Iron Status in Severe Protein-Energy Malnutrition in Children in Khartoum State

A thesis
submitted in partial fulfillment for the requirements of the Degree of Clinical MD in Paediatrics and Child Health.

By

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بسم الله الرحمن الرحيم

قال تعالى:
"وأنزلنا الحديد فيه بأس شديد ومنافع للناس
وليعلم الله من ينصره ورسله بالغيب أن الله قوي عزيز"
صدق الله العظيم
سورة الحديد (الآية 1)
To the soul of my father who taught me a lot about

Respect of human rights.

To

My mother for her continuous support and

motivation

To my husband Mr. Mohd El-Rashid for his

continuous inspiration and encouragement

My daughter Mayar and my son Mazin

Their loves make everyday

Worth-while

To all Sudanese children I wish them nice future

And peace............
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My colleagues and friends for their continuous support.
Abstract

This is descriptive cross sectional A hospital based study to asses the iron status in children with protein energy malnutrition (PEM) compared to normal controls.

The study was conducted in Gaffer Ibn Ouf Hospital, Dept. of paediatric in Khartoum Teaching Hospital and Omdurman Teaching Hospital from Sep. 2006 to March. 2007.

The study tools included: interview and clinical examination using structured questionnaire and laboratory investigations focused on haematology and iron status parameters. One hundred and four children were included, out of whom 74 had severe PEM; 34 with marasmus, 26 with kwashiorkor and with marasmic – kwashiorkor. 30 were controls.

The study showed that low level of Hb was found 86.5% of children with PEM compared to 20% of the controls (P value <0.05).

The study showed all children less than 2 years old were anemic in the study group, also all female (26) in the study were anemic compared to (38) 79.2% of male. Low level of Hb was found in all children who presented with pallor, nail changes, smooth tongue or jaundice.

Iron studies in children with PEM showed different correlation with Hb level, there were children with low serum iron and low Hb level but with normal or low TIBC also S-ferritin was normal or high in the presence of low Hb level.
There is significant number of children with low red cell indices 12 (40%) in the presence of normal Hb level in the control group.

The main recommendations include:

Iron status of children with PEM must be evaluated by methods which are not influenced by infection or inflammatory processes and hypoproteinemia such as transferrin receptors or percentage of hypochromic red cell.

1- References values of iron status parameters in our population should be studied.

2- Detection haemoglobinopathies must be included in future studies.

3- Low red cell indices in the presence of normal Hb values must be carefully interpreted (haemo-globinopathies should excluded in such cases).
مستخلص الدراسة

هذه دراسة وصفية مقطعية على مستوى المستشفى وتم تسجيل الحالات عند الأطفال المصابين بمرض التغذية، وتمت مقارنتها بمجموعة ضابطة أجريت هذه الدراسة بمستشفى جعفر بن عوف التخصصي وقسم الأطفال في كل من مستشفى الخرطوم التعليمي ومشفى أدرمان التعليمي في الفترة ما بين سبتمبر 2006 وحتى فبراير 2007

النتائج:
- اختلفت هذه الدراسة على أدوات البحث التالية:
  - المعادن والفحصات السريرية المستخدمين في ذلك استبان مفصل وفحصات معمارية ركزت على فحص خياب الخلايا ودالات كريات الدم الحمراء وقييم حالة الجديد في الجسم.
  - شملت هذه الدراسة 104 طفل 74 منهم يعانون من سوء التغذية (34 منهم يعانون من الهزال 26 مصابين بالكواشرورك و19 منهم مصابين بالهزال مع الكواشرورك) و30 يمثلون المجموعة الضابطة.

- دلت النتائج على أن 86.5% من مرضى سوء التغذية يعانون من نقص في مستوي خياب الدم مقارنة مع 6% (20%) من المجموعة الضابطة. كما توصلت الدراسة إلى أن كافة الأطفال الذين يعانون من سوء التغذية في الفترة العمرية أقل من سنتين لديهم نقص في خياب الدم.
- كل الأطفال الثلاثين شملتهم الدراسة (26) كن يعانون من فقر الدم مقابل 38% من مجموع الذكور.

- وجد ان خياب الخلايا كان منخفضا في جميع الأطفال الذين كان لديهم شحوب غير طبيعي أو تغير في ظاهرهم أو نعومة في الشتائم أو مصابين باليرقان.

- أوضحت الدراسة ان الأطفال المصابين بمرض التغذية لديهم تناسب مختلف بين معدل خياب الخلايا ودراسات الحديد يوجد أطفال لديهم نقص في عنصر الحديد وعوز في خياب الدم ولكن سعة ارتباط الحديد الكللي طبيعية أو ناقصة كما أن مخزون الحديد (الفيبريتين) طبيعي أو عالي مع وجود نقص في خياب الدم.

- كما دلت النتائج أيضا على أن نسبة 12% (40%) من أطفال المجموعة الضابطة كان لديهم نقص في دالات كريات الدم الحمراء ولكن معدل خياب الدم كان طبيعي لديهم.

- اشتملت التوصيات على الآتي:
  - يجب اجراء تقدير مستوى الحديد لدى الأطفال المصابين بمرض التغذية بطرق لاتباث بالحالات الالتهابية أو نقص البروتين في الدم مثل تعيين نسبة الخلايا الدموية الشاحبة اللون وتعيين مستقبلات البروتين الناقل لل الحديد.
يجب تحديد القيم المرجعية المثلى لفحوصات الحديد وخصاب الدم في اطفالنا.

الدراسات المستقبلية في هذا المجال يجب أن تشمل على استبعاد الاختلال الاختلاسي.

النقص في دوائر الكريات الحمراء مع وجود المعدل الطبيعي لخصاب الدم يتطلب استبعاد الاختلال الاختلاسي بواسطة الفحوصات المخبرية الأخرى.
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<table>
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<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra – acetic acid</td>
</tr>
<tr>
<td>H/A</td>
<td>Height for age</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>Hct</td>
<td>Haematocrit</td>
</tr>
<tr>
<td>ID</td>
<td>Iron Deficiency</td>
</tr>
<tr>
<td>IDA</td>
<td>Iron Deficiency Anaemia</td>
</tr>
<tr>
<td>IDD</td>
<td>Iodine Deficiency Disorders</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IRP</td>
<td>Iron Regulatory Protein</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean Cell Haemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean Cell Haemoglobin Concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Cell Volume</td>
</tr>
<tr>
<td>MUAC</td>
<td>Mid-Upper Arm Circumference</td>
</tr>
<tr>
<td>NCHS</td>
<td>National Center for Health Survey</td>
</tr>
<tr>
<td>PEM</td>
<td>Protein-Energy Malnutrition</td>
</tr>
<tr>
<td>Pg</td>
<td>Pictogram</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>TfRs</td>
<td>Transferring Receptors</td>
</tr>
<tr>
<td>TIBC</td>
<td>Total Iron Binding Capacity</td>
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<tr>
<td>W/A</td>
<td>Weight for Height</td>
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<tr>
<td>WHO</td>
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Chapter One

1- INTRODUCTION AND LITERATURE REVIEW

1.1 Definition and General Concept:

Protein-energy malnutrition (PEM) is not just the result of lack of protein & calorie intake but is due to complex biological and social adverse processes.

The World Health Organization (WHO) defined PEM as cellular imbalance between supply of nutrient and energy and the body's demand for them to ensure normal growth, maintenance and specific tissue function \(^{(1,2,3)}\).

According to the WHO 149.6 million under 5 years of age ie 26.7% of the world's children of this age group are malnourished in term of Weight - for - age geographically over two third (72%) live in Asia, while 25% are found in Africa and 2.3% in Latin America \(^{(4)}\).

The right to good nutrient is a matter of international law articulated in international declarations and human rights instruments dating back to the adoption of the declaration of the right of the child in 1924. The right to nutrient received its fullest expression in 1989 Convention of the Right of the Child. Under this convention, virtually every government in the world recognized the right of all children to the
highest attainable standard of health including the right to good nutrition and its three vital components: food, health & care\(^{(5)}\).

1.2 Pathophysiology:

In marasmus, energy intake is insufficient for the body's requirements, and the body draws on its own stores. Liver glycogen is exhausted within a few hours, and skeletal muscle protein is then used via gluconeogenesis to maintain adequate plasma glucose. At the same time, triglycerides in fat depots are broken down into free fatty acids, which provide some energy for most tissues, but not for the nervous system. When near starvation is prolonged, fatty acids are incompletely oxidized to ketone bodies, which can be used by the brain and other organs for energy\(^{(1)}\). Thus, in the severe energy deficiency of marasmus, adaptation is facilitated by high cortisol and growth hormone levels and depression of insulin and thyroid hormone secretion. Because amino acids are mobilized from muscle to provide the liver with substrate for protein synthesis, plasma protein levels decrease less in marasmus than in kwashiorkor\(^{(2,3)}\).

In kwashiorkor, relatively increased carbohydrate intake with decreased protein intake leads to decreased visceral protein synthesis. The resulting hypoalbuminemia causes dependent edema, and impaired β-lipoprotein synthesis causes fatty liver\(^{(3,4)}\). Insulin secretion is initially
stimulated but is reduced later in the disease. Fat mobilization and amino acid release from muscle are reduced, so that less amino acid substrate is available to the liver. In marasmus and kwashiorkor, the insulin response to a glucose load is poor, possibly due to chromium deficiency.(1,3)

In protein deficiency, adaptive enzyme changes occur in the liver, amino acid synthesis increase, and urea formation diminishes, thus conserving nitrogen and reducing its loss in urine. Homeostatic mechanisms initially operate to maintain the level of plasma albumin and other transport proteins. The rates of albumin synthesis eventually decrease, and plasma levels fall, leading to reduced oncotic pressure and edema. Growth, immune response, repair, and production of some enzymes and hormones are impaired in severe protein deficiency (1,2,3).

