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.(Nassar*et al* .,2010).

Proteins are polymers of amino acids that are linked covalently through peptide bonds. The use of serum protein measurement is widespread for the assessment of nutritional status.It is important markers of malnutrition.(Chandra and Shweta; 2003)

Albumin is synthesized in the liver from 585 amino acids at the rate of 9–12 grams per day with no reserve or storage. It is the protein present in highest concentration in the plasma. Albumin helps identify chronic protein deficiency under conditions of adequate non protein-calorie intake, which leads to marked hypoalbuminemia. This may result from the net loss of albumin from both the intravascular and extra vascular pools, causing kwashiorkor and albumin concentrations may help define marasmus.(Michael *et al* ., 2010).

Serum protein and albumin concentrationsis reduced in malnourished children due to insufficiency nutrition.(Sana *et al* .,2013).

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. Albumin and protein concentrations have been used to help detect and monitor protein nutritional status in malnourished children, however little information is available on assessment for severe acute malnutrition so it is very important to evaluate albumin and protein.
To my knowledge there are few published studies about this in Sudan, so this study may help to provide the monitoring of protein and albumin in malnourished children.

1.3. Objectives:

1.3.1. General objective:

To evaluate plasma levels of protein and albumin in Sudanese malnourished children in Khartoum state.

1.3.2- Specific objectives:

1- To measure plasma levels of protein and albumin and compare mean in malnourished children and control group.

2-To calculate and compare mean of BMI in both study groups.

4-To correlate between total protein and albumin and study variables (age and duration of disease) .

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 (Soetrs*etal* ., 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 - Maramus:

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 maramus . The clinical manifestation is a combination of maramus 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 marasmickwashiokor. 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 :

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

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).

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

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).

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

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 unintendedweight 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 l (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 manmade 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. Protein :

Proteins are polymers of amino acids that are linked covalently through peptide bonds. Very short chains of linked amino acids are designated as dipeptides, tripeptides, tetrapeptides, or pentapeptides. Chains more than five residues in length are called oligopeptides. Longer chains (6 to 30 residues) are referred to as polypeptides. When the number of amino acids linked together exceeds 40 (molecular mass -5 kDa), the chain takes on the physical properties associated with proteins. The different R groups found in amino acids provide peptides and proteins with their diversity in both structure and function. (Carl *et al* ., 2008).

2.2.1. Structure of protein:

There are four distinct levels of a protein’s structure:

primary, secondary, tertiary, and quaternary.

- Primary structure

represents the number and types of amino acids in the specific amino acid sequence. In order to function properly, proteins must have the correct sequence of amino acids. For example, when the amino acid valine is substituted for glutamic acid in the chain of hemoglobin A, hemoglobin S is formed, which results in sickle cell disease.

- Secondary structure

is regularly repeating structures stabilized by hydrogen bonds between the amino acids within the protein. Common secondary structures are the helix, pleated sheet, and turns with most serum proteins forming a helix. Secondary structures add new properties to a protein such as strength and flexibility.

- Tertiary structure

refers to the overall shape, or conformation, of the protein molecule. The conformation is known as the fold, or the spatial relationship of the secondary structures to one another. Tertiary structures are three dimensional. Tertiary structure results from the interaction of side chains and is stabilized through the hydrophobic effect, ionic attraction, hydrogen bonds, and disulfide bonds. The function, physical, and chemical properties of a protein depend on its tertiary structure.

- Quaternary structure

is defined as the shape or structure that results from the interaction of more than one protein molecule, or protein subunits, held together by noncovalent forces such as hydrogen bonds and electrostatic interactions, which are part of the larger protein complex with a precise three-dimensional configuration.(Michael *et al* ., 2010).

2.2.2. Biomedical importance:

Proteins perform multiple critically important roles. An internal protein network,

the cytoskeleton, maintains cellular shape and physical integrity.Actin and myosin filaments form the contractile machinery of muscle. Hemoglobin transports oxygen, while circulating antibodies search out foreign invaders. Enzymes catalyze reactions that generate energy, synthesize and degrade biomolecules, replicate and transcribe genes, process mRNAs. Receptors enable cells to sense and respond to hormones and other environmental cues. An important goal of molecular medicine is the identification of proteins whose presence, absence, or deficiency is associated with specific physiologic states or diseases. The primary sequence of a protein provides both a molecular fingerprint for its identification and information that can be used to identify and clone the gene or genes that encode it. (Robert *et al* ., 2003).

