بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Plasma Lipid Profile Among Sudanese Children with Malnutrition in Khartoum State

مستوى الدهون في البلازما لدى الأطفال السودانيين المصابين بسوء التغذية في ولاية الخرطوم

A dissertation submitted in a partial fulfillment of the requirement for the master degree in medical laboratory science (clinical chemistry)

By

Eman Abd Alhafeez Rahmtalla

B.Sc Clinical Chemistry - College of Medical Laboratories Science
Sudan University of Science and Technology

2014

Supervisor

Dr. Nuha Eljaili Abobaker

Assistant Professor of Clinical Biochemistry

May 2017

Sudan University of Science & Technology

College of Graduate Studies

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

كلية الدراسات العليا

Ref: SUST/ CGS/A11

Approval Page

(To be completed after the college council approval)

Name of Candidate: Eman | Abd | Alhafez | Rahmtalla

Thesistitle: Plasma Lipid Profile among Sudanese Children With Malnutrition in Khartoum State

Degree Examined for: Master

Approved by:

1. External Examiner

Name: Dr. Salih Abdelgadir Elmadi

Signature: ..... Date: 22/5/2017

2. Internal Examiner

Name: Mariam Abbas Ibrahim

Signature: ..... Date: 22/5/2017

3. Supervisor

Name: Nuha Elgali Abubaker

Signature: Nuha Elga Date: 22/5/2017

الآيــــة

قال تعالى :

بسم الله الرحمن الرحيم

" فَفَهَّمْنَاهَا سُـلَيْمَانَ وَكُلًّا آتَيْنَا حُكْمًا وَعِلْمًا وَسَـخَّرْنَا مَـعَ دَاوُودَ الْجِبَالَ يُسَـبِّحْنَ وَالطَّيْرَ وَكُنَّا فَاعِلِينَ "

صدق الله العظيم

سورة الانبياءالآيه(٧٩)

Dedication

To……..

My family… father, mother, sisters and brother

To……..

My friends and colleagues for sharing life with me

To…….

My teachers who help and support me

To……..

My supervisor Dr. Nuha Eljaili Abobaker

Acknowledgments

All great thanks are firstly to Allah.

I would like to express my gratitude and thank to my supervisor **Dr.Nuha Eljaili Abobaker** for her guidance, solving problems and her precious advice as well as continuous assistance through the whole process of research.

Thank also extend to members of clinical chemistry department and least thanks to the children and families who Participate in this study.

Abstract

This study was carried out to measure plasma levels of lipid profile (Total cholesterol, triglyceride, HDL-c and LDL-c) in malnourished children. Sixty samples were collected from malnourished children in period between January to April 2017, chosen randomly from Mohammed AL Amin Hamid for pediatric teaching hospital in Khartoum State, and Sixty apparently healthy individuals as control group, to assess the effect of malnutrition on plasma lipid profile levels.

Estimation of plasma Total cholesterol, triglyceride, HDL-c and LDL-c levels was done by using Spectrophotometer biosystem-310, and results were analyzed using (SPSS) computer program.

The result of this study showed that, PEM most common among age between (6-9) years (85%), and malnutrition most abundant in females (56 .7%) than males (43.3%).

The study results showed that, the plasma levels of lipid profile (Total cholesterol, triglyceride, HDL-c and LDL-c) were significantly decreased in Sudanese malnourished children when compare with control group. (Mean ± SD for cases versus control). For Total cholesterol (mean ± SD: 58.25 ±3.15 versus 128.33 ±5.68 mg/dl, p. value=0.000), Triglyceride (35.12± 4.63 versus 82.32 ±11.56 mg/dl, p. value=0.000), HDL-c (25.02±4.44 versus 55.12±3.70 mg/dl, p. value=0.000) and LDL-c (26.18±4.28 versus 56.85±5.39 mg/dl, p. value=0.000).

Also the finding of this study showed that, there was significantly decreased in the mean of BMI in malnourished children group compared to control group. Mean ± SD for case versus control (15.28 ± 2.28 kg/m2 versus 19.25 ± 2.48 kg/m2).

There were no correlation between duration of malnourished children and levels of cholesterol, HDL-c (r= 0.031, p=0.812), (r= 0.000, p=0.998) respectively, there was insignificant weak negative correlation between triglyceride and duration of disease. (r= -0.085, p=0.519) and there was insignificant weak positive correlation between LDL-c and duration of disease. (r= 0.176, p=0.817).

Person correlation showed that, there were no correlation between age of malnourished children and the level of cholesterol and HDL –c (r=0.046, p=0.729), (r=0.044, p=0.740) respectively, there were insignificant weak positive correlation between triglyceride, LDL-c and age (r=0.164, p=0.212), (r= 0.167, p=0.203) respectively.

It is concluded that: the plasma levels of cholesterol, triglyceride, HDL-c and LDL-c were significantly decreased in Sudanese malnourished children.

مستخلص الدراسة

أجريت هذه الدراسة لقياس مستويات الدهون (الكولستيرول الكلي،ثلاثي الجليسريد،الكولستيرول عالي الكثافه وكولستيرول منخفض الكثافه) في البلازما لدى الأطفال الذين يعانون من سوء التغذية. تم جمع 60 عينة من الأطفال الذين يعانون من سوء التغذية خلال الفترة من يناير إلى أبريل 2017، تم اختيارهم عشوائيا من مستشفى محمد الامين حامدالتعليمي للاطفال في ولاية الخرطوم ، و 60 من الأفراد الأصحاء كمجموعة ضابطة، لتقييم تأثير سوء التغذية على مستويات مستويات الدهون في البلازما.

تم قياس مستويات الكولستيرول الكلي،ثلاثي الجليسريد،الكولستيرول عالي الكثافه وكولستيرول منخفض الكثافه بإستخدام جهاز سبكتروفوتوميتربيوسستم-310 ، وتم تحليل النتائج باستخدام برنامج نظام الحزمة الإحصائية للعلوم الاجتماعية (SPSS)، برنامج الكمبيوتر.

وأظهرت نتائج الدراسة أن معدل الإصابة بمرض سوء التغزية اكثر شيوعا بين سن (6-9) سنوات (85٪)، وسوء التغذية اكثر وفرة لدى الإناث (56.7٪) مقارنة بالذكور (43.3٪).

وأظهرت الدراسة أن مستويات الدهون في البلازما (الكولستيرول الكلي، ثلاثي الجليسريد، الكولستيرول عالي الكثافه وكولستيرول منخفض الكثافه) انخفضت بشكل ملحوظ في الأطفال السودانيين الذين يعانون من سوء التغذية."

