

بسم الله الرحمن الرحيم



Sudan University of Science and Technology

College of Graduate Studies



**Evaluation of Plasma Levels of Calcium and Phosphate
among Sudanese Children with Malnutrition
in Khartoum State**

تقويم مستوي الكالسيوم والفوسفات في البلازما لدي الاطفال السودانيين المصابين
بسوء التغذية في ولاية الخرطوم

A dissertation submitted in a partial fulfillment of the requirement for

The master degree in clinical chemistry

By

Abubakr Ahmed Gasim Ahmed

B.Sc Clinical Chemistry – College of Medical Laboratories Science
Omdurman Islamic University – 2013

Supervisor

Dr. Nuha Eljaili Abubaker

Assistant Professor of Clinical Biochemistry

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وَنُقَرِّفُ فِي الْأَرْحَامِ مَا نَشَاءُ إِلَىٰ أَجَلٍ مُّسَمًّى ثُمَّ نُخْرِجُكُمْ

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صدق الله العظيم

سورة الحج الآية (5)

Dedication

To my mother

To my father

To my sisters

To my brothers

To my friends

Acknowledgments

I would like to express my thanks to my supervisor

Dr. Nuha ElJaili Abubaker for her kind guidance and support and all through the process of this study. Thanks for the clinical chemistry staff in Sudan University of science and technology for their help and contribution, here I would love to express my gratitude to my parents for her Encouragement and support before and during my MSc Journey.

Last, but not least thanks to the children and families who Participate in this study.

Abstract

Protein-energy malnutrition (PEM) is currently the most widespread and serious health problem of children in the world. at any time approximately hundred million children suffer from the moderate or severe forms of PEM. This study was carried out to measure plasma levels of calcium and phosphate in malnourished children sixty samples were collected from malnourished children in period between January to March 2017, chosen randomly from Mohammed AL Amin Hamid Hospital for Pediatric in Khartoum State, and sixty apparently healthy individuals as control group, to evaluate the effect of malnutrition on plasma levels of calcium and phosphate.

Plasma calcium and phosphate were measured by Spectrophotometer biosystem-310 and Colorimeter instruments respectively and the results were analyzed using statistical package of social science (SPSS), computer program.

The study showed that, the plasma levels of calcium and phosphate were significantly decreased in malnourished children. mean \pm SD for cases versus control. calcium: (6.66 ± 1.41 versus 9.16 ± 0.48 mg/dl, p-value =0.000). phosphate: (3.51 ± 1.24 versus 5.63 ± 0.99 mg/dl, p-value =0.000).

Also the finding of this study showed that, there was a significant decrease in the mean of BMI in malnourished children group compared to control group. mean BMI \pm SD for case versus control (15.28 ± 2.28 kg/m² versus 19.25 ± 2.48 kg/m²). the result of this study showed that protein energy malnutrition most common among age between (6-9) years (85%), and malnutrition most abundant in females (56.7%) than males (43.3%).

The results showed that, there were no correlation between age of malnourished children and the levels of calcium and phosphate ($r = - 0.129$, p-value= 0.325) ($r = 0.148$, p-value= 0.259) respectively.

Also there were no correlation between the levels of calcium, phosphate and the duration of malnourished children ($r = - 0.135$, p-value = 0.305) ($r = - 0.157$, p-value = 0.231) respectively.

It is concluded that: the plasma levels of calcium and phosphate were significantly decreased in Sudanese malnourished children.

مستخلص الدراسة

مرض سوء التغذية يعتبر من اخطر الامراض التي تصيب الاطفال انتشارا في العالم , حوالي مائة مليون طفل في العالم يعاني من سوء التغذية الحادة او المتوسطة .

أجريت هذه الدراسة لمعرفة مستوي الكالسيوم والفوسفات في البلازما لدي الأطفال السودانيين المصابين بسوء التغذية. تم جمع ستين عينة دم من المرضى في الفترة ما بين يناير وحتى مارس 2017م، تم اختيارهم عشوائيا من مستشفى محمد الامين حامد للاطفال في ولاية الخرطوم و ستين من الأفراد الأصحاء كمجموعة ضابطة، لتقييم تأثير سوء التغذية على مستويات الكالسيوم والفوسفات في بلازما الاطفال المصابين.

تم قياس مستويات الكالسيوم والفوسفات باستخدام جهاز الطيف الضوئي ، وتم تحليل النتائج باستخدام الحزمة الإحصائية للعلوم الاجتماعية (SPSS)، برنامج الكمبيوتر.

وأظهرت الدراسة أن مستويات الكالسيوم والفوسفات انخفضت بشكل ملحوظ في الأطفال السودانيين الذين يعانون من سوء التغذية. " المتوسط \pm الانحراف المعياري للمرضى مقارنة بمجموعة التحكم " .

بالنسبة للكالسيوم : ($1,41 \pm 6,66$ مقابل 48 ± 9.16 , ملجرام / ديسلتر وكان الاحتمال الاحصائي للمقارنة 0.000). الفوسفات: (1.24 ± 3.51 مقابل 0.99 ± 5.63 ملجرام / ديسلتر وكان الاحتمال الاحصائي للمقارنة 0.000). كما أظهرت نتائج الدراسة أن هناك انخفاض معنوي في مؤشر كتلة الجسم لدى الأطفال الذين يعانون من سوء التغذية مقارنة بمجموعة التحكم. متوسط مؤشر كتلة الجسم \pm الانحراف المعياري للمرضى (2.28 ± 15.28 كجم / م 2) مقارنة بمجموعة التحكم (19.25 ± 2.48 كجم / م 2) وأظهرت نتائج الدراسة أن معدل الإصابة بمرض سوء التغذية اكثر شيوعا بين سن (6-9) سنوات (85%)، وسوء التغذية اكثر شيوعاً لدى الإناث (56.7%) مقارنة بالذكور (43.3%).

أظهر مستوى المعنوية ليس هناك علاقة معنوية بين عمر الأطفال الذين يعانون من سوء التغذية ومستويات الكالسيوم والكالسيوم (معامل بيرسون للإرتباط = -0,129 ومستوى المعنوية = 0,325) (معامل بيرسون للإرتباط = 0,148 ومستوى المعنوية = 0,259) علي التوالي . كما انه ليس هناك علاقة معنوية بين مستويات الكالسيوم و الفوسفات ومدة إصابة الأطفال بمرض سوء التغذية (معامل بيرسون للإرتباط = -0,135، مستوى المعنوية = 0,305)، (معامل بيرسون للإرتباط = -0,157، مستوى المعنوية = 0,231) علي التوالي .

وخلصت الدراسة إلى أن مستويات الكالسيوم والفوسفات انخفضت بشكل ملحوظ لدى الأطفال السودانيين الذين يعانون من سوء التغذية.