Aflatoxien poisoning has been proposed as a cause of kwashiorkor, later the Golden hypothesis of free radical(5,6) has been advanced to explain the changes seen in kwashiorkor. According to this hypothesis, oedema in kwashiorkor result from over production of free radicals due to (infection, toxins, iron,...etc)and break down of protected mechanism (7,8).

Why, some children develop edematous PEM and others develop noneedematous form is unknown, but Gopalan proposed theory of disadaptation to explain that. Whenever a child is starved certain mechanism operate to protect vital tissues at the expense of less vital,
failure of these adaptive mechanism result in kwashiorkor whereas well adaptation result in marasmus. Marasmic-kwashirkor follows disadaptation in a previously marasmic child.\(^8\)

1.3 Clinical Signs of Protein Energy Malnutrition:

1.3.1 Kwashiorkor:

An African word used by Ga tribe meaning "the disease of the displaced child"). It refers to the observation that the first child develops PEM when the second child is born and replaces the first child at the breast \(^9\). It has been known for centuries However, it was not until the 1930s when Cicely Williams who was working in Ghana, described it in detail in 1950s kwashiorkor began to get a great deal of attention. It was often described as the most important form of malnutrition, and it was believed to be caused mainly by protein deficiency.

The presenting symptom usually include oedema. Wasting of muscles is also typical but may not be evident because of oedema\(^{10}\), unhappiness and apathy.

Hair changes include dyspigmentation and hair loss.

*Skin changes consist of hyper or hypo pigmented areas, desquamation and ulceration. Cheilosis and angular stomatitis are common*\(^{1,2}\)
The liver is enlarged with fatty infiltration \(^{(1,2,5)}\)

### 1.3.2 Nutritional marasmus:

Marasmus is the Greek word meaning wasting\(^{(9)}\). It may occur at any age, most commonly up to about three and a half years, but in contrast to kwashiorkor it is more common during the first year of life. Nutritional marasmus is in fact a form of starvation, and the possible underlying causes are numerous. The most important precipitating causes of marasmus are infectious and parasitic diseases of childhood.

The main sign of marasmus is *poor growth*. The muscles are always extremely wasted. The skin hangs in wrinkles, especially around the buttocks and thighs. Children with marasmus are quite often not disinterested like those with kwashiorkor. The child often has a good appetite\(^{(10)}\).

*Hair changes* similar to those in kwashiorkor can occur. There is more frequently a change of texture than of colour.

### 1.3.3 Marasmic- kwashiorkor:

Children with marasmic kwashiorkor have all the features of nutritional marasmus including severe wasting, lack of subcutaneous fat and poor growth, and in addition to oedema, which is always present,
they may also have any of the features of kwashiorkor described above\(^{(10)}\).

### 1.4 Evaluation of Malnourished Children:

#### 1.4.1 Anthropometry:

Anthropometric assessment involves the use of growth standard. They provide a useful tool for assessing nutritional status and well being of children the term growth standard and growth reference are use interchangeably, though the meaning is different. A growth standard reflect an optimum level of growth, suggesting that all children have the potential to achieve it, while growth reference is simply used for comparison. The National Center for Health Survey (NCHS/WHO) growth reference is widely used all over the world. \(^{(11)}\). Anthropometry includes:

**1.4.1.1 Weight:** is a very important measurement

The children should be weighted with light dresses and without shoes, the Wt. should be measured in kilograms and the reading should be taken to the nearest 0.1kg and compared to the international NCHS standards.

Growth chart\(^{(12)}\) which imply sequential weight is more important than single one the best and simplest way to do this is to plot the weight
of the child on weight chart on which these reference weights are already
drawn\(^3\).

### 1.4.1.2 Length and height:

Most severe retardation in height occurs when malnutrition occurs during 1\(^{st}\) year of life (stunting)

**Length:** To measure the length of the patients, the child will be laid in a flat wooden table with the head within the same horizontal plane of the feet and the body straight. The feet and the head will be perpendicular to the table and marks will be done on both sides of the head and feet. The distance between the marks will be read to nearest 0.1 cm with a non-stretchable tape\(^{13}\).

**Height:** To measure the height the child will be without shoes with the child standing with heels and back in contact with an upright wall using height stadiometer, the height should be measured in Cm and the reading taking to nearest 0.1 Cm, the height should be expressed to the NCHS standard\(^{13}\).

### 1.4.1.3 Anthropometric status indicators:

The most used anthropometric indicators are stunting (H/A), wasting (W/H), and underweight (W/A), and mid-upper arm circumference (MUAC) in children under five years of age and Body Mass Index (BMI) in adults\(^{13}\).
1.4.3.1 **Height-for-age (H/A):**

The term “stunting” is used to describe a condition in which children fail to gain sufficient height, given their age. Stunting is an extremely low “height-for-age” (H/A) score. Often is associated with long-term factors such as chronic malnutrition, especially protein-energy malnutrition, and frequent illness. (H/A) is very sensitive to socio-economic inequalities\(^{12,13}\).

1.4.1.3.2 **Weight-for-height (W/H):**

The term “wasting” refers to a situation where a child has failed to achieve sufficient weight for height (W/H). Weight-for height is normally used as an indicator of current nutritional status. Wasting may be the consequence of starvation or severe disease. It can also be due to chronic conditions or a combination of both\(^{13,14,15}\).

1.4.1.3.3 **Weight-for-age (W/A):**

The term “underweight” is used to describe a situation where a child weighs less than expected, given his or her age. Underweight is thus an extremely low “weight-for-age” (W/A) score. W/A reflects body mass relative to age. Unlike height, weight fluctuates over time and therefore reflects current and acute as well as chronic malnutrition. W/A is commonly used for monitoring growth and to assess changes in the magnitude of malnutrition over time\(^{13,16}\).
The recommended reporting system of H/A, W/H and W/A is in terms of Z-scores—a statistical measure of the distance from the median (mean) expressed as a proportion of the standard deviation. The most common cutoff point is –2 Z-score, i.e., two standard deviations below the median values of the international reference. This is the cutoff risk level used to differentiate malnourished children from those adequately nourished. Children whose H/A, W/H and W/A scores fall below this point are therefore considered, stunted, underweight and wasted, respectively. The WHO has proposed a classification scheme for population-level malnutrition.\(^{(13)}\)

1.4.1.3.4 Mid Upper Arm Circumference (MUAC): It remains constant between 1-5 years because progressive decrease in subcutaneous fat is associated with corresponding increase in muscle mass and does not identify the same children as malnourished compared to W/H. Despite being simple, MUAC may be prone to errors, since even half a centimeter error in measurement will make a big difference in classification, so MUAC is not an accurate indicator of PEM and it cannot be used to monitor the progress of it\(^{(13,14,16)}\).

**Shakers tape:** It's used by primary health worker and WHO field worker has three colored zones:

- 13.5 - 12.5 cm  *mild*
- 11.5-12.5  *moderate*
1.4.2 Classification:

For practical purposes the two approaches, clinical and publish health, require different types of classification. The distinction between kwashiorkor and marasmus is qualitative. The assessment of subclinical PEM in the community must be quantitative and is based on weight and height\(^{(17,18)}\).

1.4.2.1 Gomez classification (1956):

Is the oldest classification which characterize weight deficit in degrees, considering (WT- for - age) < 60 as sever, 60 – 74% as moderate and 75 – 89% as mild form of malnutrition\(^{(17,18)}\).

1.4.2.2 Mclaren classification (1967):

In which the Midarm circumference for age is calculated according to Walansky standard\(^{(18)}\).

Normal   > 85
Mild malnutrition  80 - 85
Moderate malnutrition  75 - 80
Severe malnutrition  < 75

1.4.2.3 Welcome Trust classification:

According to W/A and the presence or abscence of nutritional oedma the nutritional status classified as follow\(^{(17,18)}\).
• Weight for Age < 60% without oedema is marasmus, with oedema is marasmic kwashiorkor

• Weight for Age between 60 – 80% without oedema is underweight, with oedema is kwashiorkor

1.4.2.4 Waterlow classification:

Classified stunting, which represent chronic malnutrition in to three degrees using height for age: (17,18, 19,20,21)

1- More than 95% is normal .

2- First degree 95- 90%

3- Second degree 89 -85%

4- Third degree less than 85%

Wasting which represent acute malnutrition, measured by loss of weight related to height if the W/H :

1- More than 90% there is no wasting

2- 90-80% first degree wasting .

3- 80-70% second degree wasting .

4- Less than 70% third degree wasting (19,20,21)

1.4.2.5 FAO/WHO expert committee classified PEM to five groups (7):

(1) Underweight: body weight 60-80% of standard for age without edema.
(2) Nutritional dwarfism: body weight <60% of standard for age without edema & without signs of marasmus or kwashiorkor.

(3) **Marasmus**: body weight <60% of standard for age without edema.

(4) Marasmic kwash: body weight <60% of standard for age with edema.

(5) Kwashiorkor: body weight 60-80% of standard for age with edema.

For this study the classification of PEM is according to Welcome classification (1970), the percentage of Harvard standard of weight between 60-80 with edema is defined as Kwashiorkor & <60% without edema is defined as marasmus & marasmic -kwashiorkor stands for weight <60% with edema.