المتوسط ± الإنحراف المعياري للمرضى مقارنة بمجموعة التحكم ".

بالنسبة للكوليسترول: (58.25± 3.15 مقابل 128.33 ± 5.68 / مليجرام\ديسيلتر، وكان الاحتمال الاحصائي للمقارنة 0.000).ثلاثي الجليسريد: (35.12 ± 4.63 مقابل 82.32 ± 11.56 مليجرام/ ديسيلتر ، وكان الاحتمال الاحصائي للمقارنة 0.000)، كوليسترول عالي الكثافه: (25.02 ± 4.44 مقابل 55.12± 3.70مليجرام/ ديسيلتر، وكان الاحتمال الاحصائي للمقارنة 0.000) والكوليسترول منخفض الكثافه:(26.18 ± 4.28 مقابل 56.85 ± 5.39 مليجرام/ ديسيلتر، وكان الاحتمال الاحصائي للمقارنة 0.000).

كما أظهرت نتائج الدراسة أن هناك انخفاض معنوي في مؤشر كتلة الجسم لدى الأطفال الذين يعانون من سوء التغذية مقارنة بمجموعة التحكم. متوسط مؤشر كتلة الجسم ± الإنحراف المعياري للمرضى مقارنة بمجموعة التحكم (15.28 ± 2.28 كجم / م 2 مقابل 19.25 ± 2.48 كجم / م 2).

لم يكن هناك ارتباط معنوي بين مدة إصابة الأطفال بمرض سوء التغذية ومستوي الكوليسترول وكوليسترول عالي الكثافه (معامل بيرسون للإرتباط=0.031،مستوى المعنوية=0.812)، (معامل بيرسون للإرتباط=0.000،مستوى

المعنوية=0.998) علي التوالي. ويوجد ارتباط ضعيف سالب بين مستوي ثلاثي الجليسريد ومدة إصابة الأطفال بمرض سوء التغذية (معامل بيرسون للإرتباط=-0.085،مستوى المعنوية= 0.519)، وارتباط ضعيف موجب بين مستوى كوليسترول منخفض الكثافه ومده الاصابه بالمرض (معامل بيرسون للإرتباط=0.176،مستوى المعنوية=0.817).

أظهر مستوى المعنوية ليس هناك علاقة معنوية بين عمر الأطفال الذين يعانون من سوء التغذية ومستويات الكوليسترول و كوليسترول عالي الكثافه (معامل بيرسون للإتباط 0.046،مستوى المعنوية=0.729)، (معامل بيرسون للإرتباط=0.044،مستوى المعنوية=0.740) علي التوالي، ويوجد ارتباط ضعيف موجب بين عمر الأطفال الذين يعانون من سوء التغذية ومستويات ثلاثي الجليسريد وكوليسترول منخفض الكثافه (معامل بيرسون للإرتباط=0.164،مستوى المعنوية=0.212)، (معامل بيرسون للإرتباط=0.167،مستوى المعنوية=0.203) علي التوالي.

وخلصت الدراسة إلى أن مستويات البلازما من الكولستيرول الكلي،ثلاثي الجليسريد،الكولستيرول عالي الكثافه وكولستيرول منخفض الكثافه انخفضت بشكل ملحوظ لدى الأطفال السودانيين الذين يعانون من سوء التغذية.

List of contents

List of tables

List of figures

List of abbreviations

Abbreviation	Full terms
ATP	Adenosine Tri Phosphate
BMI	Body Mass Index
CHD	Coronary Heart Disease
FFMI	Fat Free Mass Index
HDL-c	High Density Lipoprotein cholesterol
LDL-c	Low Density Lipoprotein cholesterol
LP	Lipoprotein
PCM	Protein Calorie Malnutrition
PEM	Protein energy malnutrition
R.P.M	Round per minute
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

CHAPTER ONE

Introduction

1. Introduction

1.1. Introduction:

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.(Jancen and Mannet.,1982).

Malnutrition due to starvation, disease or ageing can be defined as a state resulting from lack of uptake or intake of nutrition leading to altered body composition (decreased fat free mass) and body cell mass leading to diminished physical and mental function.(Jancen and Mannet.,1982).

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).

Protein energy malnutrition is global public health problem affecting children from Africa, Asian, Latin American and Caribbean regions. PEM is directly or indirectly responsible for about half of 10.8 million deaths per year in children in developing countries. (Nassar *et al* .,2010)

Lipid applies to a class of compounds that are soluble in organic solvents, but nearly insoluble in water.(Carl *etal*., 2008).

Measurement of serum lipids forms one of the special tests in most clinical chemistry laboratories worldwide, this important in the management of patients with cardiovascular diseases and monitoring patients with diabetes mellitus, as well as in assessment malnutrition in children. (Low *et al*., 1996).

Malnutrition has a great impact on the health care delivery system, resulting in reduced quality of life for the affected patients and added financial costs to the hospital where the patients are receiving care.

So it is very important to evaluate essential parameters as plasma total cholesterol, triglyceride, HDL-c and LDL-c which decreased in malnourished children.(Nascimento *et al*., 2012).

1.2. Rationale:

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

There are few published studies about this in Sudan, so this study may help to provide the monitoring of plasma total cholesterol, triglyceride, HDL-c and LDL-c in malnourished children.

1.3. Objectives:

1.3.1. General objective:

To study plasma lipid profile among Sudanese children with malnutrition.

1.3.2. Specific objectives:

1- To measure plasma levels of lipid profile (total cholesterol, triglyceride, HDL-c and LDL-c) in malnourished children and control group.

2- To compare concentrations of total cholesterol, triglyceride, HDL-c and LDL-c in both study groups.

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

4-To correlate between parameters and study variables (age and duration of disease) .

CHAPTER TWO

Literature review

2. Literature review

2.1. Malnutrition:

Malnutrition is a broad term that can be used to describe any imbalance in nutrition; from over-nutrition often seen in the developed world, to under-nutrition seen in many level hoping countries, but also in hospitals and residential care facilities in developed nations. Malnutrition can develop as a consequence of deficiency in dietary intake, increased requirements Associated with a disease state, from complications of an underlying illness such as poor absorption and excessive nutrient losses, or from a combination of these aforementioned factors (Soetrs *et al* ., 2008).

2.1.1. Protein energy malnutrition:

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

i) The relative severity of protein or energy deficit

ii) The severity and duration of the deficiencies.

iii) The age of the host

iv) The cause of the deficiency

v) The association of the deficiency with other physiological problems such as infectious diseases and pregnancy (Torun and Chew., 1994).