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List of abbreviations

Abbreviation	Full term
ATP	Adenosine tri phosphate
BMI	Body mass index
ECF	Extra cellular fluids
FFMI	Fat Free mass index
FMI	Fat mass index
PTH	Parathyroid hormone
PCM	Protein calorie malnutrition
PEM	Protein energy malnutrition
WHO	World health organization

Chapter one

Introduction

1. Introduction

1.1 Introduction:

Malnutrition is a complex problem which results from a long chain of interrelated events. It continues to be a major health burden in developing countries and affects mostly infants, young children and lactating mother. (Muller and Krawinkel, 2005) Protein-energy malnutrition (PEM) previously referred to as protein-calorie malnutrition (PCM) describes the severe form of malnutrition seen in childhood (kwashiorkor, marasmic-kwashiorkor, marasmus and underweight) it is the most common nutritional disorder affecting children in developing countries and the third most common disease of childhood in such countries, it manifests primarily by inadequate dietary intake of protein and energy and always accompanied by deficiencies of other nutrients.(Nassaret al .,2010)

Calcium is the most abundant mineral in the body. about 99% of the body's calcium is present in the bone, mainly as the mineral hydroxyapatite, where it is combined with phosphate about 85% of the body's phosphate content is in the bone. the total concentration of calcium in plasma or urine is shown as plasma or urine (calcium),whereas plasma (ca+2) refers specially and solely to the concentration of ionized calcium.(Geoffrey et al.,2005)

Eighty-five percent of the body phosphorus is located in the mineral phase of bone. The remainder is present outside bone, largely in an intracellular location as phosphate compounds. In ECF, phosphate is mostly inorganic. Intracellular phosphate has vital functions in macromolecular structure (e.g. in DNA), energy metabolism (e.g. energy-rich phosphate such as ATP),cellsignaling and enzyme activation by phosphorylation.Intracellular phosphate is largely organic as a component of phospholipid,phospho-protein,nucleic acid and nucleotides (e.g. ATP).(Geoffrey et al.,2005)

Serum calcium concentrations in PEM is reduced and the levels correlated with weight-for-height.(Mishra et al .,2009)The low serum calcium and phosphorus explains the radiological findings of poor mineralization of long bones PEM patients as reported by Soliman et al (Solimanet al .,1996)

1.2 Rationale:

Malnutrition is a major problem globally (Mesham and chatterjee, 1999) it interacts with diarrhea in a vicious circle leading to high morbidity and mortality in children in developing countries, Sudan being one of developing countries and malnutrition is widely distributed among children. Malnutrition is an important public health problem, however little information is available on assessment for acute malnutrition so it is very important to evaluate essential parameters of electrolytes.

To my knowledge there are few published studies about this in Sudan, so this study may help to provide the monitoring of calcium and phosphate in malnourished children

1.3 Objectives:

1.3.1 General objective:

To evaluate plasma levels of calcium and phosphate among Sudanese children with malnutrition in Khartoum state.

1.3.2 Specific objectives:

- 1- To measure the plasma calcium and phosphate level among children with malnutrition and control group. compare mean concentration of plasma calcium and phosphate in both study groups .
- 3- To correlate between calcium and phosphate and study variable (age, BMI and duration of disease)

Chapter two

Literature Review

2. Literature review

2.1. Malnutrition:

Malnutrition is a broad term that can be used to describe any imbalance in nutrition; from over-nutrition often seen in the developed world, to under-nutrition seen in many level hoping countries, but also in hospitals and residential care facilities in developed nations. Malnutrition can develop as a consequence of deficiency in dietary intake, increased requirements

Associated with a disease state, from complications of an underlying illness such as poor absorption and excessive nutrient losses, or from a combination of these aforementioned factors (Soetrset *al.* , 2008).

2.1.1. Protein energy malnutrition:

Protein Energy Malnutrition (PEM) results when the body's need for protein, energy or both cannot be satisfied by the diet. It includes a wide spectrum of clinical manifestations conditioned by:

- i) The relative severity of protein or energy deficit
- ii) The severity and duration of the deficiencies.
- iii) The age of the host
- iv) The cause of the deficiency
- v) The association of the deficiency with other physiological problems such as infectious diseases and pregnancy (Torun and Chew, 1994).

Protein Energy Malnutrition (PEM) or protein calorie malnutrition (PCM) generally referred to simply as malnutrition is an imbalance between the supply of protein and energy and the body's demand for them to ensure optimal growth and function (WHO, 1997).

The World Health Organization (WHO) defines Protein Energy Malnutrition as “the cellular imbalance between the supply of nutrient and energy and the body's demand for them to ensure growth, maintenance and specific function” (Pauline, 2008).

Protein Energy Malnutrition (PEM) or Protein calorie malnutrition is also a deficiency syndrome caused by inadequate intake of macro-nutrients as well as micro-nutrients (Pauline, 2008). It is a syndrome that represents one of the various levels of inadequate protein and or energy intake between starvation (no food intake) and adequate nourishment.

2.1.1.1. Types of Protein Energy Malnutrition:

Clinically PEM has four forms. These forms depend on the balance of non-protein and protein sources of energy. The origin of these three forms can be primary, when it is the result of inadequate food intake or secondary, when it

is the result of other diseases that lead to low food ingestion, inadequate nutritional absorption or utilization and or increased nutrient losses. Also these forms of PEM can be graded as mild, moderate or severe (Pauline, 2008).

A-Under nutrition:

Under nutrition is a consequence of consuming little energy and other essential nutrients or using or excreting more rapidly than they can be replaced. This state of malnutrition is often characterized by infectious and diseased children who are already under nourished can suffer from protein energy malnutrition who rapid growth, infectious or disease

Increases the need for protein and essential nutrients (Pauline, 2008)

B - Marasmus:

This is the dry, thin desiccated form of PEM. It results from near starvation with deficiency of energy, protein and non protein nutrients. The marasmic individual consumes very little food. In children it is often because the mother is unable to breastfeed. Marasmus is characterized by stunted growth. Usually the children are thin from loss of muscle and body fat. It develops in children between 6-12 months who have been weaned from breast milk or who are suffering from weakening conditions like chronic diarrhea (Pauline, 2008).

C- Kwashiorkor:

This is the wet edematous and swollen form. “Kwashiorkor” is a Ghanaian word meaning “first child-second child”. It refers to the observation that this is a disease the first child develops when the second child is born and replaces the first child at the breast. This is because the weaned child is fed with a thin gruel of poor nutritional quantity compared with breast milk and as a consequence the child fails to thrive. This condition is marked with protein deficiency more marked than energy deficiency, and Oedema results. Children with Kwashiorkor tend to be older than those with marasmus and tend to develop the disease after weaning.

Adults develop kwashiorkor as a result of under-nutrition from diets rich in carbohydrate than protein. This may be as a result of poverty, wars, famine etc. Kwashiorkor is characterized by fluid retention, oedema, dry peeling skin, hair discolorations, etc (Pauline, 2008).