2.2.3. Synthesisof protein:

Most plasma proteins are synthesized in the liver and secreted by the hepatocyte into the circulation. The immunoglobulins are exceptions because they are synthesized in plasma cells. It is the information encoded in genes, specified by the nucleotide sequence, that provides each protein with its own unique amino acid sequence. This amino acid sequence of a polypeptide chain is determined by a corresponding sequence of bases (guanine, cytosine, adenine, and thymine) in the DNA contained in the specific gene. This genetic code is sets of three nucleotides known as codons with each three-nucleotide combination standing for a specific amino acid. Because DNA contains four nucleotides, the total number of possible codons is 64; therefore, some redundancy in the genetic code allows for some amino acids to be specified by more than one codon. (Michael *et al* ., 2010).

2.2.4. Classification of protein:

Proteins are classified into:

2.2.4.1. Simple proteins:

Simple proteins contain peptide chains composed of only amino acids. Simple proteins may be globular or fibrous in shape. Globular proteins are globe like, symmetrical proteins that are soluble in water. Globular proteins are transporters, enzymes, and messengers. Examples of globular proteins are albumin, hemoglobin, and the immunoglobulinsIgG, IgA, and IgM. Fibrous proteins form long protein filaments or subunits, are asymmetrical and usually inert, and are generally water insoluble due to their hydrophobic R groups. Fibrous proteins are structural, such

as connective tissues, tendons, bone, and muscle. Examples of fibrous proteins include troponin and collagen.(Michael *et al* ., 2010).

2.2.4.2. Conjugated Proteins:

Conjugated proteins consist of a protein and a nonprotein (prosthetic) group.The prosthetic group is the nonamino part of a conjugated protein.The prosthetic group may be lipid, carbohydrate, porphyrins, metals, and others. It is the prothetic groups that define the characteristics of these proteins. Examples of conjugated proteins are the metalloproteins, glycoproteins, lipoproteins, and nucleoproteins. Metalloproteins have a metal ion attached to the protein, either directly, as in ferritin (which contains iron) and ceruloplasmin (which contains copper), or as complex metals (metal plus another prosthetic group), such as hemoglobin and flavoproteins. Lipoproteins have lipids such as cholesterol and triglyceride linked to proteins, such as high-density lipoproteins (HDL) and very low density lipoproteins (VLDL). Several protein groups are used to describe carbohydrates joined to proteins. Generally, those molecules with 10%–40% carbohydrate are called glycoproteins. Examples of glycoproteins are haptoglobin and antitrypsin. When the percentage of carbohydrate linked to protein is higher, the proteins are called mucoproteins or proteoglycans. An example of a mucoprotein is mucin, a lubricant that protects body surfaces from friction or erosion. Increased mucin production occurs in many adenocarcinomas, including cancer of the pancreas, lung, breast, ovary, and colon. Moreover, mucins are also being investigated for their potential as diagnostic markers. Nucleoproteins are those proteins that are combined with nucleic acids, DNA or RNA. Chromatin is an example of a nucleoprotein that is the complex of DNA and protein that makes up chromosomes.(Michael *et al* ., 2010).

2.2.5. Plasma protein:

The plasma proteins are the most frequently analyzed ofall the proteins. The major measured plasma proteins aredivided into two groups: albumin and globulins. There are four major types of globulins, each with specific properties and actions. A typical blood panel will providefour different measurements—total protein, albumin, globulins, and the albumin/globulin ratio.(Michael *et al* ., 2010).

2.2.6. Clinical significance of protein:

2.2.6.1. Increased level of total protein:

The protein level increased in cholelithiasis, liver carcinoma and rheumatic fever.

2.2.6.2. Decreased level of total protein:

The protein level decreased in chronic renal failure, diabetes mellitus ,insuficient dietary protein, epidemic typhus and gastrointestinal carcinoma. (Jacobs, 2005).