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

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

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

2.1.1.1. Types of Protein Energy Malnutrition:

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

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

A-Under nutrition:

Under nutrition is a consequence of consuming little energy and other essential nutrients or using or excreting more rapidly than they can be replaced. This state of malnutrition is often characterized by infectious and diseased children who are already under nourished can suffer from protein energy malnutrition who rapid growth, infectious or disease
Increases the need for protein and essential nutrients (Pauline., 2008)

B - Marasmus:

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

C- Kwashiorkor:

This is the wet edematous and swollen form. “Kwashiorkor” is a Ghanaian word meaning “first child-second child”. It refers to the observation that this is a disease the first child develops when the second child is born and replaces the first child at the breast. This is because the weaned child is fed with a thin gruel of poor nutritional quantity compared with breast milk and as a consequence the child fails to thrive. This condition is marked with protein deficiency more marked than energy deficiency, and Oedema results.
Children with Kwashiorkor tend to be older than those with marasmus and tend to develop the disease after weaning.
Adults develop kwashiorkor as a result of under-nutrition from diets rich in carbohydrate than protein. This may be as a result of poverty, wars, famine etc. Kwashiorkor is characterized by fluid retention, oedema, dry peeling skin, hair discolorations, etc (Pauline., 2008).

D- Marasmic Kwashiorkor:

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 :

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

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

B - Gomez Classification:

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

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

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

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

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

C-Water Low Classification:

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

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

Therefore patients fall into four categories:

1) Normal.

2) Wasted but not stunted (suffering from acute PEM).

3) Wasted and stunted (suffering from acute and chronic PEM).

4) Stunted but not wasted (nutritional dwarfs with past PEM with present adequate nutrition).

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

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

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

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

2.1.1.3. Causes:

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

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

A- Main symptom:

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

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

Someone could be malnourished if:

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

B- Other symptoms:

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

C-Symptoms in children:

Symptoms of malnutrition in a child can include:

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

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

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

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

2.1.1.5. Diagnosis of malnutrition:

A- Weight loss:

Weight loss trajectories differ with clinical condition. Nevertheless, involuntary weight loss is a strong predictor of negative (Who, 1995) outcomes irrespective of magnitude, speed and underlying cause. Naturally, a massive and fast weight loss due to an aggressive cancer disease imposes a higher risk than a smaller and slower weight loss due to ageing. Thus, consensus was reached to propose two optional cut-offs for unintentional weight loss; i.e. either >5% over the last 3 months to cover for acute illnesses, or >10% of habitual weight indefinite of time to be relevant for chronic conditions (Who ,1995).

B- Body mass index (BMI) :

WHO advocates BMI <18.5 kg/m2 as a general cut-off for underweight. This cut-off is justified at a public health population Level l (Who, 1995), 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 3-6 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 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. Lipids:

The term lipid applies to a class of compounds that are soluble in organic solvents, but nearly insoluble in water. Chemically, lipids contain primarily non polar carbon-hydrogen (C-H) bonds and typically yield fatty acids and or complex alcohols after hydrolysis. Some lipids also contain charged or polar groups (Carl *et al*., 2008).

2.2.1. Classification of lipid:

2.2.1.1. Fatty Acids:

Are simply linear chains of C-H bonds that terminate with a carboxyl group (-COOH). In plasma, only a relatively small amount of fatty acids exists in the free or unesterified form, most of which is bound to albumin.Fatty acids are covalently attached to the glycerol backbone of triglycerides and phospholipids by an ester bond that forms between the carboxyl group on the fatty acid and the hydroxyl group (-OH) on glycerol. . (Michael *et al*.,2010). Fatty acids can be classified as being saturated (no doublebonds), monounsaturated (one double-bond), or polyunsaturated (two or more double-bonds). The C = C doublebonds of unsaturated fatty acids are typically arranged in the cis form. (Michael *et al*.,2010).

2.2.1.2. Triglycerides:

Triglycerides contain three fatty acid molecules attached to one molecule of glycerol by ester bonds. Each fatty acid in the triglyceride molecule can potentially be different in structure, most triglycerides from plant sources, such as corn, sunflower seeds, and safflower seeds, are rich in polyunsaturated fatty acids and are oils, whereas triglycerides from animal sources contain mostly saturated fatty acids and are usually solid at room temperature. (Michael *et al*.,2010).

2.2.1.3. Phospholipids:

Phospholipids are similar in structure to triglycerides except that they only have two esterified fatty acids. The third position on the glycerol backbone instead contains a phospholipid head group. There are several types of phospholipid head groups, such as choline, inositol, serine, and ethanolamine, which are all hydrophilic in nature. The various types of phospholipids are named based on the type of phospholipid head group present. The two fatty acids in phospholipids are normally 14 to 24 carbon atoms long, with one fatty acid commonly saturated and the other unsaturated. (Michael *et al*., 2010).

2.2.1.4. Cholesterol:

Cholesterol is an unsaturated steroid alcohol containing four rings (A, B, C, and D), and it has a single C-H side chain tail similar to a fatty acid in its physical properties. The only hydrophilic part of cholesterol is the hydroxyl group in the A-ring. Cholesterol is therefore, also an amphipathic lipid and is found on the surface of lipid layers along with phospholipids. (Michael *et al*.,2010).

Cholesterol can be converted in the liver to primary bile acids, such as cholic acid and chenodeoxycholic acid, which promote fat absorption in the intestine by acting as detergents. A small amount of cholesterol can also be converted by some tissue, such as the adrenal gland, testis, and ovary to steroid hormones such as glucocorticoids, mineralocorticoids, and estrogens. Finally, a small amount of cholesterol after first being converted to 7-dehydrocholesterol, can also be transformed to vitamin D3 in the skin by irradiation from sunlight. . (Michael *et al*., 2010).

2.2.2. Lipoproteins:

Lipids synthesized in the liver and intestine are transported in the plasma in macromolecular complexes known as lipoproteins.

Chemistry Lipoproteins are typically spherical particles with nonpolar neutral lipids (miglycerides and cholesterol esters) in their core and more polar amphipathic lipids (phospholipids and free cholesterol) at their surface. They also contain one or more specific proteins, called apolipoproteins on their surfaces. The association of the core lipids with the phospholipid and apolipoproteins is noncovalent, occurring primarily through hydrogen bonding and van der Waals forces. The binding of lipids to apolipoproteins is weak and allows the exchange of lipids and apolipoproteins among the

plasma lipoproteins and between cell membranes and lipoproteins. (Carl *et al*., 2008).

