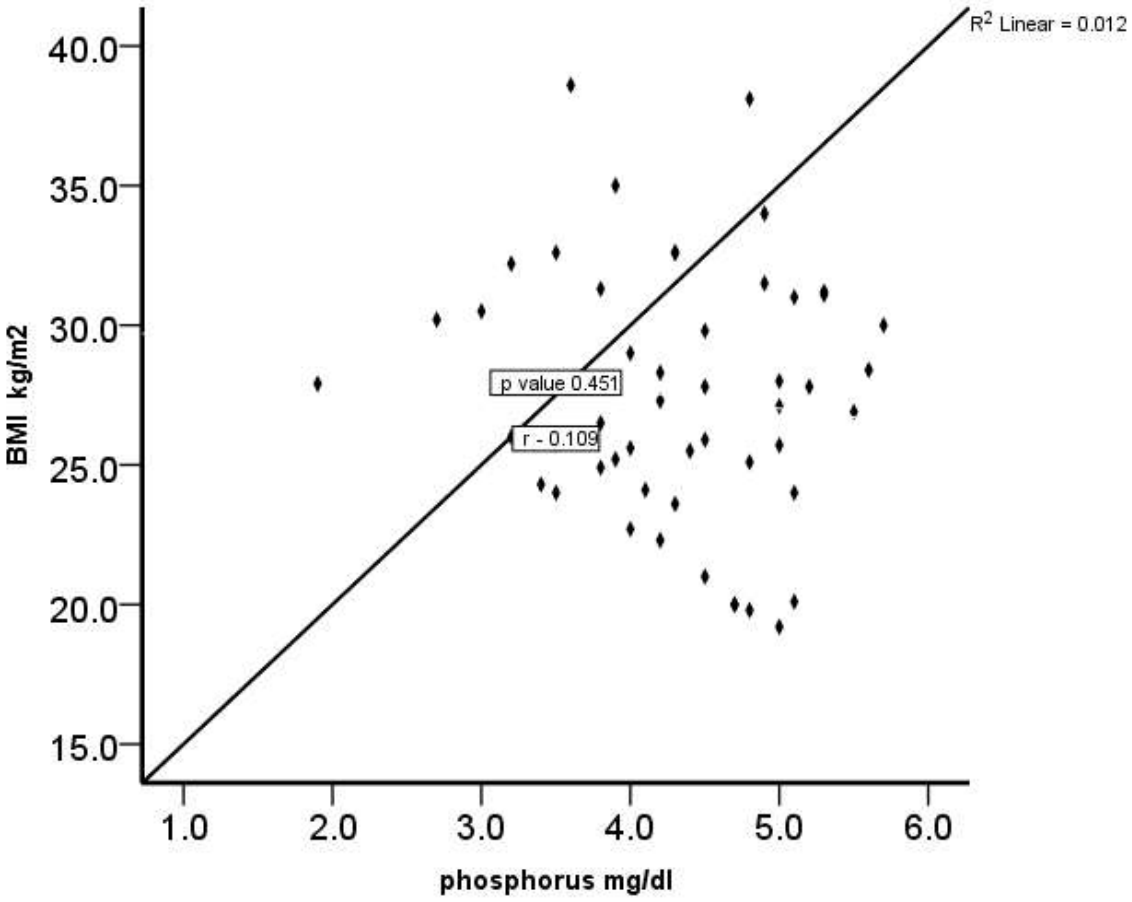
Correlation between calcium and BMI(r - 0.355,pvalue 0.011)