2.3. Albumin:

Albumin is a small globular protein with a molecular mass of 66.3 kDa. It is the most abundant protein found in plasma from midgestation until death, accounting for approximately one half the plasma protein mass. Because of its high plasma concentration and relatively small size, albumin is also the major protein component of most extravascular body fluids, including (1) CSF, (2) interstitial fluid, (3) urine, and (4) amniotic fluid. Approximately 60% of the total body albumin is in the extravascular space. It has no carbohydrate side chains but is highly soluble in water due to its high net negative charge at physiological pH. (Carl *etal* ., 2008)

2.3.1. Biochemistry and Functionof albumin:

Albumin is synthesized primarily by the hepatic parenchymal cells. The synthetic reserve of the liver is enormous. For example, in the nephrotic syndrome, the synthetic rate may be 300% or more of its normal rate. The synthetic rate of albumin is controlled primarily by colloidal osmotic pressure (COP) and secondarily by protein intake. In addition, synthesis is decreased by inflammatory cytokines. Catabolism occurs primarily by pinocytosis in all tissues, with reuse of the resulting free amino acids for synthesis of cellular proteins. The normal plasma half life of albumin is 15 to 19 days. Albumin's primary function is the maintenance of COP in both the vascular and extravascular spaces. Albumin also binds and transports a large number of compounds, including (1) free fatty acids, (2) phospholipids, (3) metallic ions, (4) amino acids, (5) drugs, (6) hormones, and (7) bilirubin. (Carl *et al* ., 2008).

2.3.2. Clinical Significance of albumin:

2.3.2.1. Increased concentration of albumin:

are present only in acute dehydration and have no clinical significance.

2.3.2.2. Decreased concentration of albumin:

are seen in a multitude of clinical conditions.

- Analbuminemia

Individuals with this rare genetic deficiency have plasma albumin concentrations less than 0.5 g/L but mild if any edema. Major clinical manifestations are related to abnormal lipid transport.

- Inflammation

Acute and chronic inflammation are the most common causes of hypoalbuminemia, resulting from (1) hernodilution, (2) ioss into the exnavascular space, (3) increased consumption by cells, and (4) decreased synthesis.

- Hepatic Disease

The decreased concentrations of albumin present in most cases of hepatocellular disease result from (1) increased immunoglobulin concentrations, (2) loss into the extravascular space, and (3) direct inhibition of synthesis by toxins and alcohol. The liver is capable of synthesizing increased amounts of albumin until hepatic parenchymal damage or loss is severe, with the loss of approximately 95% of function.

- Urinary Loss

The renal glomerulus acts as a molecular sieve, excreting any substance at a rate inversely proportional to its molecular radius. Because albumin is relatively small and globular, significant amounts filter into the glomerular urine. However, most of it is reabsorbed by the proximal tubular cells. Normal excreted urine contains up to 20 mg albumin per gram of creatinine. Excretion above this amount suggests

(1) increased glomerular filtration,

(2) tubular damage or hernaturia, or

(3) a combination of these.

- Increased filtration

also occurs with physical exercise and fever; therefore urinary albumin should be assayed under controlled conditions and repeated if a clinical question exists as to the cause of increased concentrations. Mildly increased excretion (20 to 300 mgIL), or microalbuminuria, appears to predict future development of clinical renal disease in individuals with hypertension or diabetes mellitus. Except for hereditary analbuminemia, the lowest concentrations of plasma albumin are present in individuals with active nephrotic syndrome, associated with markedly increased loss of most small and medium size proteins into the glomular urine.

- Gastrointestinal Loss

Inflammatory disease of the intes- tinal tract is associated with increased gastrointestinal (GI) loss of albumin. This increase usually is of little concern unless the loss is excessive or persists. Chronic protein-losing enteropathy may result in loss similar to that present in the nephrotic syndrome. (Carl *et al* ., 2008)

2.4. Relationship between plasma (Protein and albumin) and malnutrition:

Albumin and protein concentrations have been used to help detect and monitor protein nutritional status. However, low concentrations generally do not correlate with the degree of malnutrition.(Carl *et al* ., 2008).

3. Materials and Methods

3.1. Materials:

3.1.1. Study approach:

Quantitative methods were used to estimate protein and albumin in Sudanese children with malnutrition in Khartoum state during the period from January to April 2017.