2.2.2.1. Classification of lipoproteins:

I. Chylomicrons:

Chylomicrons, which contain apo B-48, are the largest and the least dense of the lipoprotein particles, Because of their large size, they reflect light and account for the turbidity of postprandial plasma. Because they are so light, they also readily float to the top of stored plasma and form a creamy layer, which is a hallmark for the presence of chylomicrons. Chylomicrons are produced by the intestine, where they are packaged with absorbed dietary lipids. Once they enter the circulation, triglycerides and cholesteryl esters in chylomicrons are rapidly hydrolyzed by lipases and, within a few hours, they are transformed into chylomicron remnant particles, which are recognized by proteoglycans and remnant receptors in the liver, facilitating their uptake. The principal role of chylomicrons is the delivery of dietary lipids to hepatic and peripheral cells. . (Michael *et al*., 2010).

II. **Very Low Density Lipoproteins VLDL:**

is produced by the liver and contains apo B-100, apo E, and apo Cs; like chylomicrons, they are also rich in triglycerides. They are the major carriers of endogenous (hepatic-derived) triglycerides and transfer triglycerides from the liver to peripheral tissue. Like chylomicrons, they also reflect light and account for most of the turbidity observed in fasting hyperlipidemic plasma specimens, although they do not form a creamy top layer like chylomicrons, because they are smaller and less buoyant. Excess dietary intake of carbohydrate, saturated fatty acids, and trans fatty acids enhances the hepatic synthesis of triglycerides, which in turn increases VLDL production. . (Michael *et al*., 2010).

III. Low-Density Lipoproteins LDL:

primarily contains apo B-100 and is more cholesterol rich than other apo B–containing lipoproteins. They form as a consequence of the lipolysis of VLDL. LDL is readily taken up by cells via the LDL receptor in the liver and peripheral cells. In addition, because LDL particles are significantly smaller than VLDL particles and chylomicrons, they can infiltrate into the extracellular space of the vessel wall, where they can be oxidized and taken up by macrophages through various scavenger receptors. Macrophages that take up too much lipid become filled with intracellular lipid drops and turn into foam cells. (Michael *et al*., 2010).

IV. Lipoprotein(a):

Lipoprotein (a) particles are LDL-like particles that contain one molecule of apo (a) linked to apo B-100 by a disulfide bond. Lp(a) particles are heterogeneous in both size and density, as a result of a differing number of repeating peptide sequences, called kringles, in the apo (a) portion of the molecule. The concentration of Lp(a) is inversely related to the size of the isoform. Elevated levels of Lp(a) are thought to confer increased risk for premature coronary heart disease and stroke. Because the kringle domains of Lp(a) have a high level of homology with plasminogen, a protein that promotes clot lysis, it has been proposed that Lp(a) may compete with plasminogen for binding sites, thereby promoting clotting, a key contributor to both myocardial infarction and stroke. (Michael *et al*., 2010).

V. High-Density Lipoproteins:

HDL, the smallest and most dense lipoprotein particle, is synthesized by both the liver and intestine. HDL can exist as either disk-shaped particles or spherical particles. Discoidal HDL typically contains two molecules of apo A-I, which form a ring around a central lipid bilayer of phospholipid and cholesterol.The ability of HDL to remove cholesterol from cells, called reverse cholesterol transport.

HDL is highly heterogeneous separable into as many as different subfractions. There are two major types of spherical HDL based on density differences: HDL2 and HDL3. HDL2 particles are larger in size and richer in lipid than HDL3 and may reflect better efficiency in delivering lipids to the liver. . (Michael *et al*., 2010).

2.2.2.2 Metabolism of lipoproteins:

Lipoprotein metabolism is commonly divided into the (1) exogenous, (2) endogenous, (3) intracellular-cholesterol transport, and (4) reverse-cholesterol transport pathways.

2.2.3. Lipid disorders:

2.2.3.1. Hypercholesterolemia:

Hypercholesterolemia is the lipid abnormality most closely linked to heart disease, which is associated with genetic abnormalities that predispose affected individuals to elevated cholesterol levels, is called familial hypercholesterolemia.*(* Calderon *et al., 1999).*

Causes of hypercholesterolemia:

Hypercholesterolemia is typically due to a combination of environmental and genetic factors. Environmental factors include obesity, diet, and stress.

A number of other conditions can also increase cholesterol levels including diabetes mellitus type 2, obesity, alcohol, monoclonal gammopathy, dialysis, nephrotic syndrome, hypothyroidism, Cushing's syndrome, anorexia nervosa, medications (thiazide diuretics, ciclosporin, glucocorticoids, beta blockers, retinoic acid). (Bhatnagar *et al.,* 2008*)*

2.2.3.2. Hypocholesterolemia:

Is the presence of abnormally low (*hypo*-) levels of cholesterol in the blood.

Causes of hypocholesterolemia:

Possible causes of low cholesterol are:

Statins, Hyperthyroidism, or an overactive thyroid gland, Adrenal insufficiency, Liver disease, Malabsorption (inadequate absorption of nutrients from the intestines), such as in celiac disease, Malnutrition, Abetalipoproteinemia (a rare genetic disease that causes cholesterol readings below 50 mg/dl. It is found mostly in Jewish populations), Manganese deficiency, Marfan syndrome, Leukemias and other hematological disease. (Moutzouri*etal*., 2011*).* And*(* Marini *et al.,* 1989*).*

2.2.3.3. Hypertriglyceridemia:

High blood levels of triglycerides, the most abundant fatty molecule in most organisms. Elevated levels of triglycerides are associated with atherosclerosis, even in the absence of hypercholesterolemia

Causes of hypertriglyceridemia:

High carbohydrate diet , High fat diet, Idiopathic (constitutional), Obesity, Diabetes mellitus and insulin resistance - it is one of the defined components of metabolic syndrome (along with central obesity, hypertension, and hyperglycemia), Excess alcohol consumption, renal failure (Nephrotic syndrome), Genetic predisposition; some forms of familial hyperlipidemia such as familial combined hyperlipidemia i.e. Type II hyperlipidemia, Lipoprotein lipase deficiency - Deficiency of this water-soluble enzyme, that hydrolyzes triglycerides in lipoproteins, leads to elevated levels of triglycerides in the blood., Lysosomal acid lipase deficiency or Cholesteryl ester storage disease.