Figure 4:5



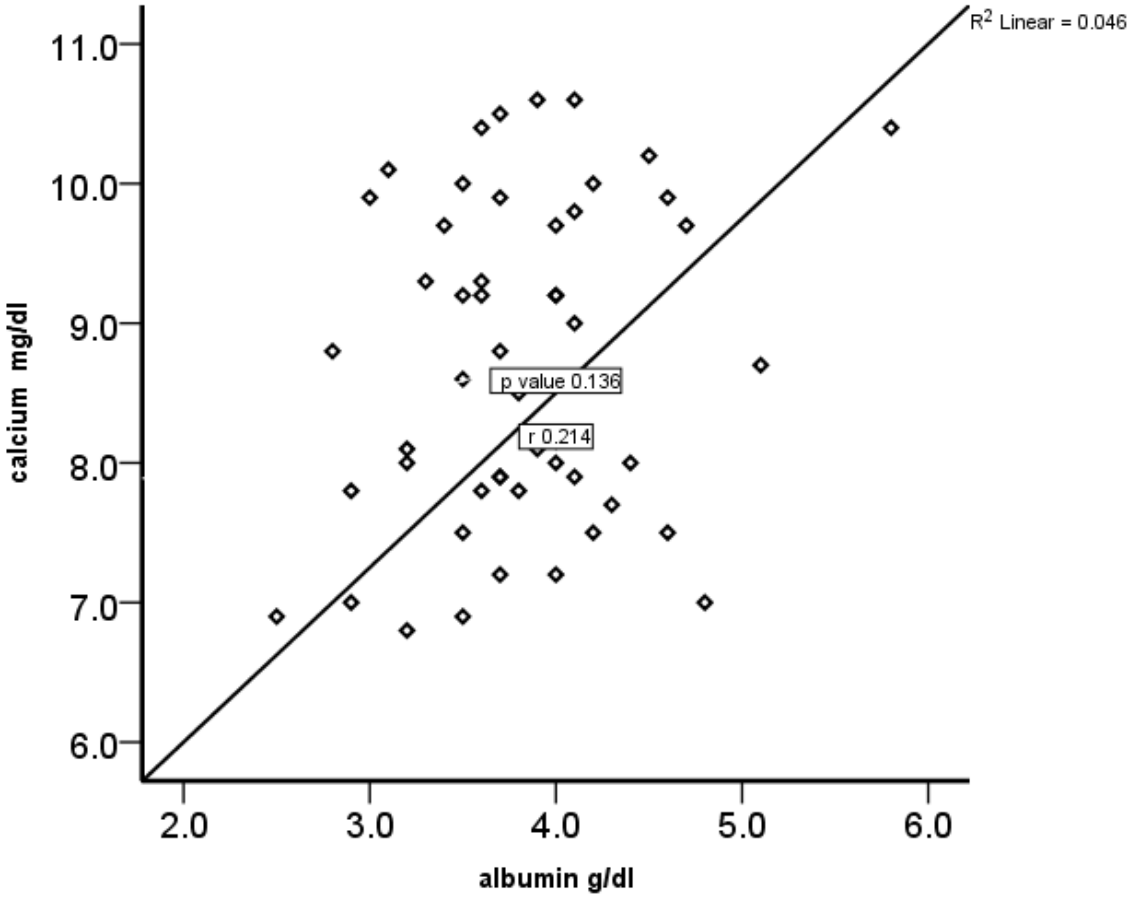
Correlation between Albumin and BMI(r - 0.262,pvalue 0.067)

Figure 4:6



Correlation between phosphorus and BMI(r - 0.109, value 0.451)

Figure: 4.7



Correlation between calcium and albumin(r 0.214,pvalue 0.136)

CHAPTER FIVE
DISSCUTION-CONCLUSION AND RECOMMENDATION

CHAPTER FIVE

DISCUSSION-CONCLUSION AND RECOMMENDATION

5.1 Discussion

Polycystic ovary syndrome (PCOS) is a condition characterized by increased production of androgen. case control study was carried out to assessment status of essential elements calcium, phosphorus and albumin among Sudanese's female with PCOS.