**1.4.2.6 WHO Classification (Z score):**

The recommended reporting system of H/A, W/H and W/A is in terms of Z- scores— a statistical measure of the distance from the median (mean) expressed as a proportion of the standard deviation. The most common cutoff point is –2 Z-score, i.e., two standard deviations below the median values of the international reference. This is the cutoff risk level used to differentiate malnourished children from those adequately nourished. Children whose H/A, W/H and W/A scores fall below this point are therefore considered, stunted, underweight and wasted,
respectively. The WHO has proposed a classification scheme for population-level malnutrition.\(^{(13)}\)

### 1.4.3 Causes and risk factors of protein-energy malnutrition:

The cause of PEM should not however, be viewed simply in terms of inadequate intake of nutrients; the correct balance of food and nutrient must be fed at the right interval and the child must have an appetite to consume the food. There should be no conditions that prevent body cells from utilizing the nutrient or that result in abnormal losses. Factors that adversely influence any of these requisites can be causes of malnutrition and the etiology therefore can be complex\(^{(7)}\).

Certain factors that contribute to PEM in young children are related to the host, the diet and environment\(^{(22)}\). The child nutritional status is also influenced by social factors such as: poverty, lack of awareness and knowledge regarding food requirements of children and customs and beliefs.

The available data suggests that the most common causes include poor maternal nutrition at conception and in utero resulting in low birth weight, and development of nutritional marasmus in infancy\(^{(23)}\).

Failure of breast feeding because of death or separation from the mother or insufficient breast milk in case where the breast feeding is the only feasible way for mother to feed her baby adequately and prolonged breast feeding without introduction of other foods after 6 month of age is
the leading cause to PEM. The global strategy for infant and young child feeding was developed by the WHO and UNICEF in 2003 it provide comprehensive frame work for promoting appropriate feeding practice and reducing malnutrition\(^{(24,25)}\). High prevalence of malnutrition among young children is also due to famine resulting from droughts, natural disasters, war and civil disturbances.
1.5 LITERATURE REVIEW

1.5.1 Protein-Energy Malnutrition in Sudan:

Historical aspect:

Several studies were conducted to determine the prevalence of PEM in Sudanese children. The first was done by Krockill in the village of Abu Deleig in 1934\(^{(26)}\).

The percentage of PEM admitted to one pediatric unit in 1960 was 10% of total admission. In Medani hospital, one third of admitted children were found to have PEM with a mortality rate of 20%\(^{(27)}\).

Nutritional survey in Hag Yusif done by Yousif MA. In 1967, it was found that a poor socio-economic status and lack of mother education as a major factor in the etiology of PEM\(^{(28)}\).

In a study done in Eastern Sudan (1967) by Hassan MM, more than 50% of children were found to be underweight.

In a study done in 1982 by Hendrickse et al, high levels of aflatoxins were found in children with kwashiorkor\(^{(29)}\).

In a survey conducted by the Ministry of Health between 1986-1987, it was found that 32.1% of children under 5 years were stunted, 14.1% were underweight, and 1.7% having severe malnutrition\(^{(30)}\).
Anemia in Sudanese children with PEM was found to be associated with iron and folic acid deficiency in a study done in 1986 by Abdalla L\textsuperscript{(31)}.

Mineral and some trace element were studied by Ahmed and Hashim (1991) found selenium level to be low in children with the oedematous forms of PEM\textsuperscript{(32, 33)}.

Hussain (1992) found that 29% of hospitalized children with PEM to have xerophthalmia\textsuperscript{(34)}.

In 1994 the plasma level of the acute phase protein was studied in 81 children with sever PEM by Omer Suliman, he found that children with PEM have high level of Acute Phase Protein (APP)\textsuperscript{(35)}.

Ibrahim SA in 1994 studied the correlation between the laboratory findings and clinical presentation in 94 children with PEM and he found that the total plasma protein, albumin and triglyceride were significantly lower in the malnourished compared to the normal children\textsuperscript{(36)}.

Salih MA, Mohamed EF, Galagan V et al in 1999 studies plasma selenium, Hb, and PCV in 53 malnourished Sudanese children aged 6-36 months and 11 children who had tuberculosis, 12 healthy children served as control. The mean plasma selenium ranged from 0.05-0.08 in children with Kwash, marasmic-Kwash, marasmus, tuberculosis and healthy control\textsuperscript{(37)}.
In 2001 Ibtihal Siddiq evaluated Standardized Protocol Management in severely malnourished children with diarrhea\(^{(38)}\)

According to the nutrition information system in Sudan (Jan .2006) Prevalence of choronic PEM in children under 5 years was found to be 43% and acute malnutrition is 16% for north sudan while they were 43% and 21 respectively among the same age group for southern Sudan\(^{(39)}\).

1.6 Nutrient Deficiencies in PEM:

Nutritional anaemias are extremely prevalent worldwide. Unlike protein-energy malnutrition (PEM), vitamin A deficiency and iodine deficiency disorders (IDD). These anaemias occur frequently in both developing and industrialized countries. The most common cause of anaemia is a deficiency of iron, although not necessarily a dietary deficiency of total iron intake. Deficiencies of folates (or folic acid), vitamin B12 and protein may also cause anaemia. Ascorbic acid, vitamin E, copper and pyridoxine are also needed for production of red blood cells (erythrocytes). Vitamin A deficiency is also associated with anaemia.

Anaemias can be classified in numerous ways, some based on the cause of the disease and others based on the appearance of the red blood cells.
Anaemias may be classified as microcytic (having small red blood cells), macrocytic (having large red blood cells), haemolytic (having many ruptured red blood cells) or hypochromic (having pale-coloured cells with less haemoglobin). Macrocytic anaemias are often caused by folate or vitamin B12 deficiencies\(^{(40)}\).

The cut off points for definition of anaemia according to the WHO suggested criteria using haemoglobin (Hb) and haematocrit (PCV).\(^{(40)}\)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Hb below (g/dl)</th>
<th>PCV below (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children 6-60 months</td>
<td>11.0</td>
<td>33</td>
</tr>
<tr>
<td>Children 5-11 years</td>
<td>11.5</td>
<td>34</td>
</tr>
<tr>
<td>Children 12-15 years</td>
<td>12.0</td>
<td>36</td>
</tr>
</tbody>
</table>

1.6.1 Iron deficiency (ID):

Is the most widespread micronutrient deficiency in the world affecting more than a billion people. ID is a serious concern for young children. Research now indicates that iron deficiency has very important implications, including poorer learning ability and behavioural abnormalities, motor development, coordination, language development. As well as it is associated with increased morbidity from infectious diseases\(^{(41,42,43,44)}\). Children with ID are also more susceptible to poisoning from heavy metals (including lead). Children with ID have
lower ability to work hard and there was a strong association between increased risk of iron deficiency anemia and weight below the 10th centile (45) or been described as difficult to feed also IQ test showing a loss of 10 – 15 point(46).

Iron deficiency in PEM can be due to decreased intake, decreased absorption and increase loss secondary to worm infestation. Nutritional anaemias have until recently been relatively neglected and not infrequently remain undiagnosed. There are many reasons for the lack of attention, but the most important are probably that the symptoms and signs are much less obvious than in severe PEM, IDD or xerophthalmia, and that although anaemias do contribute to mortality rates they do not often do so in a dramatic way.(40)

1.6.2 Vitamin A deficiency:

Vitamin A was discovered in 1913. it was called the anti-infection vitamin because of it’s striking effect to prevent death from pneumonia and septicaemia in laboratory-animals(40)

Vitamin A (retinol) is required for the formation of rhodopsin, a photoreceptor pigment in the retina and also helps maintain epithelial tissues(4)

Vitamin A deficiency among children in developing countries remain the leading cause of preventable severe visual impairment and blindness. Xerthalmia (from the Greek word xeros meaning dry)is the
term now used to cover the eye manifestation resulting from vitamin A deficiency, measles often precipitates xerophthalmia because it leads to lower food intake and to increase metabolic demands for vitamin $A^{(40)}$.

According to WHO's micronutrient deficiency information system approximately 2.8 million children under 5 years of age currently exhibit signs of clinically xerophthalmia however over 90% of vitamin A deficiency children show 20 times greater risk of death from severe infection night blindness prevalence has been reported to be 10 -20%. Africa has the highest prevalence of vitamin A deficiency.

Recent studies highlighted the increased risk in mortality facing pregnant women who are vit A deficient and also the association of vitamin A deficiency with elevated risk of mother- to - child transmission of HIV infection.
Classification of xerophthalmia

<table>
<thead>
<tr>
<th>Ocular signs</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night blindness</td>
<td>XN</td>
</tr>
<tr>
<td>Conjunctival xerosis</td>
<td>X1A</td>
</tr>
<tr>
<td>Bitot's spots</td>
<td>X1B</td>
</tr>
<tr>
<td>Corneal xerosis</td>
<td>X2</td>
</tr>
<tr>
<td>Corneal ulceration/keratomalacia &lt;1/3 corneal surface</td>
<td>X3A</td>
</tr>
<tr>
<td>Corneal ulceration/keratomalacia &gt;1/3 corneal surface</td>
<td>X3B</td>
</tr>
<tr>
<td>Corneal scar</td>
<td>XS</td>
</tr>
<tr>
<td>Xerophthalmia fundus</td>
<td>XF</td>
</tr>
</tbody>
</table>

Vitamin A can be given to children for curative as well as preventive purposes.

1.6.3 Zinc deficiency:

Zinc is an essential nutrient in humans.

Effect of deficiency include growth retardation, diarrhea, immune deficiency, skin lesions, delayed sexual maturation and behavioral changes.