3.1.2 Study designs:

This is cross –sectional case control study.

3.1.3. Study area:

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

3.1.4. Study population:

The study included children with malnutrition and healthy children as control.

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 in this study.

3.1.7. Exclusion criteria:

Any patients with liver disease, renal disease, thyroid disease, other chronic disease and any drug that affect protein and albumin measurement were excluded.

3.1.8. Ethical consideration:

Oral Consent was taken from parents of children to participate in the study and reassurance of confidentiality. Before the sample was collected, the donors knew that this specimen for research and the purpose of the research was explained to each patient.

3.1.9. Data collection:

The clinical data were obtained from history. Clinical examinations and hospital follow up records and were recorded on a questionnaire sheet.

3.1.10. Sample collection and processing:

About 2.5 ml of venous blood were collected from each participant (both cases and control). The samples collected under aseptic conditions and placed in sterile heparin containers, and after mixing centrifuged for 5 minutes at 3000 rpm to obtain plasma, then the plasma were kept at $^{-}20^{o}$C till the time of analysis.

3.2. Methods:

3.2.1. Estimation of total protein level:

3.2.1.1. Principle of method:

Protein in the sample react with copper ion in alkaline medium forming color complex that can be measured by spectrophotomery.(Gornall*et al.,* 1949)

3.2.1.2 Procedure of total protein

	Blank	Standard	Sample
Protein standard (S)	-	0.02ml	-
Sample working	-	-	0.02ml
Reagent	1.0ml	1.0ml	1.0ml

Mixed thoroughly and let stand the tubes for 10 minutes at room temperature.

The absorbance was read in 545nm against the Blank. the color was stable for at least 2 hours.

3.2.1.3. Calculation of analyte concentration:

The protein concentration (g/dl) = (A Sample/A Standard) * Concentration of standard.

3.2.2 Estimation of albumin:

3.2.2.1 Principle of the method:

Albumin in the sample react with bromocresol green in acid medium forming acoloured complex that can be measured by spectrophotomery. (Doumas and Watson, 1971).

Procedure of albumin :

	Blank	Standard	Sample
Working reagent	1.0ml	1.0ml	1.0ml
Standard	-	0.01ml	-
Sample	-	-	0.01ml

Mixed thoroughly and let stand the tubes for 1 minute at room temperature.

The absorbance was read in 630nm against the Blank. the color was stable for at least 30 minutes.

3.2.2.3. Calculation of analyte concentration:

The albumin concentration (g/dl) = (A Sample/A Standard) * Concentration of standard.

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. Data analysis:

Data was analyzed to obtain means standard deviation and correlation of the sampling using statistical package for social science (SPSS) computer programmed version 11.5, t test and person correlation were used for comparison and correlation.

4. Results

The results of biochemical determinant of plasma protein and albumin in malnourished children are given in tables and Figures:

Table (4-1): illustrate mean concentration of protein and albumin in patients and control groups. The levels of protein and albumin were significantly decreased in malnourished children compared to control group.(mean ± SD: 5.08± 0.72 versus 7.53 ±0.43 g/dl, P.value =0.000: 2.78± 0.42 versus 4.41 ± 0.51 g/dl, P.value =0.000) respectively.

Table (4-2): Show the mean of BMI in patients and control group. The BMI was significantly decreased in patients compared to control group.(mean ± SD : 15.28 ± 2.28 kg/m2 versus 19.25 ± 2.48 kg/m2 ,P.value = 0.000). .

Table (4-3): Showagedistribution, (85%) of patient between (6-9) Years, (8%) between (10-13) and (7%) between (14-17) years and show gender Distribution, (56.7%) of patients were females while (43 .3%) were males.

Figure (4-1): Show correlation between the level of protein and age of malnourished children, (r= 0.106, p-value= 0.420) there was weak positive correlation.

Figure (4-2): Show correlation between level of albumin and age of malnourished children, (r= 0.059, p-value= 0.655) there was no correlation.

Figure (4-3): Show correlation between protein level and duration of malnourished children (r = 0.092, p-value = 0. 487) there was no correlation.

Figure (4-4): Show correlation between albumin levels and duration of malnourished children (r = 0. 255, p-value =0.052) there was weak positive correlation.