D- MarasmicKwashiorkor:

This is the combined form of the Protein Energy Malnutrition. It is a combination of chronic energy deficit and chronic or acute protein deficiency. Children with this form of PEM have some edema and or body fat than those

with marasmus . The clinical manifestation is a combination of marasmus and kwashiorkor (Stanfield *et al.*, 1978).

2.1.1.2. Classification of Protein Energy Malnutrition:

The classification scheme for PEM is useful for diagnosis and treatment as well as the application and evaluation of public health measures. Several methods have been suggested for the classification of PEM. The choice of classification depends on the purpose for which it is used, e.g. clinical studies or community surveys.

There are three main classifications of PEM based on clinical and anthropometric assessments.

- a) The welcome classification
- b) The water-low classification
- c) The Gomez classification

In order to understand these classifications, it is necessary to have a knowledge of the central chart system on which they are based (Stanfield *et al.*, 1978) .

A- Welcome Classification :

This was proposed by the Welcome Working Party. In this classification reduction in body weight below 80 percent of the Harvard Standard (50th Centile) is considered malnutrition. There is also the presence and absence of oedema as well as deficit in body weight. Therefore children with oedema with weight 60-80 percent of the expected weight for age are classified as suffering from kwashiorkor (Welcome, 1970). Those without oedema and who weigh less than 60 percent of the standard are considered as marasmic. Those with oedema and body weight less than 60 percent of the standard are diagnosed marasmickwashiorkor. However, children without oedema weighing 60-80 percent of the standard weight are classified as underweight. The Welcome classification is the most generally accepted and widely used for clinical purposes (See Table 2.1).

Table (2.1): Welcome Classification of Malnutrition :

MALNUTRITION	BODY WEIGHT % OF STANDARD	OEDEMA
Underweight	80-60	-
Marasmus	<60	-
Kwashiorkor	80-60	+
Marasmic kwashiorkor	<60	+

50th Centile of Harvard Standard (Welcome Trust Working Party, 1970).

B - Gomez Classification:

Gomez classification is based on the deficit in weight for age and the 90 percent of the Harvard Standard is used as cut-off point from normal to malnourished. Malnutrition is subdivided into three degrees, first, second and third degree malnutrition(Gomez, 1956).

First degree malnutrition is defined as 75-90 percent; second degree is defined as 60-75% while third degree is defined as less than 60% of expected weight as illustrated Table 1.2. All cases of oedema are included in third degree malnutrition regardless of body weight.

The Gomez classification is useful for community surveys and helps to access the magnitude of the problem in a community. However it does not indicate the duration or types of malnutrition (Table 2.2).

Table (2.2): Gomez Classification of Malnutrition (Gomez, 1956).

MALNUTRITION	BODY WEIGHT (% of standard)
First degree	75-90
Second degree	60-75
Third degree	<60

C-Water Low Classification:

Water low described a classification of malnutrition using both weight and height for age. This classification is useful in that it distinguishes those children with acute malnutrition (wasting) from those with chronic under-nutrition who are stunted.

It also assesses the relationship between weight and height in early childhood which is reasonably constant as indicated in (Table 2-3). Water low suggested the terms “wasting” for a deficit in weight and “stunting” for a deficit in height for age (Stanfield *et al.* ,1978).

Therefore patients fall into four categories:

- 1) Normal
- 2) Wasted but not stunted (suffering from acute PEM)
- 3) Wasted and stunted (suffering from acute and chronic PEM)
- 4) Stunted but not wasted (nutritional dwarfs with past PEM with present adequate nutrition)

The disadvantage of this method is that, although height is a far more accurate reflection of growth in the long term, it is often difficult to measure accurately in community surveys. There is also the tendency to place the genetically or

constitutionally small child or premature infants into the category of malnutrition (Stanfield *et al.*, 1978).

Table(2.3): Water low Classification of Malnutrition (Stanfield *etal.*,1978).

Height for age >80%		Weight for Age <80%	
>90%	Normal	Wasted	
<90%	Stunted	Stunted and wasted	

2.1.1.3. Causes:

inadequate food intake, infections, psychosocial deprivation, the environment (lack of sanitation and hygiene),social inequality and perhaps genetics contribute to childhood malnutrition.(Pauline, 2008).

2.1.1.4. Clinical signs and symptoms of protein-energy malnutrition (PEM):

A- Main symptom:

The main symptom of malnutrition (under nutrition) is unintended weight loss, although this isn't always obvious(Pauline, 2008).

Most people who are malnourished will lose weight, but it is possible to be a healthy weight or even overweight and still be malnourished.

Someone could be malnourished if:

They unintentionally lose 5-10% of their body weight within three to six months their body mass index (BMI) is under 18.5 (although a person with a Under 20 could also be at risk)(Pauline, 2008) .

B- Other symptoms:

- * reduced appetite.
- * Lack of interest in food and drinks.
- * Feeling tired all the time.
- * feeling weaker.
- * getting ill often and taking a long time to recover.
- * wounds taking a long time to heal .
- * Poor concentration.
- * feeling cold most of the time.
- *low mood or depression (Pauline, 2008)

C-Symptoms in children:

Symptoms of malnutrition in a child can include:

- * not growing at the expected rate or not putting on weight as would normally be expected (faltering growth).
- * changes in behavior, such as being unusually irritable, slow or anxious .

* low energy levels and tiring more easily than other children .

Clinical signs and symptoms of micronutrient deficiencies: Some of the clinical signs and symptoms of specific micronutrient deficiencies may closely resemble those observed in PEM. Deficiencies of micronutrients, including vitamins, minerals, and trace elements have been well described. The most common and clinically significant deficiencies include the following:

- Iron - Fatigue, anemia, decreased cognitive function, headache, glossitis, and nail changes.
- Iodine - Goiter, developmental delay, and mental retardation.
- Vitamin D - Poor growth, rickets , and hypocalcaemia
- Vitamin A - Night blindness, xerophthalmia, poor growth, and hair changes
- Folate - Glossitis, anemia (megaloblastic), and neural tube defects (in fetuses of women without folate supplementation)
- Zinc - Anemia, dwarfism, hepatosplenomegaly, hyper pigmentation and hypogonadism, acrodermatitisenteropathica, diminished immune response, poor wound healing. (Pauline, 2008) .

2.1.1.5. Diagnosis of malnutrition:

A- Weight loss :

Weight loss trajectories differ with clinical condition. Nevertheless, involuntary weight loss is a strong predictor of negative (Who, 1995) outcomes irrespective of magnitude, speed and underlying cause.

Naturally, a massive and fast weight loss due to an aggressive cancer disease imposes a higher risk than a smaller and slower weight loss due to ageing.