Zinc is involved in over 200 enzymes and has critical role in structured function of biomembranes. There is some evidence that zinc
deficiency may cause intrauterine growth retardation and neural tube
defect in the fetus(4).

1.6.4  Copper deficiency:

Copper is a component of many body proteins; almost all of the
body's copper is bound within copper proteins. Unbound (free) copper
ions are toxic.

Dietary deficiency rarely causes clinically significant copper
deficiency. The only reported causes are kwashiorkor, persistent infantile
diarrhea (usually associated with a diet limited to milk). Deficiency may
cause neutropenia, impaired bone calcification, and hypochromic anemia
not responsive to iron supplements. Diagnosis is based on low serum
levels of copper and ceruloplasmin(4).

1.6.5  Selenium deficiency:

Has been identified as a cause an endemic cardiomyopathy
(keshan disease ) and osteoarthropathy (Kashin – Bech disease) and
anemia( iron deficiency anemia) which does not respond to iron
supplementation(4).

1.7   Normal Iron Metabolism:

The iron status of the human body can be considered as
continuum with iron deficiency anemia at one end. Normally
approximately 73% of the body's iron is incorporated in to circulating
haemoglobin and 12% in the storage complexes of ferritin and haemosiderin 15% is incorporated into other iron-containing compounds including enzymes\textsuperscript{(44)}.

Iron is an essential element for all living cells and plays an important role in many metabolic pathways\textsuperscript{(48)}. Iron has the capacity to accept and donate electron readily, interconverting between two forms (ferric and ferrous). This makes it a useful component of cytochromes, oxygen binding molecules like haemoglobin, myoglobin and many enzymes\textsuperscript{(49)}. Iron can also damage tissues by catalising conversion of hydrogen peroxide to free radicals that attack all cell membranes\textsuperscript{(50)}.

1.7.1 Normal Iron balance:

In individuals with normal body iron, the store regulator is responsible for meeting normal iron loss as well as the additional requirement for growth\textsuperscript{(51)}.

In undeficient individuals, only enough iron to balance the daily loss is absorbed.

Requirements are higher during period of rapid growth in infancy and adolescence\textsuperscript{(52)}.

1.7.2 Iron requirement:

In the normal term infant, total body iron changes little during the four month of life. Consequently IDA in this age group is uncommon, except in the presence of gastrointestinal blood loss. The need for iron
supplementation in the first few months is therefore questionable. By four months of age neonatal iron stores have been reduced by half, and exogenous iron is required during the rapid phase of growth between 4 & 12 months. Absorption of about 0.8mg of iron per day from the diet is required, of which 0.6mg is needed for growth, and 0.2mg to replace losses.\(^{(44)}\) the reference nutrient intake for iron(mg/day) is 4.3(4-6 months) and 7.8(7-12 months)\(^{(53)}\) .

1.7.3  Iron absorption and losses:

Iron absorption depends not only on the amount of iron in the diet, but also on the bioavailability of that iron as well as the body needs\(^{(52)}\). Dietary iron exists in two forms: haem and non-haem\(^{(54)}\). Most of it is in the non-haem form which is derived from cereals, and a lesser component in the haem form which is found in haemoglobin and myoglobin in red meat and organs. Haem iron is more readily absorbed than non-haem iron. Absorption of non-haem iron is increased by ascorbic acid and citric acid. Food rich in tannins such as tea, phytates and polyphenoles inhibits iron absorption. Absorption of iron occurs in the proximal duodenum\(^{(55)}\).

The absorption of iron is regulated in several ways:

a) By the amount of iron recently consumed in the diet; a mechanism referred to as dietary regulation.
b) By mechanism that responds to total body iron; known as store regulation.

c) By erythropoietic regulation which responds to erythropoietic requirements only and has a greater capacity to increased iron absorption\(^{(50,56)}\).

Massa et al \(^{(56)}\) suggested that a transitory abnormality in iron absorption may be present in infantile malnutrition, a suggestion based on the increase in Fe in serum after oral administration of the isotope.\(^{(56)}\) However, iron absorption has been found to be unaffected in protein depleted rats \(^{(57)}\), and an earlier study of iron absorption by iron-deficient malnourished subjects had failed to provide evidence of decreased iron absorption \(^{(58)}\).

1.7.4 Iron transport:

Once it enters the circulation, iron is complexed and transported by transferrin to different tissues. Transferrin is a pink glycoprotein with an electrophoretic mobility of betaglobulin.\(^{(59)}\) It is synthesised in various tissues but mainly in the liver.\(^{(60)}\) Its plasma concentration is about 230 mg/dl,\(^{(61)}\) and its half disappearance time is about 10 days.\(^{(60)}\) In addition to iron, transferrin is also involved in the transport of other minerals such as aluminium, copper, magnesium and cadmium. Affinity of transferrin for iron is PH-dependent\(^{(62)}\) which stabilizes ferric iron and prevents
formation of free radicals. Cellular iron uptake is mediated by transferrin-receptors (TfRs) which are present virtually in all mammalian cells\textsuperscript{(63)}.

1.7.5 \textbf{Iron storage:}

The level of body iron stores is affected both by dietary intake and by the physiological need of iron for erythropoiesis\textsuperscript{(64)}. Iron is primarily stored in tissue as ferritin or haemosiderin\textsuperscript{(65)}.

\textbf{1.7.5.1 Ferritin:}

Ferritin protein is an iron-containing spherical rhombic dodecahedron protein of 24 repeating subunits with molecular weight of approximately 460,000\textsuperscript{(66)}.

Ferritin stores the excessive cytosolic iron and subsequently, donates the stored for iron cellular needs. Each Ferritin molecule accumulates up to 4500 iron atoms\textsuperscript{(66)}.

A new type of feritin subunit in human cells was recently discovered. This feritin was called mitochondrial feritin, and was expressed as a precursor target to mitochondria where its processed into functional protein exhibition structural and functional properties similar to those observed in cytoplasmic feritin\textsuperscript{(62)}.

\textbf{1.7.5.2 Haemosiderin:}

It is water insoluble non-crystalline protein – iron complex\textsuperscript{(52)}. 
In normal subjects, the majority of storage iron is present as feritin, and haemosiderin is predominantly found in the macrophages rather than hepatocytes\(^{(52)}\).

1.8 Diagnostic Methods for Assessment of Body Iron Status:

In order to assess body iron status, the three main compartments of iron: storage iron, transport iron, and functional iron compartment, particularly hemoglobin in red cells, should be considered\(^{(52)}\).

1.8.1 Assessment of iron storage:

1.8.1.1 Serum ferritin:

In normal people < the serum ferritin concentration correlates with hepatic and macrophage iron stores, assessed by quantitative phlebotomy or tissue biopsy \(^{(52)}\). In a recent study, the conventional reference range for ferritin was found to be considerably lower than the optimal decision limit for iron deficiency, these value have generally been calculated of haemoglobin values only, this is, however, an inadequate measure to exclude the effect of latent iron deficiency on the reference value of ferritin, because anaemia is know to develop as a late manifestation of iron deficiency \(^{(67)}\). Measurement of serum ferritin level is generally accepted as the best non-invasive means to determine body iron stores, but only if serum level of ferritin and serum level of iron ran the same direction\(^{(64)}\).
Quantitated serum ferritin measured using antibody to ferritin protein does not reflect iron content of the ferritin, serum ferritin is an acute phase reactant, and a proferritin (a ferritin protein with almost no iron in it, and not in equilibrium with body stores), is elevated in any inflammatory state such as infection, rheumatoid arthritis, hepatitis and cancer, due in part to interleukin enhancing the translation of apoprotein mRNA\(^{(66)}\).

Elevation of serum ferritin levels may occur without elevation of iron stores. Serum ferritin levels \(>400\text{ug/l}\) defines iron overload in most clinical laboratories, but in fact, such interpretation require confirmation by finding high percentage of saturation of iron binding capacity, thus, high serum ferritin accompanied by high percentage of saturation of serum transferrin usually indicate iron overload. However, high serum ferritin accompanied by a percentage saturation of transferrin \(<45\) usually indicated that inflammation caused the the high ferritin\(^{(66)}\).

Serum ferritin \(<12\text{ug/l}\) is diagnostic of iron deficiency, but it's sensitivity is compromised in the presence of inflammation\(^{(66)}\).

Although serum ferritin has high specificity for iron deficiency especially when combined with other markers such as Hb, the test is expensive and has limited available in a clinic setting, therefore it is not used commonly for screening addition to that it can be elevated in the setting of inflammation, chronic infection and other diseases\(^{(68)}\).
1.8.1.2 **Tissue biopsy:**

Liver biopsy (in diagnosis of iron overload) or bone marrow biopsy (in the differential diagnoses of iron deficiency) allows the direct examination of iron by histological staining\(^{(66)}\).

Stainable marrow is the reference standard for iron deficiency, although the absence of iron is not absolute proof of iron deficiency, the presence of stainable marrow is the reference standard for iron deficiency. The presence of stainable marrow iron reliably excludes iron deficiency\(^{(65)}\). Bone marrow examination shows normal iron despite actual iron deficiency when megaloblastic anemia is present\(^{(66)}\). However, examination of stained aspirate of bone marrow for haemosiderin is still considered the gold standard method for evaluation of iron status, this technique is invasive and not suitable for screening purposes\(^{(65)}\).

Liver biopsy is the gold standard for quantifying iron patient with iron overload\(^{(50)}\).