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Table (4-1): Mean of plasma levels of protein and albumin in malnourished children and control group.

Variable	Controls N=60 Mean ± SD	Patients N=60 Mean ± SD	p-value
Protein(g/dl)	7.53 ±0.43	5.08± 0.72	0.000
Albumin(g/dl)	4.41 ± 0.51	2.78± 0.42	0.000

*Result given in mean ± SD.

* P-value ≤ 0.05 Consider significant.

Table (4-2):Mean of Body mass index (BMI) in malnourished children and control groups.

Variables	Control group N=60	Case group N=60	p-value
BMI Kg/m2	19.25 ± 2.48 kg/m2	15.28 ± 2.28 kg/m2	0.000

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

* Independent sample T test was used for comparison.

Table (4-3): 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	43.3%
Sex female	34	56.7%

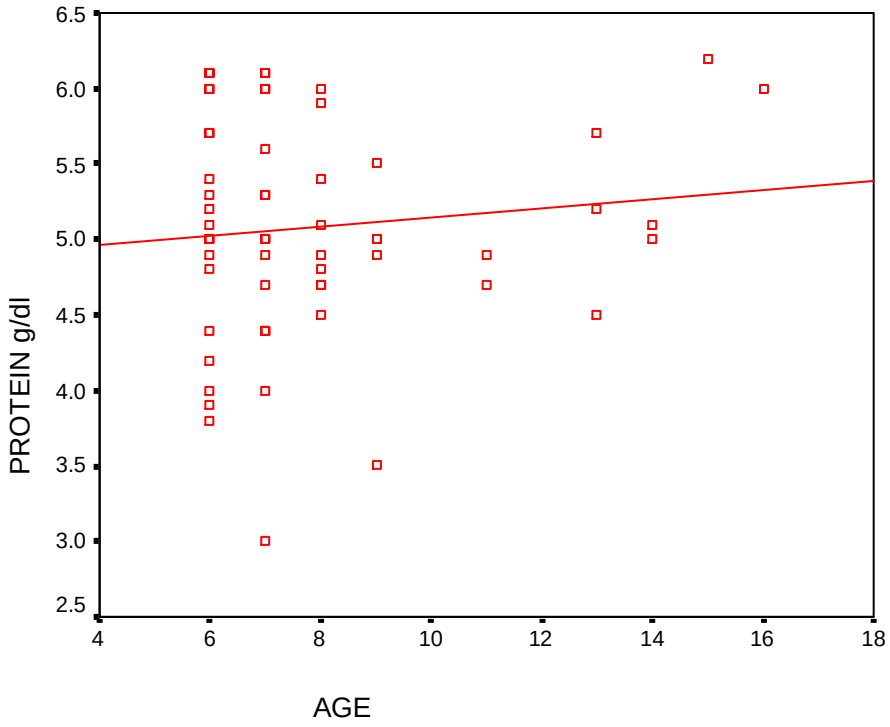

Figure (4-1): Correlation between protein level and age of malnourished children (r= 0.18, p-value= 0.168) .