The present study revealed that,The mean of body mass index was significantly increased among PCOS patient in comparison with control group with p- value 0.015 this finding indicate that the PCOS is associated with increased BMI or weight gain when compared with women without PCOS. The finding was in agreement with some studies who stated that, PCOS is associated with increased BMI , weight gain (*Danish, 2010 ; Genazzani, 2008; William, 2012; martinez et al 2007).*

The result also finding that the mean concentration of calcium was significantly decreased among PCOS patient in comparison with non PCOS p value 0.031. This data is similar to data obtain by some studies which reveal that decreased mean concentration of calcium among PCOS patient when compared with non PCOS duo to vitamin D deficiency in PCOS patient (*Rashidi et al 2009; Pal et al;* 2012, *Firouzabadiet al;* 2012, *Mohamed ;2014, Elmugadam ;2018).*

The findings of the study showed that, there were insignificant differnt in the mean of phosphorus level in PCOS patient versus non PCOS female with p-value 0.133.this finding agreed with another study carried by (*Mohamed; 2014).*Which showed that insignificant differnt in phosphorus

and within the normal limit.

Also This results revealed insignificant differnt in mean concentration of albumin in PCOS patient in Comparison with non PCOS with(p value=0.068)is .this finding agreed with another study carried by *(Elmugadam ;2018)*the mean concentration of albumin was insignificant different in woman with PCOS .

Person's correlation revealed correlation between serum calcium and BMI showed, serum calcium level inversely correlated with body mass index of PCOS patient (r=-0.355, p-value 0.011), this Data is similar to data obtain by (*Firouzabadi et al;*2012). Study which reveal calcium correlated inversely with BMI.

Also when associate phosphorus with BMI the result showed; weak insignificant negative correlation observed when associate serum phosphorus with BMI of PCOS patient(r - 0.109, p- value 0.451), this data is similar to data carried by *(Obeid; 2013*) Low phosphorus status might contribute to the onset of obesity.

Also no correlation observed when associate albumin with BMI of PCOS patient(r – 0.262 p- value 0.067),this is agree with(*Wlodek;2003)* High protein albumin diets were constantly found to induce weight loss, probably because of their capacity to decrease energy intake and increase energy expenditure .

Also no correlation observed when associated serum calcium with age of PCOS patient(r 0.241, p value 0.092). may comment: Normally no correlation between serum calcium and age. But serum calcium is changed in sum disease or post-menopausal.

Also no correlation observed when associate serum phosphorus and albumin with age of PCOS patient(r – 0.027, p-value 0.851)(r - 0.110 p-value

0.449)respectively . No previous study's available.

Also serum calcium not correlated with serum albumin of PCOS patient(r 0.214 p value 0.136).

5.2 Conclusion

The study was concluded that, serum calcium is lower among

PCOS patient while phosphorus and albumin is not significantly changed.

5.3 Recommendations:

From the findings of this study it is recommended that:

- Biochemical test calcium, should be done as early screening test for diagnosis of PCOS, especially for female have irregular menstrual cycle or over weight .
- Further studies should be done with increased sample size to investigate phosphorus and albumin.

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APPENDIX

Appendix I


Sudan University of sciences and technology

Collage of graduate studies

Questionnaires of

Serum level of calcium, albumin and phosphorus in women with polycystic ovary syndrome

Serial no ( )

(1)Demographic data:

A/age -------- years

(2)Clinical Data:

A/present of male like characteristics

Yeas (specify) ---------- NO ( )

B/body mass index (BMI)

Weight————k/g height————m

(3) Investigation.

Calcium --------mg/dl

Albumin--------mg/dl

Phosphorus-------mg/dl

Appendix II

SPECTRUM

The Creative Approach to Bioscience

Calcium
Arsenazo III (Single Reagent)

REF: 227 001 (2 x 30 ml) 60 test
REF: 227 002 (2 x 100 ml) 200 test
REF: 227 003 (4 x 30 ml) 120 test
REF: 227 004 (4 x 100 ml) 400 test

SYMBOLS IN PRODUCT LABELLING

EC REP Authorised Representative
IVD For in-vitro diagnostic use
LOT Batch Code/Lot number
REF Catalogue Number
Consult instructions for use
Temperature Limitation
Use by/Expiration Date
CAUTION. Consult instructions for use
Manufactured by

Intended Use

Spectrum Diagnostics calcium reagent is intended for the in-vitro quantitative, diagnostic determination of calcium in human serum on both automated and manual systems.