Thus, consensus was reached to propose two optional cut-offs for unintentional weight loss; i.e. either >5% over the last 3 months to cover for acute illnesses, or >10% of habitual weight indefinite of time to be relevant for chronic conditions (Who ,1995).

B- Body mass index (BMI) :

WHO advocates BMI <18.5 kg/m² as a general cut-off for underweight.

This cut-off is justified at a public health population Level I (Who, 1995), whereas its relevance for clinical and care settings may

be questioned. As already mentioned the trend of increasing BMI in all populations world-wide make this acknowledged BMI cut-off value difficult to use for the purpose of defining malnutrition. Patients struck with highly

catabolic diseases may in 3e6 months lose substantially more than 10% of their weight and still have BMI values well above “normal” ranges. Another issue to consider is that epidemiological evidence indicates that older populations display higher optimal BMI intervals (e.g. for survival) than younger people (Who, 1995).

Partly due to the strong global acceptance of the WHO cut-off of 18.5 kg/m² it was decided unanimously to accept the WHO recommended cut-off of as a criterion that in its own right will be enough to diagnose malnutrition.

With this latter decision it was easy to come to consensus for a complementary suggestion for relevant BMI cut-off values; namely <20 kg/m² for subjects <70 years of age, and <22 kg/m² for subjects 70 years and older, remembering the fact that these BMI levels need to be linked to weight loss as defined above. The choices of 20 and 22 kg/m², respectively, were based on consensus in the group. Ethnic and regional variability in BMI may need to be considered (Who,1995) .

C- Fat Free mass index (FFMI):

Cut-offs for FFMI need to be linked to the decided cut-offs for BMI on one hand, and to the fact that women have lower FFMI (and higher FMI) than men on the other hand. Based on Swiss reference material (Schutz, *et al* ., 2002) .

it was decided to suggest FFMI <15 and <17 kg/m² in women and men, respectively. It has to be emphasized that reference values, like for BMI, should be relevant for the specific ethnic and cultural context that is at hand (Schutz, *et al* ., 2002) .

D- Biochemical Methods:

Serum biochemical markers are primarily and non proteins used in establishing the nutritional status of patients. They are used to determine whether they are at risk of complications and also in monitoring their nutritional treatment (Heymsfield *et al.*, 1994).

2.1.1.6. Prevention of Malnutrition:

Poverty, Ignorance, frequent infection, cultural norms/customs, severe cyclic climatic conditions, natural and man made disasters are among the main causes of PEM. Therefore, its control and prevention require multi-sectoral approaches that include food production and distribution, preventive medicine, education, social development and economic improvement. At a national or regional level, control and prevention can only be achieved through short-term 41 and long-term political commitments and effective

actions to enforce the measure to eradicate the underlying causes of malnutrition(Who,1995).

The most likely victims of PEM are children and women, especially those within child- bearing age from low socioeconomic strata. Children whose parents have misconceptions concerning the use of food, who come from broken or unstable families, whose families have a high violence, alcoholism and drug abuse, who live under poor sanitary conditions in urban slums or in rural areas frequently subject to droughts or floods, whose societal beliefs prohibit the use of nutritious foods. Special attention must be given to the following for the prevention of PEM (Who, 1995).

2.2 Calcium:

Calcium is the fifth most common element in the body and the most prevalent action, the skeletons contains 99% of the body's calcium, predominantly as extracellular crystals of unknown structure with a composition approaching that of hydroxyapatite (Carla *et al.*, 2008)

2.2.1 Biochemistry and physiology:

In the blood, virtually all of the calcium is in the plasma, which has a mean normal calcium concentration of approximately 9.5mg/dl (2.38mmol/l).

Calcium exists in three physiochemical states in the plasma, with approximately

- (1) 50% free (ionized)
- (2) 40% bound to plasma proteins primarily albumin and
- (3) 10% complexed with small anions.

Calcium also is redistributed among the three plasma pools, acutely or chronically by:

- (1) Alteration in the concentration of protein and small anion
- (2) Change in pH
- (3) Change in the quantities of free calcium and total calcium in the serum

The free calcium fraction is biologically active form; its concentration is tightly regulated by the calcium regulating hormones, PTH and 1,25dihydroxyvitamine D (Carla *et al.*, 2008)

2.2.2 Clinical significance:

Disorders of calcium metabolism are separated into those causing hypocalcaemia and hypercalcemia (Carla *et al.*, 2008).

2.2.2.1 Hypocalcaemia:

Low total serum calcium (hypocalcaemia) may be due to either reduction in the albumin-bound calcium, the free fraction of the calcium or both, hypoalbuminemia is the most common cause of decreased total calcium (pseudohypocalcemia), serum calcium is lower when serum albumin is low (Carla *et al.*, 2008)

2.2.2.2 Hypercalcemia:

Hypercalcemia is commonly encountered in clinical practice and results when the flux of calcium into the extracellular fluid compartment from the skeleton, intestine, or kidney is greater than the efflux. (Carla *et al.*, 2008)

2.3 Phosphate:

Phosphorus in the form of inorganic and organic phosphate is an important and widely distributed element in the human body. inorganic phosphate is the fraction measured in serum and plasma by clinical laboratories (Carla *et al.*, 2008)

2.3.1 Biochemistry and physiology:

Phosphate in plasma exists as both the monovalent and divalent phosphate anions. In blood, organic phosphate esters are located primarily within cells. Inorganic phosphate is a major component of hydroxyapatite in bone; in the soft tissue most phosphate is cellular. Most of the phosphate in cells is organic and incorporated into nucleic acids, phospholipids, phosphoproteins, and "high-energy" compounds such as adenosine triphosphate (ATP) (Carla *et al.*, 2008)

2.3.2 Clinical significance:

2.3.2.1 Hypophosphatemia:

Hypophosphatemia, defined as the concentration of inorganic phosphate in the serum below the normal reference interval, usually 2.5 mg/dL.

Hypophosphatemia may be present when cellular concentrations are normal, and cellular phosphate depletion may exist when serum concentrations are normal or even high. Hypophosphatemia or phosphate depletion may be caused by

- (1) a shift of phosphate from extracellular to intracellular spaces
- (2) renal phosphate wasting
- (3) decreased intestinal absorption
- (4) loss from intracellular phosphate. Box 38-5 lists the commonly encountered causes of hypophosphatemia and phosphate depletion (Carla *et al.*, 2008)

2.3.2.2 Hyperphosphatemia

Hyperphosphatemia is usually secondary to the inability of the kidneys to excrete phosphate, as in renal failure. Moderate increases of serum phosphate occur in individuals with

- (1) low PTH (hypoparathyroidism)
- (2) PTH resistance (pseudohypoparathyroidism)
- (3) Acromegaly (increased growth hormone) caused by an increased renal phosphate threshold.