Certain medications e.g. isotretinoin, estrogen, hydrochlorothiazide diuretics, beta blockers, protease inhibitors, Hypothyroidism (underactive thyroid), Systemic Lupus Erythematosus, Glycogen storage disease type 1, and HIV medications. (Silva *et al.,* 1987*;* Mccarty*etal.,*2004 *;*Garg *et al.,* 1992and ;Pejicand, Lee., 2006*).*

2.2.3.4. Hypolipoproteinemia:

Hypolipoproteinemias, or low levels of lipoproteins, exist in two forms: hypoalphalipoproteinemia and hypobetalipoproteinemia. Hypobetalipoproteinemia is associated with isolated low levels of LDL cholesterol but not associated with CHD.

HypoalphalipoproteinemiaHypoalphalipoproteinemia indicates an isolated decrease in circulating HDL which it concentration less than 40 mg/dL without the presence of hypertriglyceridemia, the term alpha denotes the region in which HDL migrate on agarose electrophoresis. (Michael *et al*., 2010).

CHAPTER THREE

Materials and Methods

3. Materials and methods

3.1. Materials:

3.1.1. Study approach:

Quantitative methods were used to estimate lipid profile in Sudanese malnourished children in Khartoum state.

3.1.2. Study design:

This was hospital case-control study.

3.1.3. Study area:

This study was conducted from Mohammed AL Amin Hamid for Pediatric Teaching Hospital in Khartoum State during the period from January to April 2017.

3.1.4. Study populations:

The study included 120 individuals, 60 malnourished children as cases and 60 apparently healthy subjects serve as control with normal nutrition (age and sex match with test group) .

3.1.5. Inclusion criteria:

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

3.1.6. Exclusion criteria:

Any patients with liver disease, hyperlipidemia , cardiovascular disease , ,other chronic disease, any patients taking drug that affect in lipid metabolism , Nephrotic Syndrome and End Stage Renal Disease were excluded.

3.1.7. Ethical consideration:

Verbal 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 them.

3.1.8. Data collection:

The clinical data were obtained from clinical examinations and hospital follow up records and were recorded on a questionnaire sheet.(Appendix 1).

3.1.9. Samples collection and processing:

About 4 ml of venous blood were collected from each fasting participant (both cases and controls). 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 cholesterol:

3.2.1.1. Principle of the method:

Ester cholesterol hydrolyzed in present of cholesterol esterase to free fatty acid and free cholesterol which oxidized by atmospheric oxygen in presence of cholesterol oxidize to cholestene-3,1 and hydrogen peroxide , which converted by peroxidase to H_2O and oxygen then oxygen accepted by para-amino phenazone in presence of phenol to produce quinoninmine pink color measured by spectrophotometry. (Allain*et al*., 1974) .

3.2.1.2. Procedure:

	Blank	**Standard**	**Sample**
Cholesterol STD	-	10 µl	-
Sample	-	-	10 µl
Reagent	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubate for 10 minutes at room temperature.

The absorbance of standard and sample were measured at 500 nm against the blank.

3.2.2. Estimation of triglyceride:

3.2.2.1. Principle of the method:

Triglycerides hydrolyzed enzymatically in the presence of lipase to 3fatty acid and glycerol , which phosphorylated in the presence of ATP and glycerol kinase to glycerol-3-phosphate that oxidized in presence of glycerol-3-phospate oxidase to dihydroxyacetone phosphate and hydrogen peroxide which converted by peroxidase to H_2O and oxygen then oxygen accepted by para-amino phenazone in presence of phenol to produce quinoninmine pink color measured by spectrophotometry. (Fossati and Prencipe., 1982).

3.2.2.2. Procedure:

	Blank	**Standard**	**Sample**
Triglycerides STD	-	10 µl	-
Sample	-	-	10 µl
Reagent	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubate for 15 minutes at room temperature.
The absorbance of standard and sample were measured at 500 nm against the blank

3.2.3 Estimation of high density lipoprotein (HDL-c):

3.2.3.1. Principle of method:

Very low density lipoproteins, chylomicrons and low density lipoproteins in the sample precipitate with phosphotungstate and magnesium ions, after centrifugation the supernatant contains high density lipoproteins which measured by cholesterol oxidase method spectrophotometrically. (Burstein *et al.,* 1980).

3.2.3.2. Procedure:

Precipitation:

Sample	0.2 mL
Reagent cholesterol HDL	0.5 mL

Mix thoroughly and let stand for 10 minutes at room temperature
Centrifuge at minimum of 4000 r.p.m for 10 minutes.
Carefully collect the supernatant

Colorimetry:

	Blank	Standard	Sample
D.W	100 µL	-	-
HDL cholesterol standard	-	100 µL	
Sample supernatant	-	-	100 µL
Reagent cholesterol	1.0 mL	1.0 mL	1.0 mL

The test tubes were mixed thoroughly and incubate for 30 minutes at room temperature.
The absorbance of standard and sample were measured at 500 nm against the blank

3.2.4. Calculation of low density lipoprotein (LDL-c):

LDL-c calculated from Fried-Wald's equation:
LDL-c = Total cholesterol – HDL-c –Triglyceride\5

3.3. Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample (control normal and pathogen) 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 applied for correlation between variables.

CHAPTER FOUR

Results

4. Results

The results of biochemical determinant of plasma lipid profile (total cholesterol, triglyceride, HDL-c and LDL-c) in malnourished children are given in tables and Figures.

Figure (4-1): Shows gender distribution, (56.7%) of patients were females while (43 .3%) were males.

Figure (4-2): Shows age distribution, (85%) of patients between (6-9) years, (8%) betweens (10-13) years and (7%) between (14-17) years.

Table (4-1): illustrates mean concentration of plasma lipid profile (total cholesterol, triglyceride, HDL-c and LDL-c) and BMI in patients and control groups. The levels of plasma lipid profile (total cholesterol, triglyceride, HDL-c, LDL-c) and BMI were significantly decreased in malnourished children compared to control group. Total cholesterol (mean ± SD: 58.25 ±3.15 versus 128.33 ±5.68 mg/dl, p. value=0.000), triglyceride (35.12± 4.63 versus 82.32 ±11.56 mg/dl, p. value=0.000), HDL-c (25.02±4.44 versus 55.12±3.70 mg/dl, p. value=0.000) LDL-c (26.18±4.28 versus 56.85±5.39 mg/dl, p. value=0.000) and BMI (mean ± SD: 15.28 ± 2.28 kg/m2 versus 19.25 ± 2.48 kg/m2. p. value=0.000).