1.8.2 **Assessment of iron transport compartment:**

1.8.2.1 **Serum iron:**

Serum iron concentration can be measured directly, and generally decreases as iron stores depleted. However, serum iron may not reflect iron stores accurately because it's influenced by several factors including; iron absorption from meals, infections, inflammations and diurnal
variation (68). Morning levels are generally assumed to be higher than after noon or evening levels (69).

Circulating iron, bound to transferrin, comprises only a very small amount (0.01%) of the total body iron and has a very high turnover rate of 10 – 20 times /day in normal subject, these factor contribute to the variability of measurement encountered and severely limit the diagnostic usefulness of an individual serum iron measurement (70).

1.8.2.2 Total iron binding capacity (TIBC):

TIBC indirectly measures transferrin levels, which increase as serum iron concentration and stored iron decreased (68).

It is more stable measurement than serum iron. Unfortunately this test is also affected by factors other than iron status (malnutrition, inflammation, infection and cancer) (68). Saturation level less than 16% reflects inadequate transport iron to sustain erythropoiesis (70).

1.8.2.3 Transferrin Receptors (TfRs):

In the human body, TfRs can be found in two forms: the cell surface bound form, which can be detected by cytometry using monoclonal antibody against CD71, and the circulating form in serum, which can be detected by immunoassay (48).

The Serum TfRs concentration, in contrast to serum ferritin, provides direct information about any deficit in the adequacy of iron supply to erythropoiesis (71). Recent studies have shown that the
measurement of serum TfRs is dependable test for the diagnosis of iron deficiency and is useful for the differentiation of iron deficiency anaemia from the anaemia of chronic disease and inflammation\textsuperscript{(48)}.

TfRs are unaffected by underlining acute or chronic inflammation. Therefore, serum TfRs measurement is particularly promising for evaluation of iron status when iron deficiency is simultaneously present with infection or inflammation\textsuperscript{(72)}. Serum TfRs is elevated earlier than erythrocyte protoporphyrin or reduction in MCV\textsuperscript{(73)}.

The combination of serum TfRs and serum ferritin provide complete information about storage and functional iron compartment, using this combination along with Hb concentration, it is possible to define the iron statues completely but it is use limit because limitation in methodology and definition of reference range\textsuperscript{(71)}.

1.8.2.4 \textbf{Red cell protoporphyrin:}

Zinc protoporphyrin (ZPP) is synthesized by developing erythrocytes, when there is insufficient iron available for haem production\textsuperscript{(70)}.

ZPP can be measured easily and cheaply using haemoflurometry with the laboratory cut off point off 1.2mol\,l\textsuperscript{-1}.

Values are standardized by expressing the result as the ratio to heme\textsuperscript{(70)}. ZPP \, heme reflect and status during hemoglobin synthesis and detect iron deficiency before the onset of anaemia\textsuperscript{(68)}. 

45
1.8.2.5 Red cell ferritin:

Red cell ferritin content reflects changes occurring in tissues both in iron deficiency and iron overload\(^{(74)}\).

1.8.2.6 Percentage of hypochromic red cells:

As iron supply to the erythron diminishes, the new red cells produced are increasingly hypochromatic. Assessment of the haemoglobin content of individual red cells, which is possible using some automated cell counter allows measurement of the percentage of hypochromic cells. Value of >10% may help in the early identification of impaired iron supply.\(^{(52)}\)

1.8.3 Assessment of function compartment:

Function iron is present in red blood cells as haemoglobin, available for oxygen storage in tissues myoglobin, and for cell aerobic metabolism in cytochromes\(^{(66)}\).

1.8.3.1 Red cell hemoglobin:

Reduced amounts of hemoglobin accompany an overall reduction in body iron or acute blood loss\(^{(52)}\).

Anaemia is diagnosed when there is reduction of haemoglobin in grams per 100 ml to at least two standard deviation below the means, adjusted for age, sex and altitude of residence\(^{(66)}\). Based on the world health organization (WHO) definition anaemia is considered when
haemoglobin is less than 11g\dl for children age 0-4 years, and haemoglobin less than 12g\dl for children age 5-12 years (75).

1.8.3.2 Definition and measurement of red cell indices:

1.8.3.2.1 Mean corpuscular volume (MCV):

It is the average volume of red blood cell expressed in femtoliter (cubic micrometer) (76). The measurement of MCV uses the average size in the Hct. It is calculated by dividing the Hct value by the RBC count (77), the normal Mcv value Is 76- 86 fl (78).

1.8.3.2.2 Mean corpuscular haemoglobin (MCH):

It is average content of haemoglobin per red blood cells expressed in picogram per cell reference value are 27-31pg (79).

1.8.3.2.3 Mean Corpuscular Haemoglobin Concentration (MCHC):

It is average concentration of haemoglobin in agiven volume of packed red blood cells expressed in g\dl (76).

The MCHC is calculated by dividing the Hb value by the Hct value, reference value are 32- 36g\l by manual methods or 31-37g\l by coulter counter (79).

The Mcv is the most useful index, it enables the classification of anaemia by red cell size as microcytic or macrocytic (80) it is useful as first step in approaching the itiologic diagnosis of anaemia (78).
MCHC is more frequently reduced in iron deficiency anaemia than with other causes microcytosis \(^{(81)}\).

MCHC most useful methods for detecting erythrocyte cellular dehydration \(^{(82)}\).

1.9 Anaemia In Protein – Energy Malnutrition:

There are three mechanisms which may be responsible for anaemia in protein malnutrition.

The First mechanism is deficiency of vital haemopoietic raw material (factor deficiency anaemia). The most common causes are iron deficiency, deficiency of folic acid, vitamins "B" subgroup which have been incriminated in many of the features of kwashiorkor such as cheilosis and angular stomatitis, riboflavin deficiency is suggested as the cause of oral and mucosal changes in malnourished children, vitamin "E" appears to act by maintaining the stability of the membrane \(^{(83,84)}\). Deficiency may cause anaemia which is hemolytic in type.

Other deficiency of trace element such as copper, selenium, and zinc can also be a cause of anaemia in PEM.

The second mechanism is failure for blood forming organ to produce or to deliver mature RBC. To the peripheral blood (production deficiency anaemia). Commonly the bone marrow shows some degree of hypoplasia in PEM \(^{(83,85)}\).
The third mechanism is RBCs loss from peripheral blood (depletion anaemia) due to hook worms infestation, malaria an infections\(^{(58)}\).

In addition to these mechanisms hemodilution due to oedema is also suggested as the cause of anaemia in oedematous form of malnutrition.

Almost every morphological type has been described in blood smear.

Anaemia of chronic disease also can explained anaemia in chronic PEM although there are deferent pathways contribute to the anaemia of chronic disease such as diversion of iron traffic, diminished erythropoiesis and blunted response to erythropoietin.

Proinflammotory and anti inflammatory cytokines, acute phase protein and free radicals are predominately involved as causing disturbances of iron homeostasis\(^{(44)}\). Antinflmmotory cytokines IL\(_4\), IL\(_{10}\) and IL\(_3\) modulate iron metabolism by two pathways, first, by opposing interferon (IFN) – mediated activation of iron regulatory protein (IRP) there by increasing ferritin translation and second by augmentating transferrin receptor mRNA expression most likely by reversing the inhibitory effect of "IFN" on transferring receptor\(^{(86)}\).
Overall, anaemia of chronic disease seems to be the product of activated immune system using defense strategy of withholding iron and essential growth factor for invading pathogen, while increasing the efficiency of cell mediated immunity\(^{(87)}\).

### 1.10 Hazards of Iron Overload:

Although iron deficiency is a serious condition, excessive iron also can contribute to disease processes in several ways. Excessive iron in certain tissues and cells can hinder the ability of proteins such as transferring and ferritin to prevent accretion of free iron. Moreover, in infectious diseases and inflammatory processes that involve ischemia and reperfusion, iron causes reactions that produce superoxide radicals\(^{(88)}\). Non-protein-bound ferric irons are reduced by superoxides and the ferrous product is reoxydised by peroxides to regenerate ferric irons and yield hydroxyl radicals which attack all classes of biologic macromolecules. The resultant depolymerisation of polysaccharides causes DNA strands breaks, inactivates enzymes and initiates lipid peroxydation\(^{(88,89)}\). Iron can also increase disease risk by functioning as a readily available nutrient for the invading microbes and neoplastic cells. To survive and replicate in hosts, microbial pathogens must acquire host iron. In such cases even microbial strains that are not ordinarily dangerous can cause
illness. Markedly invasive neoplastic cells glean host iron more easily than less malignant and normal cells\textsuperscript{(88,89)}. 
Protein energy malnutrition and iron deficiency anaemia are major nutritional problems in Sudan.

Micronutrient including iron is used in management of malnutrition; excess iron may predispose to sepsis and increase morbidity.

No similar study was done in children with severe PEM in Sudan.
OBJECTIVES

- To study iron status in children suffering from severe protein energy malnutrition. And to compare the iron status among different form of PEM.
- To relate iron status of children with severe PEM malnutrition to nutritional feeding habits, other clinical sign of micronutrient deficiencies and co-morbidities.
Chapter Two

2- PATIENT AND METHODS

2.1 Study Design:

Descriptive cross-sectional hospital based case study.

2.2 Study Area:

The study was conducted in Gaffer Ibnouf Children’s Hospital, dept. of paediatric in Khartoum Teaching Hospital and Omdurman Children Teaching Hospital.

2.3 Duration of the Study:

Data were collected in five months duration From Sep. 2006 to February 2007.