A rapid increase in serum phosphate may be associated with hypocalcaemia. Therefore symptoms may include tetany, seizures, and hypotension. Long-term hyperphosphatemia may be associated with

- (1) Secondary hyperparathyroidism
- (2) soft tissue calcification of the kidneys, blood vessels, cornea, skin, and periarticular tissue (Carla *et al.*, 2008)

2.4. Relationship between plasma electrolytes (Calcium and Phosphate) and malnutrition

Electrolyte changes were commonly seen in grade II and III malnourished patients particularly who presented with diarrhoeal episode of variable duration. If these changes are diagnosed in time and treated appropriately the morbidity and mortality could be decreased (Yasmeen, *et al.*, 2007).

serum electrolyte disturbances in malnourished children are obvious during diarrheal illness particularly in those patients with Grade III Malnutrition irrespective of Age and duration of Diarrhea. Measurement of these Serum electrolytes is helpful for immediate therapy to avoid serious life threatening situations (Yasmeen *et al.*, 2007)

Chapter three

Materials and Methods

3. Materials and Methods

3.1 Materials:

3.1.1 Study approach:

Quantitative method was used to estimate calcium and phosphate in Sudanese children with malnutrition in Khartoum state during the period from January to March 2017.

3.1.2 Study design:

This is cross sectional hospital base case control study.

3.1.3 Study area:

This study was conducted in Mohamed EL-Amin Hamid Hospital for pediatrics in Khartoum state.

3.1.4 Study population:

The study included children with malnutrition

3.1.5 Sample size:

A total of 120 samples were collected (60 patients and 60 apparently healthy individual serve as control (age and sex matched with test group).

3.1.6 Inclusion Criteria:

Sudanese children with protein energy malnourished and healthy individual serve as control were included.

3.1.7 Exclusion criteria:

Any patients with bone disease, renal disease, thyroid disease ,other chronic disease and any drug that affect calcium and phosphorus measurement were excluded.

3.1.8 Ethical consideration:

Oral informed consent was obtained from all participants.

3.1.9 Data collection:

The clinical data were obtained and recorded on questionnaire sheet.

3.1.10 Sample collection and processing

Local 70% antiseptic for the skin was used 4ml blood was collected by standard venipuncture method, directly in centrifuge tube (lithium heparin container) and the plasma was separated after centrifugation for 5 minutes at 500 r.p.m at room temperature and the plasma were used for estimation of calcium and phosphorus.

3.2 methods:

3.2.1 Estimation of calcium:

3.2.1.1 Principle of the method:

Calcium in the sample react with methyl thymol blue in alkaline medium forming color complex that can be measured by spectrophotometry. hydroxyquinoline is induced in the reagent to avoid magnesium interference. (Ginder and King, 1972)

3.2.1.2 Procedure

	Sample	Standard	Blank
Calcium standard(S)	-	0.01ml	-
Sample	0.01ml	-	-
Reagent	1.0 ml	1.0 ml	1.0 ml

Mixed thoroughly and let stand the tubes for 2 minutes at room temperature. The absorbance was read in 610nm against the Blank. the color was stable for at least 1 hour.

3.2.2 Estimation of phosphorus:

3.2.2.1 Principle of the method:

inorganic phosphorus react with molybdic acid forming a phospho molybdic complex. its subsequent reduction in alkaline medium originate blue molybdenum color.

the intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample (Farrell *at al.*, 1984)

3.2.2.2 PROCEDURE:

	Sample	Standard	Blank
WR (ml)	1.5 ml	1.5 ml	1.5 ml
Standard (ml)	-	0.05 ml	-
Sample	0.05 ml	-	-

Mixed and incubated for 10 min at 37°C or 30 min at room temperature (15-30°C).

The absorbance (A) was read and calibrate against the Blank. The colour is stable for at least 2 hours.

3.3 Quality control:

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

3.4 Statistical analysis:

Data obtained from this study was analyzed using statistical package of the social science (SPSS), T test and person correlate were used for comparison and correlation.

CHAPTER FOUR

RESULTS

4. Results

The results of the biochemical parameters of plasma electrolyte (calcium and phosphate) are given in tables and figures.

Table (4-1): Show age and gender distribution in case group . (85%) of patient between (6-9) years, (8%) between (10-13) and (7%) between (14-17) years. (56.7%) of patients were females while (43.3%) were males.

Table (4-2): Illustrate mean concentration of calcium and phosphate in patients and control groups. The levels of calcium and phosphate were significantly decreased in malnourished children compared to control group: calcium (mean \pm SD: 6.66 ± 1.41 mg/dl versus 9.16 ± 0.48 mg/dl, P.value = 0.000), phosphate (mean \pm SD: 3.51 ± 1.24 mg/dl versus 5.63 ± 0.99 , P.value = 0.000)

Table (4-3):Show the mean of BMI in patients and control group.the BMI was significantly decreased in patients compared to control group.(mean \pm SD : 15.28 ± 2.28 kg/m² versus 19.25 ± 2.48 kg/m² ,P.value = 0.000). in patients, (56.7%) of patients were female while (43.3%) were males.

Figure (4-1): Show correlation between the level of calcium and age of malnourished children, There was no correlation ($r = -0.129$, p-value= 0.325).

Figure (4-2):Show correlation between the level of phosphate and age of malnourished children, There was no correlation ($r = 0.148$, p-value = 0.259).

Figure (4-3): Show correlation between the level of calcium and BMI ($r = 0.671$, P.value = 0.000), there was positive correlation.

Figure (4-4): Show correlation between the level of phosphate and BMI ($r = 0.552$, P.value = 0.000) , there was positive correlation.

Figure (4-5): Show correlation between calcium level and duration of malnourished children ($r = -0.135$, p-value = 0.305), there was no correlation.

Figure (4-6): Show correlation between phosphate level and duration of malnourished children ($r = -0.157$, p-value = 0.231), there was no correlation.

Table (4-1) : Age and gender distribution in case group .

Variable	Number	Percentage
Age (6 -9)	51	85%
Age (10 – 13)	5	8%
Age (14 – 17)	4	7%
Sex male	26	56.7%
Sex female	34	43.3%

Table (4-2): Mean concentration of calcium and phosphate in case and control groups.

Variable	Case N=60 Mean±SD	Control N=60 Mean±SD	p.value
Calcium mg/dl	6.66± 1.41	9.16 ± 0.48	0.000
Phosphate mg/dl	3.51± 1.24	5.63± 0.99	0.000

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

Independent sample T test was used for comparison.

Table (4-3):Mean of body mass index (BMI) of patient with protein energy malnourished in case and control groups

Variables	Case group N=60	Control group N=60	p-value
BMI Kg/m ²	15.28± 2.28 kg/m ²	19.25± 2.48 kg/m ²	0.000

Result given in mean ± SD, P.value≤0.05consider significant.
Independent sample T test was used for comparison.