Background

Calcium is the fifth most common element in the body, most of which (98 %) is present in the skeleton. One half of the remaining calcium is found in extracellular fluid and the rest in tissues. Calcium has a crucial role in bone mineralization and is also vital for basic physiological processes such as blood coagulation, neuromuscular conduction, and normal muscle tone. Calcium is constantly lost from the body through excretion in feces, urine and to a small extent in sweat. The determination of serum calcium is useful for monitoring myeloma, renal failure, acid base balance,and cirrhosis. Both serum and tissue calcium in the body are controlled by parathyroid hormone, calcitonin and vitamin D. Hypocalcemia may be observed in hypoparathyroidism, steatorrhea, pancreatitis and nephrosis. Increased levels may be associated with multiple myeloma and other neoplastic diseases.

Method

colorimetric. Arsenazo III.

Assay Principle

At a neutral pH, the Ca^{2+} form with Arsenazo III a complex, the color intensity of which is directly proportional to the concentration of calcium in the sample.

Reagents

Standard Calcium (ST) 10 mg/dL	2.5	mmol/L
Reagent (R) MES, pH 6.40	100	mmol/L
Arsenazo III	200	µmol/L

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin, rinse immediately with plenty of soap and water. In case of severe injuries, seek medical advice immediately.

Reagent Preparation, Storage and Stability

Spectrum Calcium reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored sealed at 15 – 25 °C.

Deterioration

Do not use the Spectrum Calcium reagents if turbid. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation
Serum and plasma

Use nonhemolyzed serum.Heparin is the only acceptable anticoagulant. No other anticoagulant can be used. Fresh serum collected in the fasting state is the perferred specimen. Serum or plasma should be separated from cells as soon as possible, because prolonged contact with the clot may cause lower calcium values. Sera from patients receiving EDTA (treatment of hypercalcemia) are unsuitable for analysis, since EDTA will chelate the calcium and render it unavailable for reaction with O-cresolphthalein complexone. The biological half-life of calcium in blood is few hours.

Urine

Specimens should be collected in acid washed bottles. 24 hour specimens should be collected in containers containing 5 ml of 6 mol/L HCl. If the specimen is collected without acid, the pH should be adjusted < 3 with 6 mol/L HCl. Dilute urine specimen 2 times with bidistilled water (1volume urine + 1volume distilled water) before assay.

Stability (serum): 7 days at 15 – 25 °C; 3 weeks at 4 – 8 °C; 6 months at -20 °C.

Stability (urine): 2 days at 15 – 25 °C, 4 days at 4 – 8 °C; 3 weeks at -20 °C.

Stored serum or urine specimens must be mixed well prior to analysis.