Table (4-2): illustrates correlation between plasma lipid profile (levels cholesterol, triglyceride, HDL-c, LDL-c) and duration of disease in malnourished children, there were no correlation between duration of disease and plasma cholesterol level(r=0.031, p=0.812) and HDL-c(r=0.000, p=0.998), there was insignificant weak negative correlation between plasma triglyceride and duration of disease (r=-0.085, p=0.519) and there was insignificant weak positive correlation between plasma LDL-c and duration of disease (r=0.176, p=0.817).

Figure (4-3): Shows correlation between plasma Cholesterol level and age in case group. There was no correlation. (r=0.046, p=0.729).

Figure (4-4): Shows correlation between plasma triglyceride level and age in case group. There was insignificant weak positive correlation. (r=0.164, p=0.212).

Figure (4-5): Shows correlation between plasma HDL-c and age in case group. There was no correlation. (r=0.044, p=0.740).

Figure (4-6): Shows correlation between plasma LDL-c level and age in case group. There was insignificant weak positive correlation. (r= 0.167, p=0.203).

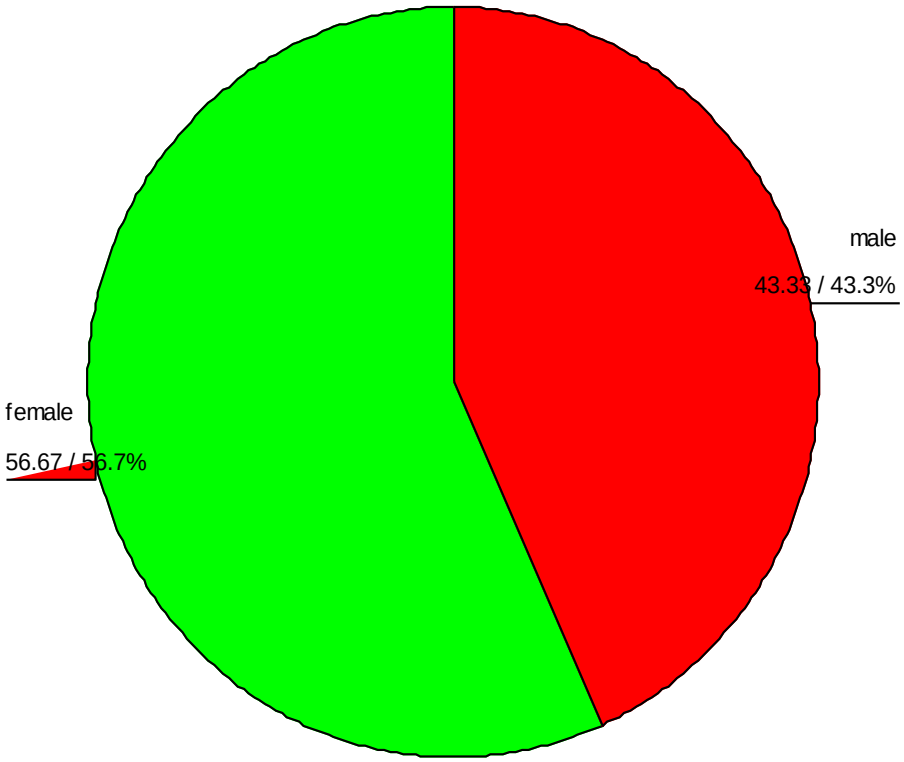

Figure (4-1): Gender distribution in case group.

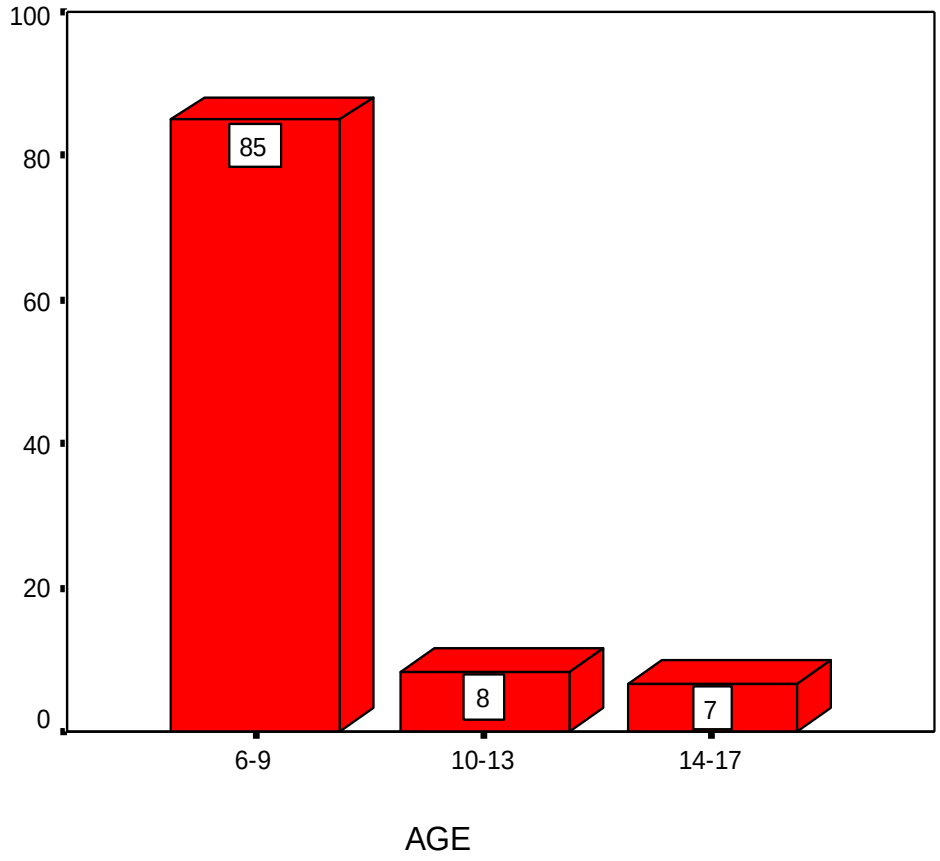

Figure (4-2): Age distribution in case group.

Table (4-1): Comparison of plasma levels cholesterol, triglyceride, HDL-c, LDL-c and BMI in malnourished children and control group.

Variable	**Case** N=60 Mean ± SD	**Control** N=60 Mean ± SD	**p. value**
Cholesterol (mg\dl)	58.25 ± 3.15	128.33±5.68	0.000
Triglyceride(mg\dl)	35.12±4.63	82.32±11.56	0.000
HDL-c (mg\dl)	25.02±4.44	55.12±3.70	0.000
LDL-c (mg\dl)	26.18±4.28	56.85±5.39	0.000
BMI (Kg/m2)	15.28±2.28	19.25±2.48	0.000

*Result given in mean ± SD.