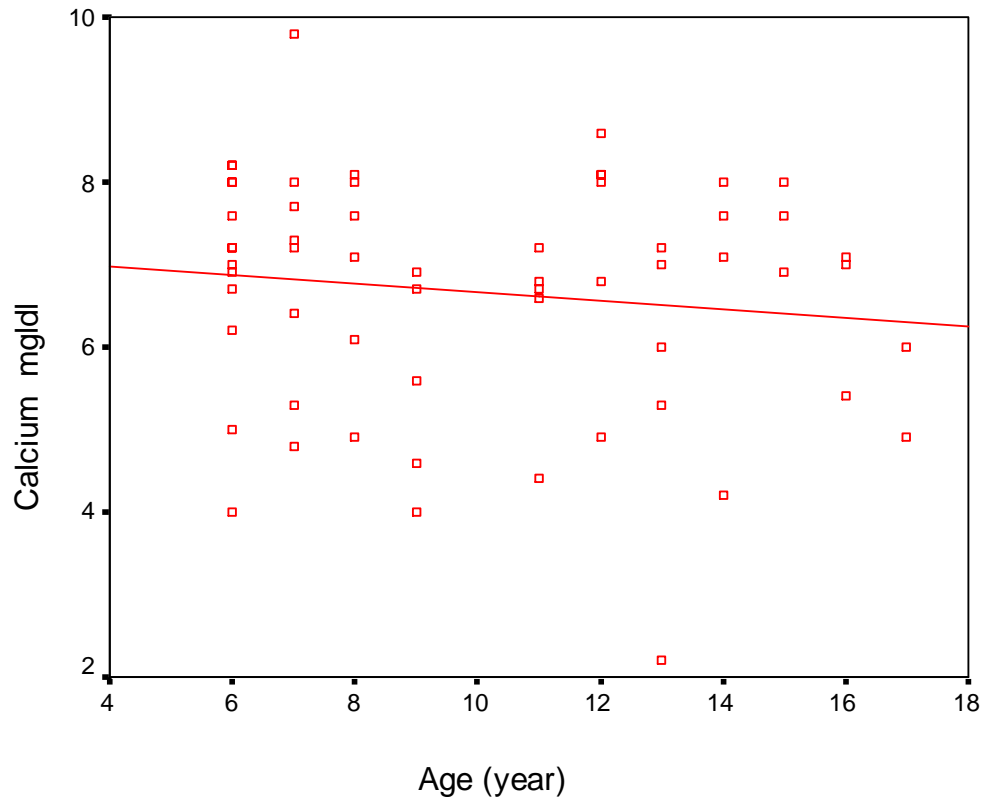


Figure (4-1):Correlation between Calcium level and age in case group ($r = -0.129$, p .value =0.325)

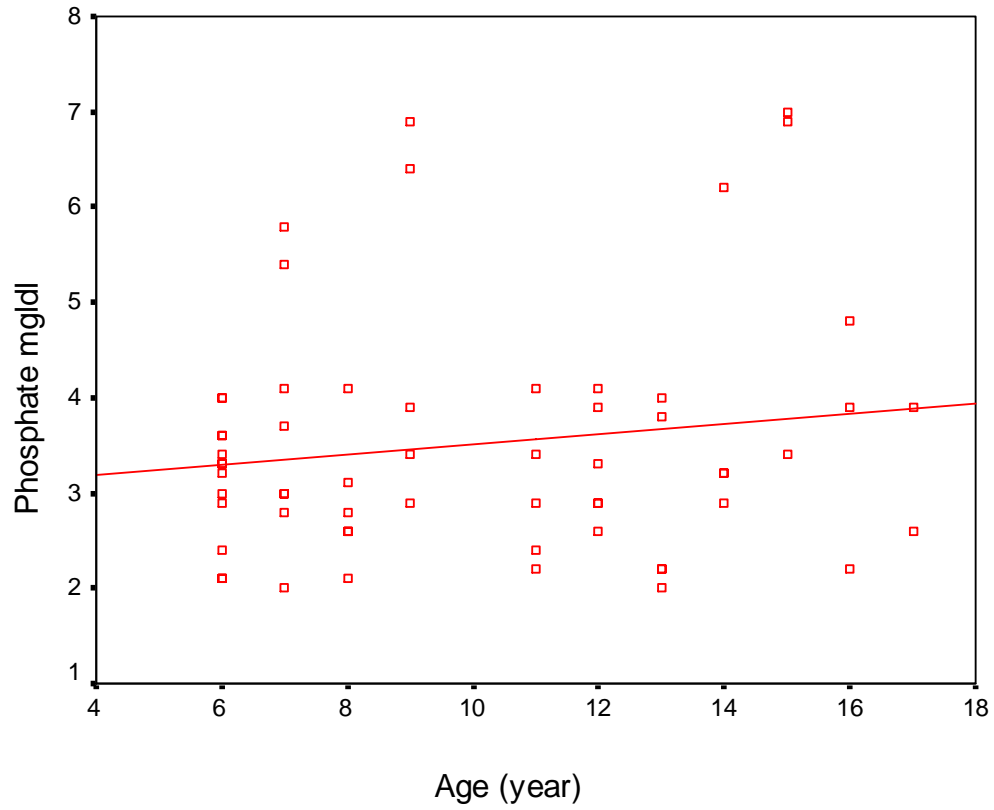


Figure (4-2): Correlation between phosphate level and age in case group ($r = 0.148$, $p\text{-value}=0.259$).