System Parameters

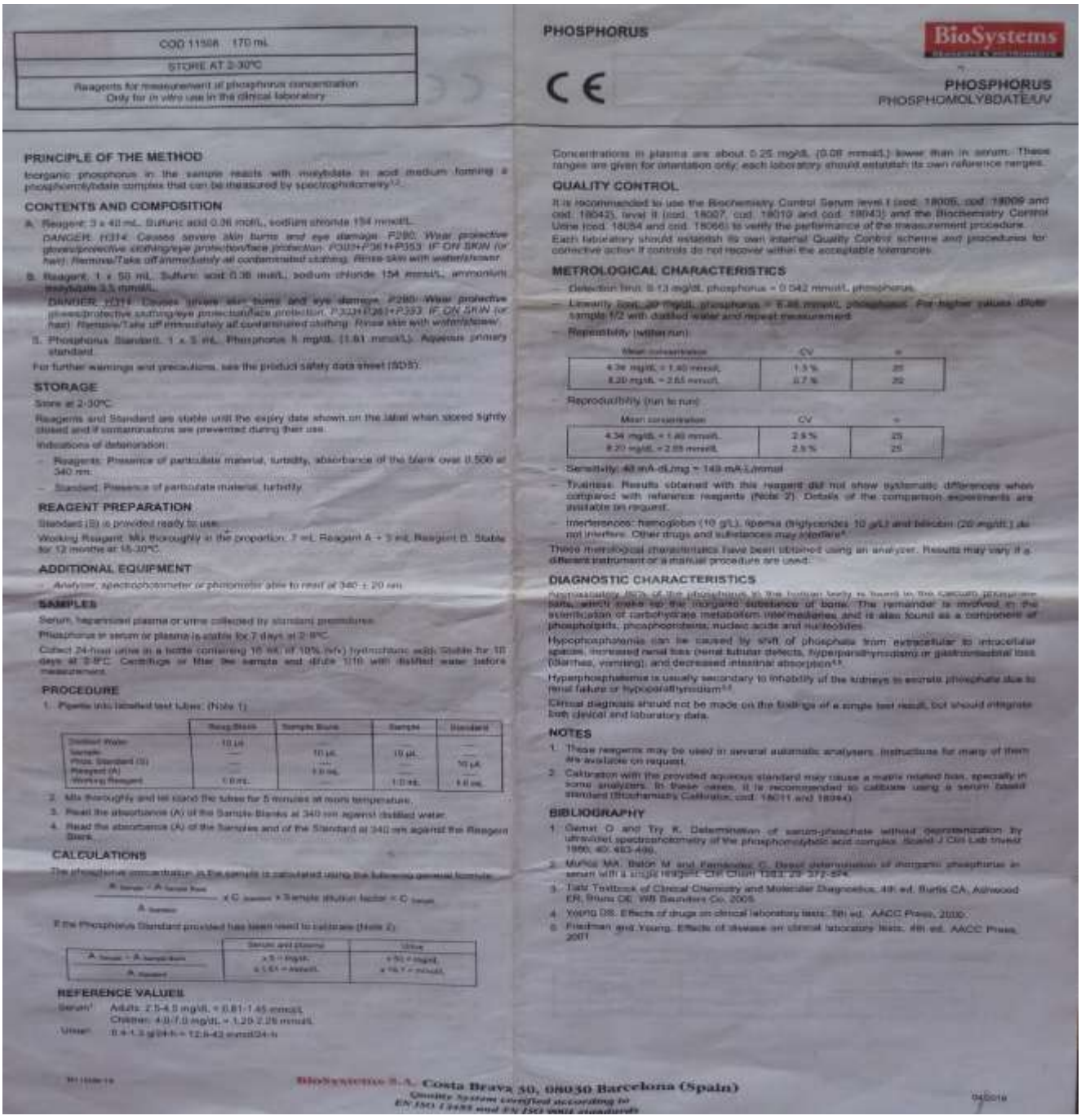
Wavelength	650 nm (600 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 100
e.g.: Reagent volume	1 ml
Sample volume	10 µl
Temperature	15 - 25 °C
Zero adjustment	Reagent Blank
Sensitivity	2 mg/dL (0.25 mmol/L)
Linearity	20 mg/dL (5 mmol/L)

Procedure

	Blank	Standard	Specimen
Standard	.....	10 µl	.....
Specimen	.....	.....	10 µl
Reagent	1 ml	1 ml	1 ml

Mix and incubate for 3 minutes at 20 - 25 °C. Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

Appendix III



COD 11508 170 mL

STORE AT 2-30°C

Reagents for measurement of phosphorus concentration
Only for in vitro use in the clinical laboratory

PHOSPHORUS

BioSystems

PHOSPHORUS
PHOSPHOMOLYBDATE/UV

PRINCIPLE OF THE METHOD

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming a phosphomolybdate complex that can be measured by spectrophotometry[1,2].