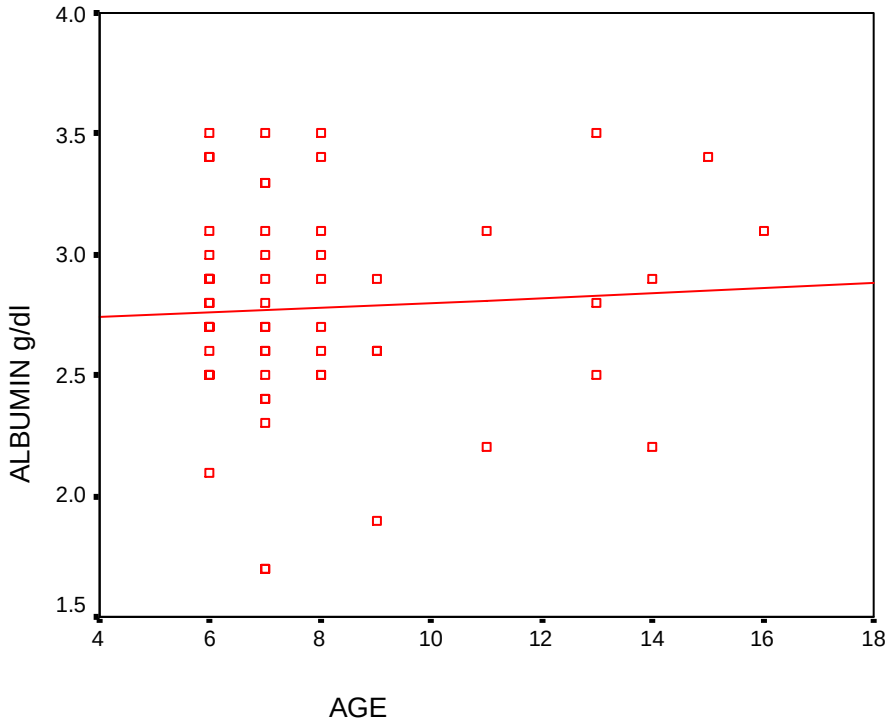

Figure (4-2): Correlation between albumin level and age of malnourished children (r= 0.09, p-value= 0.496).

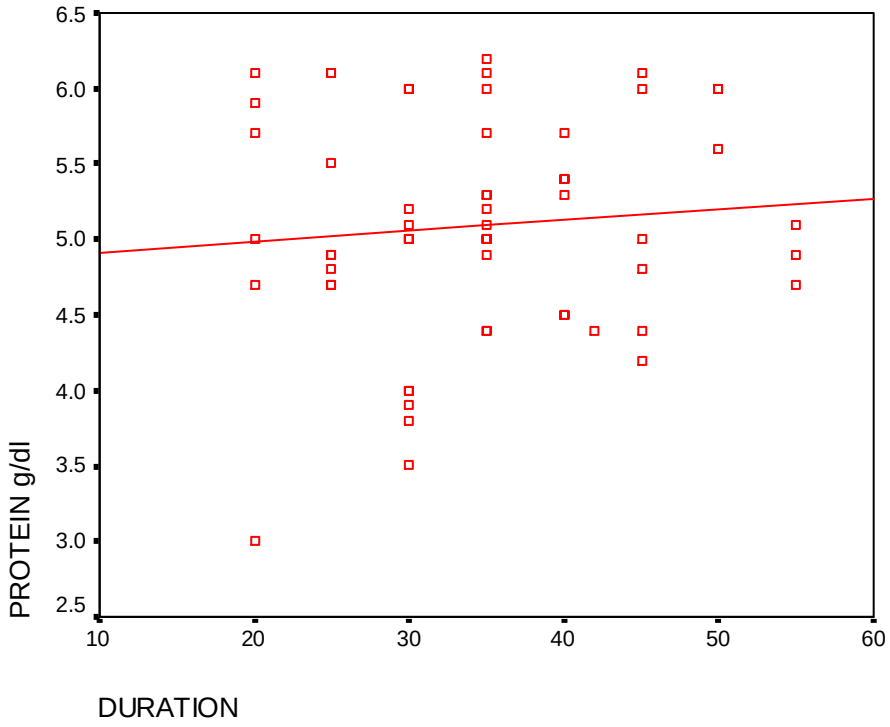

Figure (4-3): Correlation between protein level and duration of malnourished children (r = 0.092, p-value = 0. 487).