* P-value ≤ 0.05 Consider significant.

Independent sample T test was used for comparison.

Table (4-2): Correlation between plasma levels cholesterol, triglyceride, HDL-c, LDL-c and duration of disease in malnourished children .

Correlation variables	**R. Value**	**P. value**
Duration/ Cholesterol	0.031	0.812
Duration/ Triglyceride	-0.085	0.519
Duration/ HDL-c	0.000	0.998
Duration/ LDL-c	0.176	0.817

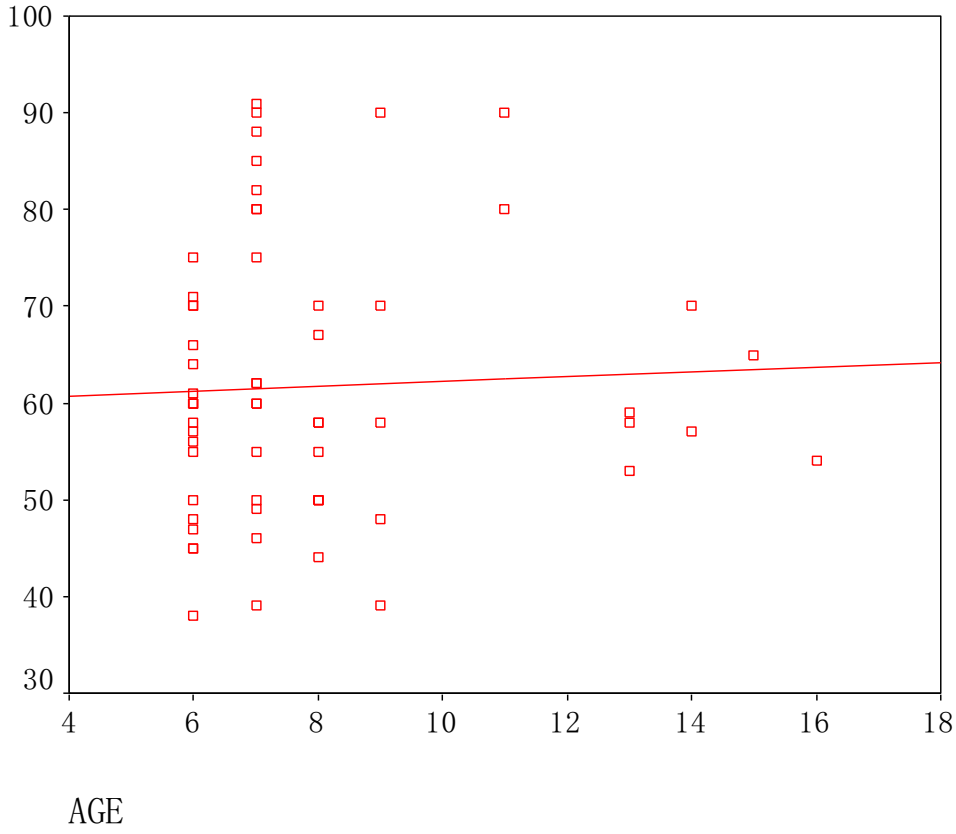

Figure (4-3): Correlation between plasma Cholesterol level and age in case group. (r=0.046, p=0.729).

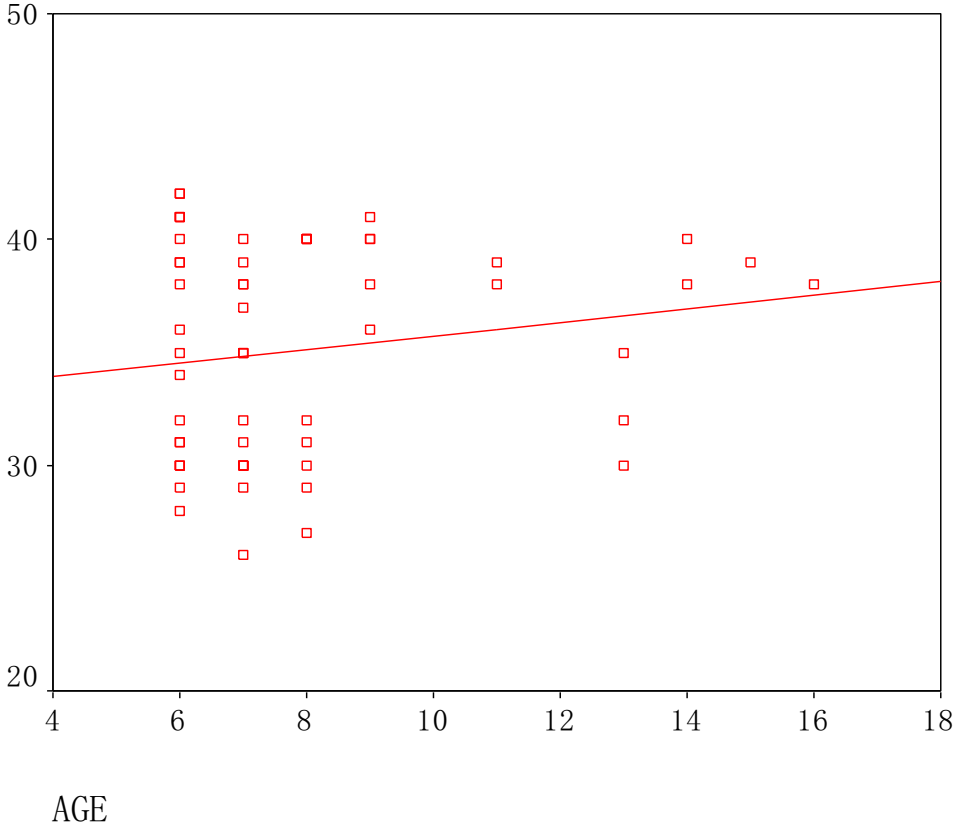

Figure (4-4): Correlation between plasma triglyceride level and age in case group. (r=0.164, p=0.212).