CONTENTS AND COMPOSITION

A. Reagent: 3 x 40 mL. Sulfuric acid 0.36 mol/L, sodium sfeonate 154 mmol/L.
DANGER. H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

B. Reagent: 1 x 50 mL. Sulfuric acid 0.36 mol/L, sodium chloride 154 mmol/L, ammonium molybdate 3.5 mmol/L.
DANGER. H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

S. Phosphorus Standard: 1 x 5 mL. Phosphorus 5 mg/dL (1.61 mmol/L). Aqueous primary standard.

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

STORAGE

Store at 2-30°C.

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

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.500 at 340 nm.
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Standard (S) is provided ready to use.

Working Reagent: Mix thoroughly in the proportion: 7 mL Reagent A + 1 mL Reagent B. Stable for 12 months at 15-30°C.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer able to read at 340 +- 20 nm.

SAMPLES

Serum, heparinized plasma or urine collected by standard procedures.

Phosphorus in serum or plasma is stable for 7 days at 2-8°C.

Collect 24-hour urine in a bottle containing 10 mL of 10% (v/v) hydrochloric acid. Stable for 10 days at 2-8°C. Centrifuge or filter the sample and dilute 1/10 with distilled water before measurement.

PROCEDURE

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

	Reag.Blank	Sample Blank	Sample	Standard
Distilled Water	10 µL	---	---	---
Sample	---	10 µL	10 µL	---
Phos. Standard (S)	---	---	---	10 µL
Reagent (A)	---	1.0 mL	---	---
Working Reagent	1.0 mL	---	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 5 minutes at room temperature.
3. Read the absorbance (A) of the Sample Blanks at 340 nm against distilled water.
4. Read the absorbance (A) of the Samples and of the Standard at 340 nm against the Reagent Blank.

CALCULATIONS

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

$$\frac{A_{sample} - A_{sample\ blank}}{A_{standard}} \times C_{standard} \times \text{Sample dilution factor} = C_{sample}$$

If the Phosphorus Standard provided has been used to calibrate (Note 2):

	Serum and plasma	Urine
$\frac{A_{sample} - A_{sample\ blank}}{A_{standard}}$	x 5 = mg/dL x 1.61 = mmol/L	x 50 = mg/dL x 16.1 = mmol/L

REFERENCE VALUES

Serum[3]: Adults: 2.5-4.5 mg/dL = 0.81-1.45 mmol/L
Children: 4.0-7.0 mg/dL = 1.29-2.26 mmol/L
Urine[3]: 0.4-1.3 g/24 h = 12.9-42 mmol/24 h

Concentrations in plasma are about 0.25 mg/dL (0.08 mmol/L) lower than in serum. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, cod. 18009 and cod. 18043), level II (cod. 18007, cod. 18010 and cod. 18043) and the Biochemistry Control Urine (cod. 18054 and cod. 18066) to verify the performance of the measurement procedure.
Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.13 mg/dL phosphorus = 0.042 mmol/L phosphorus.
- Linearity limit: 20 mg/dL phosphorus = 6.46 mmol/L phosphorus. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

Mean concentration	CV	n
4.34 mg/dL = 1.40 mmol/L	1.3 %	20
8.20 mg/dL = 2.65 mmol/L	0.7 %	20

- Reproducibility (run to run):

Mean concentration	CV	n
4.34 mg/dL = 1.40 mmol/L	2.5 %	25
8.20 mg/dL = 2.65 mmol/L	2.5 %	25

- Sensitivity: 40 mA·dL/mg = 149 mA·L/mmol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: hemoglobin (10 g/L), lipemia (triglycerides 10 g/L) and bilirubin (20 mg/dL) do not interfere. Other drugs and substances may interfere[4].