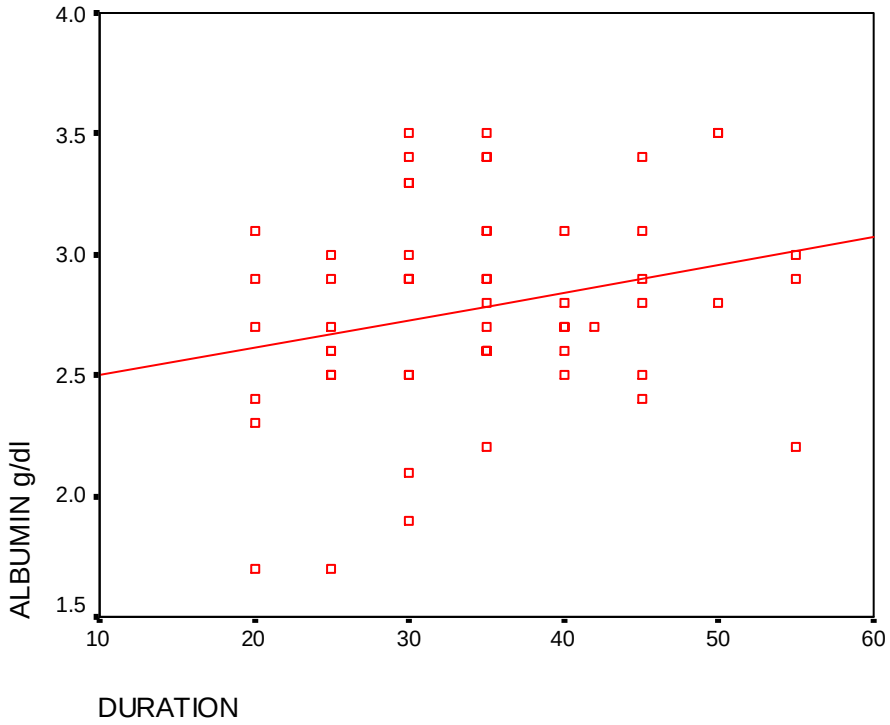

Figure (4-4): Correlation between albumin level and duration of malnourished children (r = 0. 255, p-value =0.052).

5.1. Discussion

Malnutrition is generally a nutritional problem that results from varying proportion of protein and calories deficiency in infant and young children and is a complicating factor for other illnesses in developing countries.
(Mubarak *et al.,* 2003).

Malnutrition affects many substances in the body by increasing them .This study conducted to get the effect of malnutrition on the levels of protein and albumin.

From the finding of this study ,it appears that plasma levels of protein and albumin were significantly decreased in malnourished children group compared to control group.This results agreed with a study carried by (Sana *et al* . , 2013) . which showed that ; Plasma protein and albumin concentration were decreased in malnourished children due to the decrease intake of nutrients, reduced food intake often due to decreased appetite, infection, starvation, malabsorption and increased metabolic losses of nutrients or an initial nutrients deficiency.

Also the results in agreement with another studies carried by many authors (Hsoro*et al* . , 2002; Ibrahim *et al* . , 1994) which finding significant decreased (p = 0.000) in the mean of plasma level of total protein when compared with control group.

Also the results in agreement with another studies carried by (Begum and Serajul., 2005) which finding there was significant decreased (p = 0.000) in the mean of plasma level of albumin when compared with control group.

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

The result of this study showed that PEM most common among age between (6-9) years (85%).This result agreed with another studies carried by many authors(Chukwuma, 2015;Irena , 2011;Jobia ,2008) which showed that malnutrition tendency to develop in lower group aged due to increased nutrional need for developing 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 due to traditional food sharing behaviours in some households may result in females diets being less adequate than males.

According to figure (4-5) and (4-6), the results showed that, there was no correlation between duration of PEM and level of protein and there was weak positive correlation with albumin.

5.2. Conclusion:

From the results and finding of this study, it is concluded that:

1. Protein and albumin concentration are significantly decreased in malnourished children.
2. The duration of malnutrition not correlated with plasma protein and weak positive correlated with albumin.

5.3. Recommendations:

From the findings of this study it is recommended that

1. Malnourished children should be monitored for protein and albumin regularly.
2. Uptake of adequate protein and albumin rich nutrition
3. More studies should be carried out on the effect of malnutrition on plasma protein and albumin concentration with large sample size and to cover area with high population.

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Appendix (I)

Questionnaire

Sudan University of Science and Technology
Collageof graduate studieswith
Evaluation of plasma levels of Total protein and albumin in Sudanese children with malnutrition in Khartoum state

Questionnaire:

Patient No……
Name …….
Age in years …… years
Gender male ( )female ( )
Diagnosis ……
Weight ……kg
Height …… cm
BM ……kg/m2
Duration of malnutrition …….. Days

History of disease:

Liver disease Yes ( ) No ( )
Thyroid disease Yes ( ) No ( )
Renal failure Yes ( ) No ( )
Other disease (specify) ……

Laboratory results:

Plasma protein level g/dl
Plasma albumin level g/dl