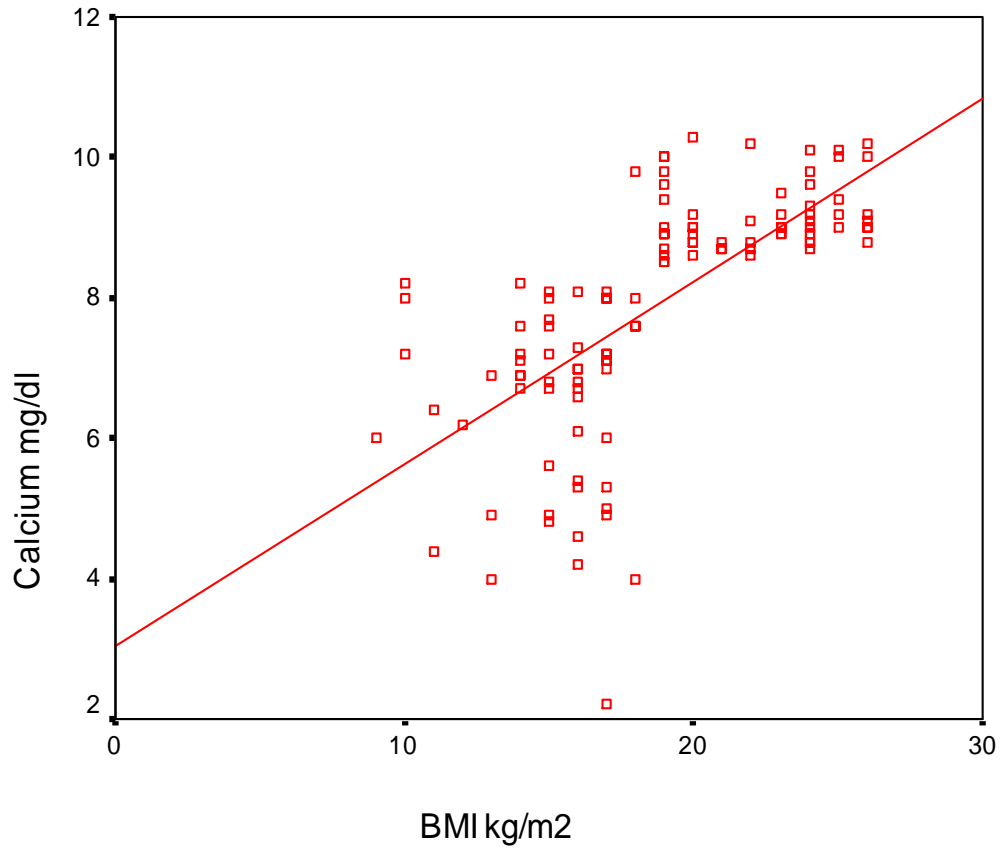


Figure (4-3):Correlation between calcium level and BMI in case group (r=0.671, p-value= 0.000).

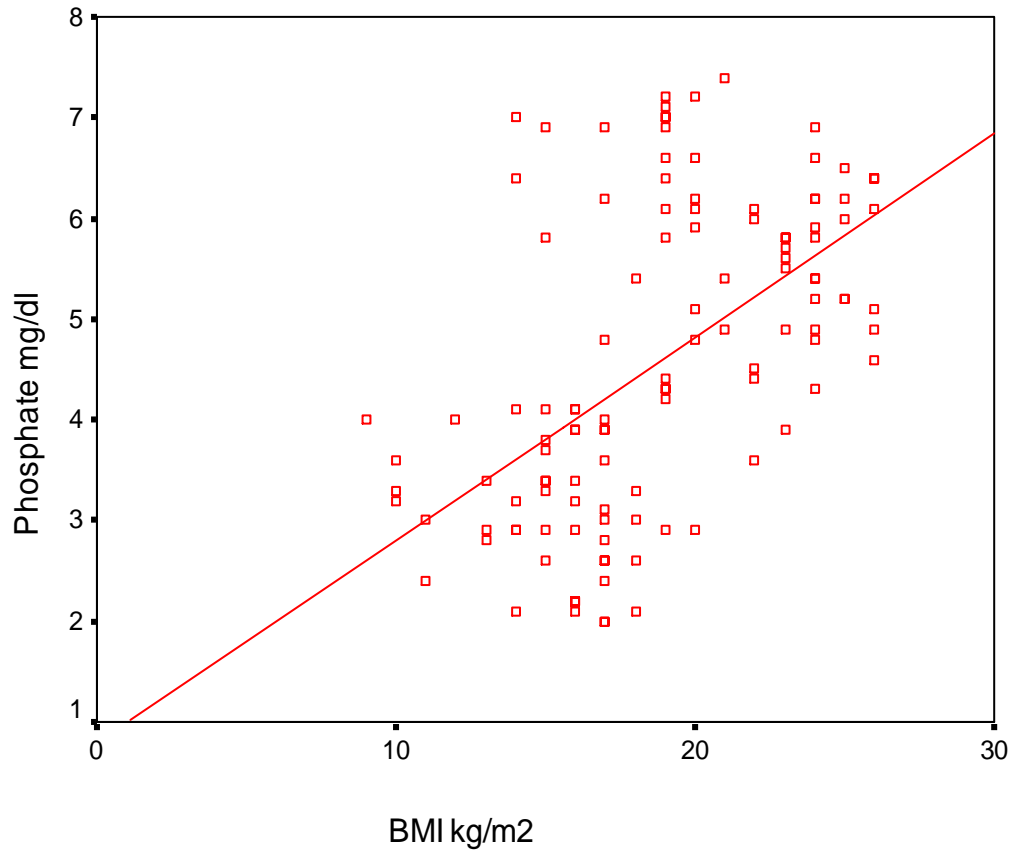
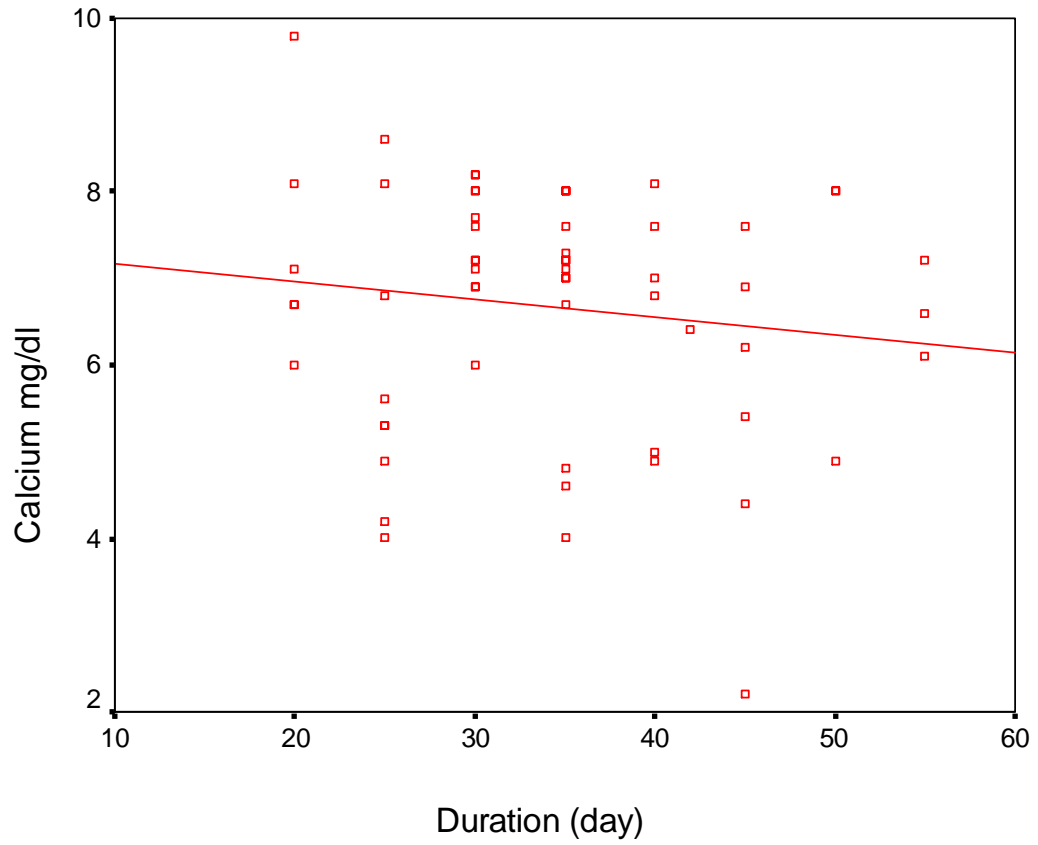
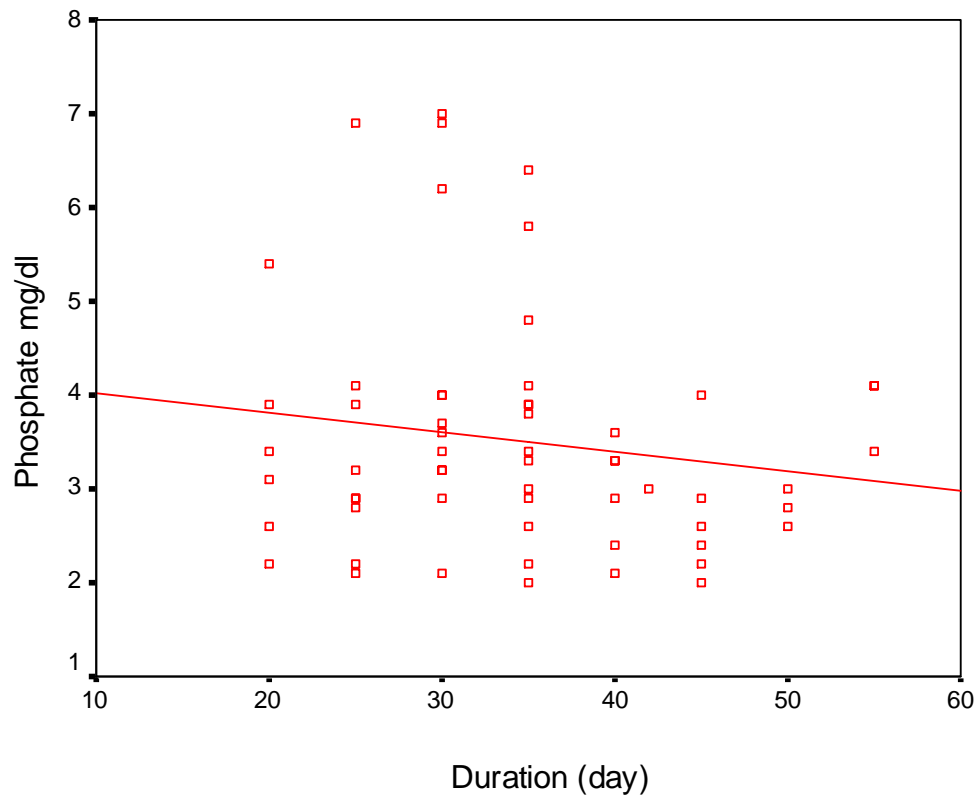