2.4 Study Population:

The study populations were severely malnourished children aged 6-60 months admitted in above mention hospitals .Patients were diagnosed severely malnourished and classified according to the Welcome classification (17'18).and the control was chosen from out-patient clinic ' the patients who were well nourished and had no clinical signs of anaemia and they were not on iron therapy.
2.5 **Sample Size:**

Sample size was determined according to the following formula and found to be 104 patients (74 malnourished and 30 controls).

\[ n = \frac{z^2 \cdot pq}{d^2} \]

- **n** = Sample size
- **z** = Statistical certainty = 1.96 at 95% confidence interval
- **p** = Prevalence
- **q** = Probability of failure (1-p)
- **d** = Designed margin of error

2.6 **Inclusion Criteria:**

Children between 6 months and 5 years with severe PEM classified as above.

2.7 **Exclusion Criteria:**

2.7.1 Age less than 6 months or more than 5 years.

2.7.2 Children with non-severe forms of PEM or failure to thrive due to non-nutritional causes.

2.7.3 Patient already on iron supplement.

2.7.4 Patient with congenital hemolytic anaemias.

2.7.5 Patient who received blood transfusion

2.7.6 Those who refuse to be enrolled in the study.
2.8 Ethical Approval:

2.8.1 Written approval will be obtained from hospital administrators.

2.8.2 Informed consent will be taken from the parents or caretakers of children, after they were briefed about the aims and methods of the study.

2.9 Study Tools:

2.9.1 Questionnaire:

A standardized questionnaire was used to obtain information concerning the personal data, anthropometric measurement, medical history, family history, sociodemographic history and detail review of nutritional history and feeding habits together with clinical signs of malnutrition and IDA.

2.9.2 Clinical examination:

Every child will be subject to a through clinical examination including general checkup for: signs of PEM associated with nutritional deficiencies and systemic examination for complication of other systems eralized, bipedal oedema, hair changes, skin changes.
2.9.3 Anthropometry:

2.9.3.1 Weight:

The children were weighted with light dresses and without shoes, the wt was measured in kilograms and the reading should be taken to the nearest 0.1kg and compared to the international NCHS standards, using standard scales for older children and baby scales for infants.

2.9.3.2 Length and height:

Length: was measured with the child lying on a flat wooden table with the head within the same horizontal plane of the feet and the body straight, the feet will be perpendicular to the table. Length was read to nearest 0.1 cm with a non-stretchable tape.

Height: The height was measured with the child bared foot, standing with heels and back in contact with an upright wall using height stadiometer, the reading taking to nearest 0.1 Cm, the height was compared to the NCHS standard.

2.8.4 Investigation:

Samples:

5 mls of blood were taken by the author from a peripheral vein.

2.5 mls were added into a bottle containing EDTA for measurement of hematological values(hemoglobin and red cell indices).
2.5 mls where collected as clotted blood to obtain 2 mls of serum, which was stored in cryo – tubes at – 20 degree centigrade for measuring the biochemical values including:

1. Serum iron
2. Total iron binding capacity (TIBC).
3. Serum ferritin.

Hb and red cell indices were measured by automatic blood counter (Sysmex Kx - 21).

**Instruments:**

**Sysmex Kx – 21:**

This is an automated multi – parameter blood cell counter for in vitro diagnostic use in clinical laboratories.

The Sysmex Kx-21 processes approximately 60 samples an hour and display on the LCD screen the particle distribution curve of WBC, RBC and platelets, along with data of 18 parameters as analytical results.

The Sysmex Kx-21 employs three detector block using the DC detecting method. The RBC count and platelets are taken by the RBC detector block also using the DC detection method. The Hb detector block measures the Hb concentration using a non – cyanide hemoglobin method.
MCV is measures directly, while MCH and MCHC are obtained by calculation. MCH is calculated by HB/RBC, and MCHC is calculated by Hb/Hct.

**BTS 370 plus (biosystem):**

This is a computer controlled, bench-top instrument designed to perform spectrometric measurements at predetermined wavelengths of analyte concentration and enzyme activity using various reagents.

Any combination of tests can be performed, on up to 60 samples per work list. The analyzer automatically performs all reagent and sample pipetting, incubations measurement and calculations.

**Principles of Methods:**

**Serum iron:**

**Principle of methods:**

Transferrin bound ferric iron in the sample is released by guanidinium and reduce to ferrous by means of hydroxylamine. Ferrous iron reacts with ferrozine forming a colour complex that can be measured by spectrophotometry.

**Iron binding capacity:**

Precipitation/spectrophotometric (Biosystem)

**Principle of method:**

An excess ferric iron is added to the sample to saturate serum transferrin. Uncomplex ferric iron is precipitated with magnesium
hydroxide carbonate and the iron bound to protein in the supernatant is then spectrophotometrically measured.

**Serum Ferritin:**

Immulyte – ferritin is an immunometric assay for the quantitative determination of ferritin in human serum.

**Principle of test:**

Specific anti-ferritin antibodies are coated on to micro – titration wells. Test sera are applied, then monoclonal anti-ferritin labeled with horse radish peroxidase enzyme (conjugate) is added. If human ferritin is present in the sample, it will combine with antibody on the well and the enzyme conjugate, resulting in the ferritin molecules being sandwiched between the solid phase and the enzyme linked antibodies.

After 40 minutes incubation at 37°C, the wells are washed with water to remove unbound antibodies. On addition of substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the present of ferritin.

The reaction is stopped by addition of dilute hypochloric acid and the absorbance is then measured at 450 nm.

The concentration of ferritin is directly proportional to the colour intensity of the test sample.
**Definition of iron status:**

a) Iron sufficient if haemoglobin more or = 110gm/L, s. ferritin ≥ 12 ug/L, serum iron more than or equal to 8 microgram/L and iron saturation more than or equal 12%.

b) Non-anemic iron deficiency if Hb is more than 110gm/L. S. ferritin less than 12 microgram/L serum iron less than 8 microgram/L iron saturation less than 12%.

c) Iron deficiency anemia, if Hb less than 110 gm/L and other iron studies less than normal\(^{40}\).

**Statistics:**

Data was analyzed by using SPSS (Statistical Package for Social Science) computer program.

**Research team:**

- Author.
- Biochemist.
- Lab technician.
- Statistician.
- State author contributor to work.
Chapter Three

3- RESULTS

3.1 Distribution of Children with Severe PEM According to Classification:

Classification of 74 children with severe malnutrition according to Wellcome Trust classification is shown in (Fig. 1).

3-2 Demographic Characteristics of Children in The Study:

3.2.1 Age:

Almost half of the children in the study group were in the age group 1-2 years compared to 40% of the control whereas 24.3% and 33.3% respectively were more than 2 years in the study and control group. Infants less than 1 years were in 27.7% in study group compared to 26.7% in the control, the differences are statistically not significant. (P value > 0.05) this is shown in (Fig. 2).

The sex distribution of the study and control group was shown in (Fig. 3).

The total male to female ratio was 1.9:1 in the study group compared to 1.1:1 in the control group (P value > 0.05).
3.2.2 Geographic location origin:

The majority of children in study were from the Western states of the Sudan, they were 38 (51.4%) in the study group and 8(26.7%) in the control followed by central Sudan 16 (21.6%) in study group and 8 (26.7%) in the control, representation from South states was 12(16.2%) in the study group whereas in the control was 2(6.2%). The Northern tribes were dominating in the control group they were 12(40%) children whereas the Eastern tribes were absent in both study and control group .(P value <0.05) (Fig. 4).

3.3 Feeding Practices:

Breast feeding:
3.3.1 Exclusive breast feeding was found in (89.1%) of the study group compared to (91.3%) of the control (P >0.05 ). (Fig. 5 ). Sudden weaning was observed in 40 (54%) in the study group whereas in the control group no children were weaned suddenly while gradual weaning was observed in 18 (60%). The difference is statistically significant (P value <0.05). (Fig. 6 ).

3.3.2 Pattern of supplementary feeding:

Adequate supplementary feeding was found in only 9 (12.1%) of the study group compared to 21 (70%) of the control .(P value <0.05 ) . This is shown in (Fig. 7)
3.3.3 Meal frequency / day:

All compared children in the control group had more than three meals /day to 21.6% of children in the study group. (P value <0.05) which is statistically significant, this is shown in (Fig. 8).

3.3.4 Frequency of meat intake /week:

Eating meat every day was observed in 16 (21.6%) of the study group compared to 20 (66.7%) of the control while 20 (27.02%) of the study group had meat twice a week and not eating meat at all was observed in 38 (51.1%) of the children in the study group whereas no children in the control group had not eat meat at all. (P value <0.05) (Fig. 9).

3.4 The Relevant Social History of The Study Group:

3.4.1 The education level of fathers:

There was relatively low education level of fathers and mothers in the study group compared to the control. there were thirty (40.5%) of fathers in the study group
had khalwa (quranic school) compared to 10% in the control group School. Only 4 (5.4%) of fathers had secondary school education compared to 46.7% in the control group.
Education level of mothers in both study and control groups was low, there were 81% of illiterate mothers in the study group while 66.7% of mothers in the control group had primary school education compared to 12 (16.2%) in the study group. (Fig. 10, 11)

3.4.2 Parents occupation and family income:

There were 8.1% of the group unemployed and 78.4% unskilled laborers in the study group compared to 26% unskilled laborer and 46.7% with civil or military jobs in the control whereas 97.2% unemployed mothers were observed in the control compared to 66.7% in the control. (Fig. 12, 13) shows that.

Family income was low in both groups, 32.4% of the study group their income was below 100.000 /month pounds, and in more than half families 54% was ranging from 100.000-300.000 pounds. (Fig. 14)
3.4.3 Family size in the:

The family members more than 6 was found in 60% of study group compared to 53.3% of the control group. The difference is statistically not significant (Fig. 15).