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

DIAGNOSTIC CHARACTERISTICS

Approximately 80% of the phosphorus in the human body is found in the calcium phosphate salts which make up the inorganic substance of bone. The remainder is involved in the esterification of carbohydrate metabolism intermediates and is also found as a component of phospholipids, phosphoproteins, nucleic acids and nucleotides.

Hypophosphatemia can be caused by shift of phosphate from extracellular to intracellular spaces, increased renal loss (renal tubular defects, hyperparathyroidism) or gastrointestinal loss (diarrhea, vomiting), and decreased intestinal absorption[3,5].

Hyperphosphatemia is usually secondary to inhability of the kidneys to excrete phosphate due to renal failure or hypoparathyroidism[3,5].

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

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analysers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18094).

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2. Muñoz MA, Balón M and Fernández C. Direct determination of inorganic phosphorus in serum with a single reagent. Clin Chem 1983; 29: 372-374.
3. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co. 2006.
4. Young DS. Effects of drugs on clinical laboratory tests. 5th ed. AACC Press, 2000.
5. Friedman and Young. Effects of disease on clinical laboratory tests. 4th ed. AACC Press, 2001.

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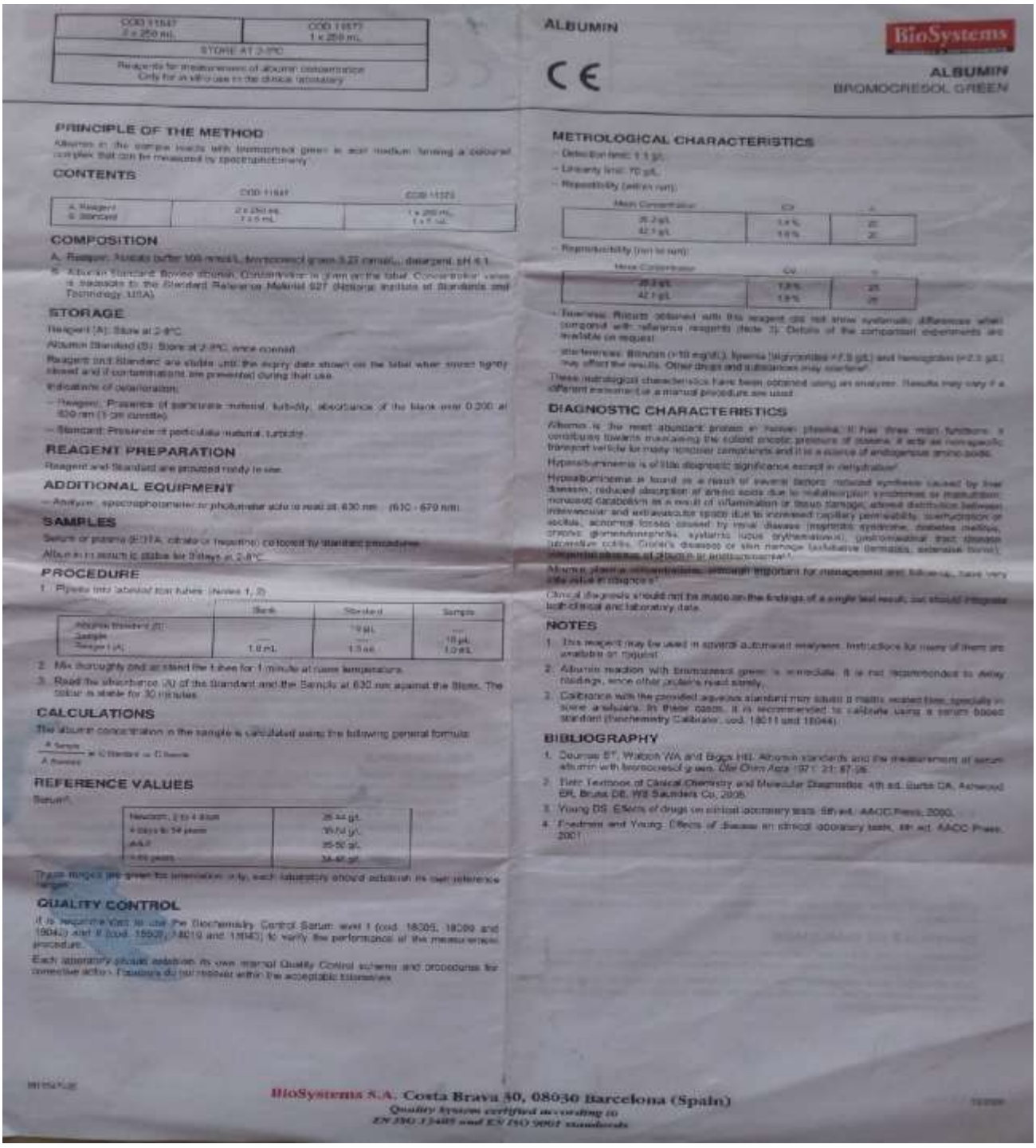
04/2018