Figure (4-4):Correlation between phosphate level and BMI in case group (r=0.552, p-value= 0.000).



Figure(4-5):Correlation between calcium level and duration in case group($r = -0.135$, $p\text{-value}=0.305$).



Figure(4-6):Correlation between phosphate level and duration in case group ($r = -0.157$, $p\text{-value} = 0.231$).

CHAPTER FIVE

**Discussion, conclusion, and
Recommendations**

5. Discussion

5.1 Discussion:

This study was carried out to evaluate plasma levels of calcium and phosphate, among Sudanese children with protein energy malnutrition in Khartoum state.

The result of this study showed that protein energy malnutrition most common among age between (6-9) years (85%). This result with agreed with another studys carried by many authers (Chukwuma, 2015;Irena , 2011;Jobia ,2008) which showed that malnutrition tendency to develop in lower aged group in African (Nigeria) and Asian (Bangladesh) countries .

The finding obtained from especially designed questionnaire revealed that,(56 .7%) of patients were females and (43.3%) of patients were males. This result agreed with another study carried by (kaneta, 2000) which showed that malnutrition most abundant in females than males .

The results of this study showed, there were significant decreased in calcium and phosphate in protein energy malnourished patients compared to control group. (P.value =0.000). This result agreed with another result carried by (Mahaman.*et al* ,2017) which showed that, calcium and phosphate levels were significant decreased, the occurrence of reduced levels of serum calcium and phosphate in malnourished children due to dietary deficiency of these nutrients or infection owing to increased metabolic loses. The dietary deficiency of calcium could be as a result of intake of calcium-free diet such as gruel which is often prepared from phytates-containing cereal state.

Also the study showed, there was significantly decreased in the mean of BMI in patients compared to control group.(p.value = 0.000)

Also the result showed that, there was no correlation between this parameters (calcium and phosphate) and age of malnourished children ($r = -0.129$, p-value= 0.325) ($r = 0.148$, p-value= 0.259) respectively. this result agreed with another result, which showed that, there were no correlation between electrolytes (calcium and phosphate) levels and age of malnourished children (Ahmad , 2016).

The result showed there were significant positive correlation between BMI , and the parameters (Calcium and Phosphate) $r = 0.671$ and 0.552 respectively (P.values =0.000) as appear in figure (4-3) and (4-4) This result agreed with another result carried by (Freedman, 2009) which showed, there were significant positive correlation between BMI and this parameters (calcium and phosphate).

According to figure (4-5) and (4-6) showed that, there were no correlation between duration of PEM and levels of calcium and phosphate.

5.2 Conclusion:

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

- 1- Calcium and phosphate are significantly reduced in patients with protein energy malnutrition children.
- 2-The body mass index was significantly reduced in patients with PEM
- 4- There are no correlations between calcium and phosphate, and study variables (age and duration).

5.3 Recommendations:

From the finding of this study it is recommended that:

- 1- Patients with PEM should be monitoring of serum electrolytes to prevent Serious complications and avoid hypocalcaemia and hyphosphatemia.
- 2- More studies should be carried out on the effect of PEM on electrolyte (calcium and phosphate) with large sample size and to cover area with high population.
- 3- estimation of albumin should be done together with calcium to correct low calcium level

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Appendices

Questionnaire Appendix (1)

Sudan University of Science and Technology collage of graduate studies

Evaluation of plasma levels of calcium and phosphate in Sudanese malnutrition in Khartoum state Children with

Questionnaire:

Patient No.....

Name

Age in years years

Gender male () female ()

Diagnosis

Weight kg

Heightcm

BM kg/m²

Duration of malnutrition days

History of disease:

Bone disease Yes () No ()

Parathyroid disease Yes () No ()

Renal failure Yes () No ()

Other disease (specify)

Laboratory results:

Plasma calcium level mg/dl

Plasma phosphate level mg/dl