3.5 Immunization Status:

The vaccination coverage in the study group was 51.4% compared to 86.6% of the control. There were 32.4% partially immunized and 16.2% not vaccinated at all in the study group, where partial immunized in the control group were 13.3% (V value < 0.05).

3.6 Clinical Presentation:

3.6.1 Symptoms:

Commonly observed symptoms in the study group were poor weight gain in 86.5%, whereas loss of appetite and pica were observed in 59.9% and 8.1% respectively (Fig. 16).

3.6.2 Clinical signs in children:

The most frequently encountered sign was pallor, it was found in 81%, skin changes was found in 39.21%, tachycardia and angular stomatitis were found in 35.1%, smooth tongue in 27%, abdominal distention was found in 24.3%, nail changes were found in 12.2%, hepatomegaly was observed in 10.8%, splenomegally and ocular signs of vitamin A deficiency was found in 2.7% (Fig. 17).
3.7 Investigations:

3.7.1 Haemoglobin:

Low level of Hb was found in 86.5% of the study group compared to 20% of the control (P value < 0.05) (Fig. 18).

Hb level in different groups of PEM was shown in (Table 1) low Hb level was found in 92.3% of kwashiorkor, 82.4% of marasmus and 85.7% of marasmic kwashiorkor, the difference are not statistically significant (P value 0.533)
The Hb levels in relation to demographic characteristics of children is shown in table (2). 70% of children under the age of 1 year were anaemic compared to 88.9% in the age group 1 - 2 year and all patient more than 2 years were anaemic (p value 0.022 ) (Table 2).

79.2% of males were found to be anaemic compared to all females in the study group (P value 0.009). (Table 3)

The relation of the Hb levels to nutritional practice of children with PEM was shown in table 3 Low Hb level was found in 84.8% of exclusively breast – fed and 8% children with mixed feeding. Low Hb also was found in 80% children who were weaned suddenly compared to 10% who weaned gradually the difference is statistically significantly (Pvalue <0.05). All children who breast – fed more than 2 years were anaemic . (P value 0.192) .
The frequency of eating meat/week was shown in (Table 4). The Hb levels were low in 75% of the group who eat meat every day compared to 84.2% of patient who did not eat meat at all, the differences is statistically significant (P value 0.024), and all patient who eat meat twice/week were anaemic. (Table 4)

The correlation between Hb level and clinical signs was shown in (Table 5). Low level was found in all children who were presented with pallor p value (0.00), nail changes (P value 0.021) smooth tongue (p value 0.026) jaundice (p value 0.028) Or cheilosis (p value 0.026), 84.6% of patients who presented with angular stomatitis (p value 0.026) and 91.3% of patients who presented with skin changes (p value 0.116) and 92% of patients who presented with hair changes (P value 0.0)

3.7.2 Red cell indices:

The MCV values was low in 68 (91.8%) of the study group compared to 24 (80%) of the control (P value >0.05) (Figure 19).

The MCV values in different groups of PEM were low 22 (84.6%) of children with kwashiorkor had low MCV compare to 31 (91%) of marasmic and all patient with marasmic kwashiorkor, the difference are not statistically significant (P value = 0.191) as shown in table 1.

The MCH values were low in 64 (86.5%) of the study group compared to 18 (60%) of the control (P value 0.05). (Fig 19)
The MCH is low in different group of PEM

Low MCH values were found in 26 (80.8%) of kwashiorkor, in 31 (91.1%) of marasmic patients and 12 (85.7%) of marasmic-kwashiorkor with no significant difference (p value= 0.917), as shown in table 1.

The MCHC values were significantly low in 64 (86.4%) in the study group compared to 18 (60%) of the control (P value < 0.05) as shown in figure 19.

The values of MCHC were low in 22 (84.6%) of kwashiorkor, 30 (88.2%) of marasmas and 12 (85%) of marasmic kwashiorkor the difference are not statistically significant (P value= 0.917), as shown in table 1.

3.7.3 Iron studies:

Low level of s–iron was founding 68 (91.8%) of study group compared to 13.3% of the control the difference is highly significant statistically (P0.05) (Fig. 20).
The low level of s-iron was found in 20 (76.9%) of kwashiorkor, 28 (82.4%) of marasmus and 12 (85.7%) of marasmic kwashiorkor the difference between the groups is statistically not significant. (P value 0.769) (Table 6).
While low serum ferritin observed in 10 (13.5%) in the study group compare to the control, normal level was found in 54 (72.9%) of the study group compared to 18 (60%) of the control, the difference is statistically significant (P value > 0.05) (Fig. 21).

All children with marasmic-kwashiorkor had normal ferritin level compared to 18 (69.2%) of kwashiorkor and 22 (64.7%) of marasmic children, whereas low or high levels were found in 4 (15.4%) and 6(17.6%) of kwashiorkor and marasmus respectively which is statistically significant (P value 0.039) as shown in Table 6.

Figure 22 shows the TIBC in the study and control groups:

56 (75.6%) in study group had normal or high level of TIBC and 18(24.4%) had low level. The difference is statistically significant (P value<0.05).

Low level was found in 4 (15.4%) of kwashiorkor, compared to 2(14.3%) of marasmic-kwashiorkor and 12 (35.5%) of marasmic children, the difference is statistically significant. High levels were found in 6(23.1%) of kwashiorkor compared to 16 (47.9%) of marasmic and 6 42.9% of marasmic-kwashiorkor and normal levels were found in 16 (61.5%) of kwashiorkor compared to 16 (47.1%) of marasmic and 6 (42.9%) of marasmic – kwashiorkor, the difference is statistically significant (P value 0.011) as shown in Table 6.
Transferrin saturation low levels were found in 53(71.6%) of the study group compared to 4 (13.3 %) of the control , normal levels were found in 12(16.2%) of study group compared to(8) 26.7% of the control and high levels were observed in 9 (12.2%) in study group compared to 18 (60%) of the control (P value <0.05) (Fig. 23)

(Table :5) comparing transferrin saturation in different subtypes of PEM ,low levels were found in 18(69.2%) of kwashiorkor, 22(64.7%) of marasmus and 12(85.7%) of marasmic- kwashiorkor and normal levels were found in 4 (15.4%) of kwashiorkor compared to 6 (17.6%) marasmus and 2 (14.3%) of marasmic-kwashiorkor . High levels were found in 4 (15.4%) and 6 (17.6%) of kwashiorkor and marasmus respectively . (P value = 0.541).

Correlation coefficients range in value from -1(negative relationship) and+1 (positive relationship) a value of 0 indicates no linear relationship .
There was correlation between Hb% and transferrin saturation (Pearson correlation 0.047) and strong correlation with Hb% level to MCV. (Pearson correlation 0.566).

There is also correlation between MCV, MCH and MCHC (Pearson correlation ranging from 0.566-0.760) s-iron is well correlated with MCV, MCH and MCHC (Pearson correlation were 0.236, 0.876 and 0.181 respectively).
s-iron correlates well with TIBC and transferrin saturation (Pearson correlation 0.003 and 0.636).

s-ferritin was not correlate with s-iron or Hb %level (Pearson correlation -0.18 and 0).

The mean values of iron status among study group and control group were calculated using Independent Sample T test, difference between means is statistically significant if (Pvalue <0.05).

The mean of Hb% in study group was 7.9% compared to 12.06 % in the control (P value 0.00)].

The MCV mean value was 59.7, MCH 25.2 and MCHC 27.4 compared to 68.2 , 23.8 and 31.8 respectively in the control group (P value 0.0).

The MCHC mean was 27 compared to 31.8 in the control group (P value 0.0).

The S-iron mean value was 4.29 and the mean value of s-ferritin was 168 in the study group compared to 16.3 and 13.5 in the control respectively. (P value 0.00) as shown in table 5.

The mean of TIBC in the study group was 62 and the mean value of transferrin saturation was 10.23 compared to 67.3 and 25 in the control group respectively. (P value 0.284). as shown in table 5.

The relation of s-ferritin level to demographic characteristics of children with severe PEM is shown in (Table 7, 8).
In the age group <1 year low level s- ferritin was found in 4 (20%), and normal in 16 (80%), whereas low level was found 4 (11.1%), normal in 24 (66.7%) and high in 8 (22.2%) in the age group 1-2 years. In the age group > 2 years the low level was observed in 2 (11.1%), normal in 14 (77.8%) and high levels in 2 (11.1%) (P value 0.08). as shown in table 5.
The s-ferritin was low in 20.8% of males, normal in 62.5% and high in 16.7% whereas in females low levels were found in 92.3% and high levels in 7.7% (P value 0.003), as shown in table 7.
Chapter Four

4- DISCUSSION

Malnutrition is a major public health problem the study was designed to assess iron status in children with severe PEM.

104 children were included in the study out of whom 74 were severely malnourished. 46% of these were marasmic, 35% had kwashiorkor and 19% were marasmic – kwashiorkor. Subtypes in this study were different from the groups in previous studies were the percentage of kwashiorkor was less than marasmic kwashiorkor in the group studied by Omer 34.7% were marasmic –kwashiorkor whereas kwashiorkor were 18.4%, this may be due to seasonal variation in the collection of the data. In this study the data was collected in the rainy seasons, where the prevalence of kwashiorkor was suggested to be higher Coulter et al in 1988 in study done in Khartoum teaching hospital in period of 2 years 416 children with PEM were included, 158 out of whom were marasmic, 145 with kwashiorkor and 113 were marasmic – kwashiorkor they found that peak incidence of kwashiorkor was in the wet and post-wet seasons (90).