Appendix IV



ALBUMIN

BioSystems

CE

ALBUMIN
BROMOCRESOL GREEN

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

PRINCIPLE OF THE METHOD

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

CONTENTS

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

COMPOSITION

A. Reagent: Acetate buffer 100 mmol/L, bromocresol green 0.23 mmol/L, detergent, pH 4.1.

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

STORAGE

Reagent (A): Store at 2-8ºC.

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

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

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 629 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer able to read at 630 nm (610 - 679 nm).

SAMPLES

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

Albumin in serum is stable for 9 days at 2-8ºC.

PROCEDURE

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

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

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

CALCULATIONS

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

$$\frac{A\ Sample}{A\ Standard} \times C\ Standard = C\ Sample$$

REFERENCE VALUES

Serum[3]:

Newborn, 2 to 4 days	28-44 g/L
4 days to 14 years	38-54 g/L
Adult	35-50 g/L
> 65 years	34-48 g/L

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

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level 1 (cod. 18305, 18099 and 19042) and II (cod. 18306, 18019 and 18942) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective actions if results do not remain within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.1 g/L.
- Linearity limit: 70 g/L.
- Repeatibility (within run):

Mean Concentration	CV	n
26.2 g/L	1.4 %	20
42.1 g/L	1.6 %	20

- Reproducibility (run to run):

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

- Interferences: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.
- Interferences: Bilirubin (<10 mg/dL), lipemia (triglycerides <7.5 g/L) and hemoglobin (<2.5 g/L) may affect the results. Other drugs and substances may interfere[4].

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

DIAGNOSTIC CHARACTERISTICS

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

Hypoalbuminemia is of little diagnostic significance except in dehydration[3].

Hypoalbuminemia is found as a result of several factors: reduced synthesis caused by liver diseases, reduced absorption of amino acids due to malabsorption syndromes or malnutrition, increased catabolism as a result of inflammation or tissue damage, altered distribution between intravascular and extravascular space due to increased capillary permeability, overhydration or ascites, abnormal losses caused by renal diseases (nephrotic syndrome, diabetes mellitus, chronic glomerulonephritis, systemic lupus erythematosus), gastrointestinal tract diseases (ulcerative colitis, Crohn's disease) or skin damage (exfoliative dermatitis, extensive burns), congenital absence of albumin or analbuminemia[3].

Albumin plasma concentrations, although important for management and follow-up, have very little value in diagnosis[3].

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

NOTES

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

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1. Doumas BT, Watson WA and Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta. 1971; 31: 87-96.
2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co; 2006.
3. Young DS. Effects of drugs on clinical laboratory tests. 5th ed. AACC Press; 2000.
4. Friedman and Young. Effects of disease on clinical laboratory tests. 4th ed. AACC Press; 2001.

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