More than half children in our study were below the age 2 years this was comparable to figures given by Ibtihal (38). in 2001 were 62.7%
of children were found in this age group, the effect of inadequate feeding practices, sudden weaning and recurrent infection are contributors as risk factors for PEM in this age group.

More than half of children in study were from the western states whereas predominating southern tribes in previous studies, because a lot of children and their families were victims of Darfur conflict due to family instability and deterioration in health and nutrition status in the last decade.

In this study sudden and late weaning were major contributors for the development of PEM and ID this was also observed by Sultan in study done in 2003 in 50 Pakistani children were the late weaning was found to be the major predictor for iron deficiency in 1-2 years old children\(^91\).

In this study the majority of mothers exclusively fed their children from (0-4 month) this is not comparable to the results of national survey in 2001 (MICS)\(^92\) where only 17% of mothers exclusively breast fed (0-3 month) this can explain improvement in the nutritional education.

In the present study anaemia was observed in all children on mixed feeding compared to 84% of those who were exclusively breast-fed this may be due to the early introduction of inappropriate breast milk substitutes with low iron bioavailability such as non-iron fortified – formula and cow milk. Anaemia was also observed in children who
breast fed more than 2 years, iron in the breast milk is well absorbed but the concentration is only modest, unfortunately breast feeding with out complementing with iron–rich food becomes an increasing risk factor for ID for second 6 month of life. In 2004 Soha et al evaluated risk factors for lower iron stores in 323 New Zealanders, 6-24 months-old found that consumption of more than 500 ml of cow milk per day associated with 25% decrease in s-ferritin\textsuperscript{(93)}.

Low education level of parents and poor family income were considered as the main risk factors of PEM and associated nutritional deficiencies including iron in this study which is comparable to previous studies. Hayat.A.O. in 1983 studied the risk factors for PEM in Sudanese children. She found low family income, poverty and illiteracy of mothers were the major risk factors\textsuperscript{(94)}. Another study (Coulter et al)\textsuperscript{(90)} evaluated socioeconomic factors and family background in children with PEM admitted to Khartoum hospital over 2 years period they found that mothers of malnourished children had poorer housing, lower income and less education than in the control\textsuperscript{(90)}.

In this study the immunization coverage of children was 51.3% in the study group whereas in the control was 86.7 this can explain by the fact that the most of the malnourished children were from periurban areas for the displaced families.
In this study anaemia was found in all children in the age group more than 2 years, this is explained by that, the late infancy and early childhood are high risk period for iron deficiency anemia because of an increase iron requirement related to rapid growth and diet are relatively low in iron content\(^{105}\). And the fact that most of children in the study group were having less than 3 meals with meat offer less than twice /week.

In this study 64 (86.5%) of children in study group had low Hb out of them 38 (79.2%) were males and 26 (100%) were females this indicate that all females in the study group were anemic. For cultural and social reasons females are more subjected to poor nutrition The socio-economic factors were studied in 66 Kenyan children with PEM by Ayoya SO et al they found the female gender, poverty and single mother were the risk factors for PEM\(^{95}\), Soha P et al evaluated risk factors for ID\(^{93}\), they found that girls are more affected than boys although in other studies no sex differences.

In the present study 60 (81%) of children with protein – energy malnutrition showed low level of serum iron. The high maternal illiteracy which observed in 67.6% of mothers in the study group, low family income which observed in 86.5% families, in addition to infection which was found in the majority of children may contributed to inadequate feeding habit and poor appetite resulting in iron deficiency. Infection is
well recognized cause of PEM because it affect three factors of nutrition: decrease food intake, impaired absorption and increase losses due to diarrhoea\(^{(96)}\).

In our study we found that 52 (70.3\%) of children with PEM had low transferrin saturation, low transferrin level may be explained by hypoproteinemia which is the main finding in PEM\(^{(97,98)}\). Antia et al\(^{(99)}\) have shown that, transferrin may be a better index of malnutrition than albumin. In the study also we observed low red cell indices, which were indicators of low serum iron. MCV was low in 68 (91.8\%) of children with PEM and in 24 (80\%) of the control whereas MCHC was low in 64 (86.5\%) of children with PEM and 18 (60\%) of the control. MCV is considered to be more specific but less sensitive indicator of iron deficiency. MCV was also specific (88\%) in identifying patients who had low ferritin with elevated TIBC, but the sensitivity is only 43\%\(^{(100)}\). In our study no patient had low MCV alone and so we were not able to calculate it's sensitivity and specificity also our serum ferritin and TIBC results are variably different.

MCHC was low in 86.5\% of the study group and 60\% of the control. MCHC is more frequently reduced in iron deficiency than with other causes of microcytosis but the drop in MCHC occurs late and the diagnostic utility of this parameter is therefore poor\(^{(81)}\).
TIBC was low in 18 (24.3%), high or normal 38 (51.4%) of children with PEM. 22 (29.8%) children had low serum iron but their TIBC was low or normal although TIBC is usually raised in iron deficiency, but it may remain normal or even low\textsuperscript{(101)} also the effect of malnutrition on TIBC and indirectly on transferrin saturation was studied in 45 Indian children with PEM 31 of those patients had iron deficiency anaemia in the basis that their Hb improved with iron therapy all the three groups had significantly low TIBC as compared to the control group with pure iron deficiency and normal serum albumin. So in our study low or normal level of TIBC can not exclude iron deficiency in this group of patient and may reflect the low protein due to malnutrition.

In our study normal serum ferritin was found in 54 (73%) of children with PEM whereas high or low level was found in 10 (13.5%) of children of PEM. The normal or high level it is likely due to concomitant infection which are usually prevalent in PEM. In this study we confirmed this suggestion.

Serum ferritin is an acute phase reactant, and a proferritin (a ferritin protein with almost no iron in it, and not in equilibrium with body stores) is elevated in any inflammatory state such as, infection, rheumatoid arthritis and cancer, due in part to interleukin 1 enhancing the translation of apoprotein mRNA\textsuperscript{(101)}. Salim S in 2003 evaluated iron status in Sudanese patients, 11 children were included in this study he
found that normal s-ferritin was found in 27.3% of patients with classical finding of IDA who presented with inflammatory processes (102).

Wickramsinghe S in 1985 studied iron status in 46 Nigerian children with PEM. 56.5% of them had elevated level of s-ferritin and there was no statistically significant correlation between the s-ferritin and amount of stainable iron in marrow (103).

In the control group, 6 children constituted 20% had low Hb, low red cell indices was found in 60 – 80% of children. Serum iron was low in 4 (13.3%) S-ferritin was low in 18 (60%) of children and TIBC was high in 12 (40%) of children. There were 12 (40%) had normal Hb and low red cell indices and low S-ferritin, this can explain by that these children had depleted iron stores (iron deficiency without anaemia).

In this study, children with kwashiorkor showed lower level of Hb (24 pt). 92.3% than marasmic (82.4%) children and marasmic–kwashiorkor (85.7%) whereas less drop in s-iron and s-ferritin which is observed in 76.9% and 15.4% respectively compared to 85.7% of marasmic–kwashiorkor with low level of s-iron and all of them had normal level of s-ferritin and 82.4% and 17.6% of children with marasmus had low level of s-iron and s-ferritin respectively. The less drop in s-ferritin in children with kwashiorkor can explain by infection which is commoner in kwashiorkor than in other subtypes of PEM due to thymus atrophy which is observed in children with kwashiorkor (104).
The TIBC is normal or even low in (20 pt) 76.9% children with kwashiorkor compared to (18 pt.) 52.% of marasmus and (8 pt) 57.4 % of marasmic-kwashiorkor, this is similar to findings in study done by Agarwad(101) who observed maximum decrease in TIBC in children with kwashiorkor while those with marasmus had the minimum this could be correlated with the degree of fall in the s –albumin level which is also maximum in the kwashiorkor group.

Regarding transferrin saturation it was low in (18pt.) 69.2% of kwashiorkor compared to (22 pt.) 64.7% and (12pt.) 85.7% of marasmic and marasmic – kwashiorkor respectively. In previous study Agarwad observed similar results apart from that children with kwashiorkor showed maximum fall in trasferrin saturation while in this study the maximum fall was observed in children with marasmic – kwashiorkor this may be due to the difference in representation of groups in the studies, there were 26 children of kwashiorkor and 14 children with marasmic - kwashiorkor in this study while the children with kwashiorkor were 10 and with marasmic - kwashiorkor were 20 in the other study.
CONCLUSION

1. Iron deficiency is highly prevalent in children with or without anaemia.

2. Children with PEM have different iron status compared to children with normal protein.

3. Low red cell indices in the presence of normal Hb values must be carefully interpreted (minor haemoglobinopathies should be excluded in such cases).

4. Concurrent infection can give erroneous serum ferritin values.
RECOMMENDATIONS

1. Organized nutritional educational programs should be placed high on the agenda for public health intervention to improve childhood nutrition in our country and must include the following:
   i. Promotion of appropriate breast-feeding and weaning practices.
   ii. Promotion of food with high iron content especially meat product and food rich in vitamin C which improved iron absorption.
   iii. Education in importance of increased frequency of meal/day

2. Iron status of children with PEM must be evaluated by methods which are not influenced by infection or inflammatory processes and hypoproteinemia such as transferring receptors or percentage of hypochromic red cell.

3. Reference values of iron status parameters in our population should be studied.

4. Detection of haemoglobinopathies must be included in future studies.
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