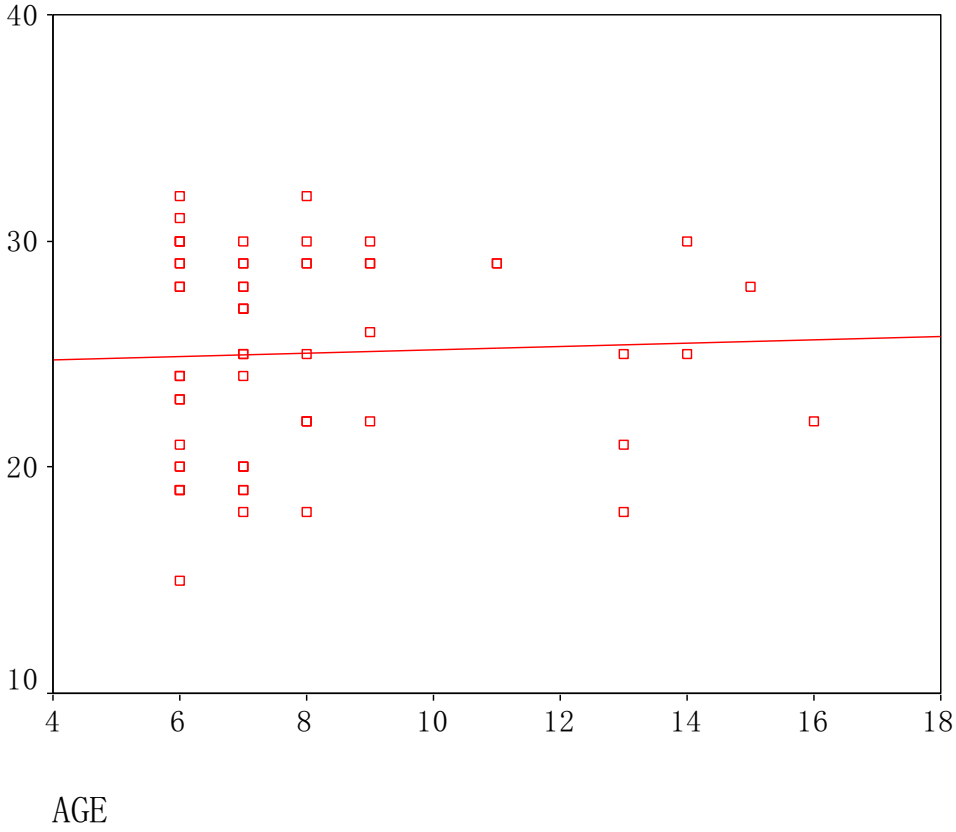

Figure (4-5): Correlation between plasma HDL-c and age in case group (r=0.044, p=0.740).

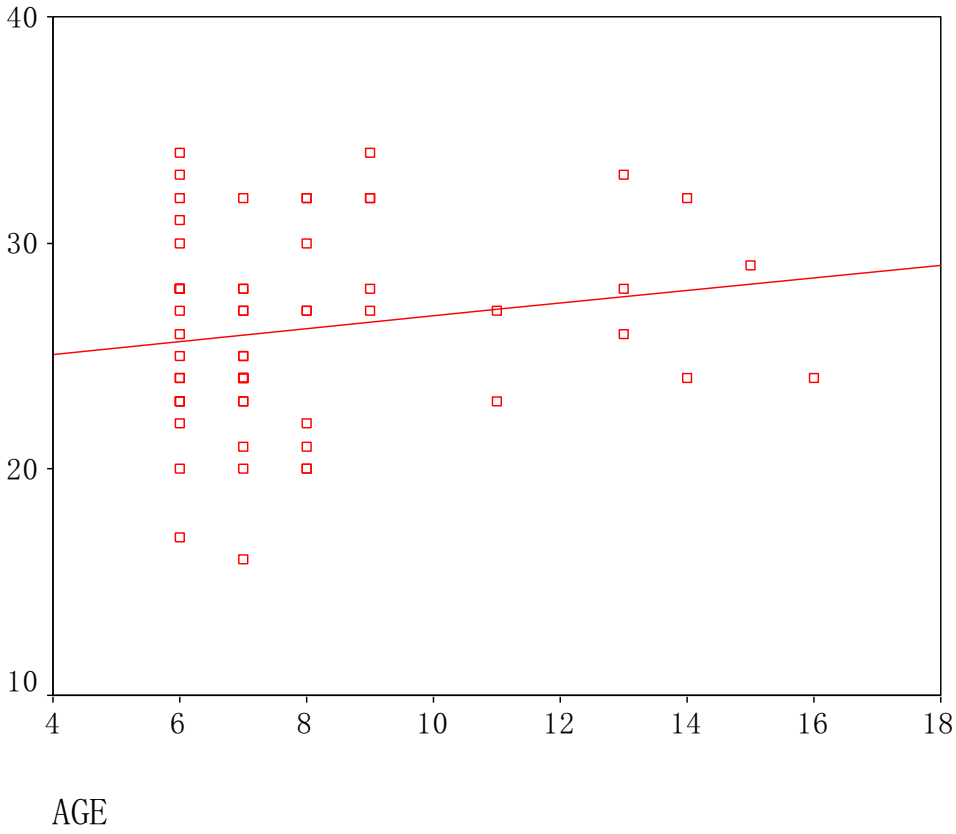

Figure (4-6): Correlation between plasma LDL-c level and age in case group. (r= 0.167, p=0.203).

CHAPTER FIVE

Discussion, Conclusion and Recommendations

5. Discussion, Conclusion and Recommendations

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 decreasing them, this study conducted to get the effect of malnutrition on the levels of plasma lipid profile (total cholesterol, triglyceride, HDL-c and LDL-c).

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 *et al*., 2000), which showed that malnutrition most abundant in females than males due to traditional food sharing behaviors in some households may result in females diet being less adequate than males.

The result of this study showed that, PEM most common among age between (6-9) years (85%).This result agreed with group studies carried by many researchers (Chukwuma ,2015 ;Irena , 2011 and Jobia ,2008) which showed that , malnutrition tendency to develop in lower aged group due to increased nutritional need for developing in African (Nigeria) and Asian (Bangladesh) countries.

From the finding of this study, it appears that plasma lipid profile levels of total cholesterol, triglyceride, HDL-c and LDL-c were significantly decreased in malnourished children group in comparison to control group (p.value=0.000), this results agreed with a study carried by (Akuyam *et al*., 2008) who reported similar finding in malnourished children group in compare to control, this due to the decreased 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 many studies carried by other researchers. (Mishra *et al*., 2009 ; Chatterjee ,K and Chaudhui,J.,1960; Ogunkeye and Ighogboja,1992; Teran ,1999 and Boulga *et al*., 2000) who had reported decreased plasma lipid profile in malnourished children, the reduction due to decreased ability of the liver cells to metabolize lipid in form of lipoproteins. Also the findings of this study showed that there were no correlation between duration of disease and concentration of plasma total cholesterol, HDL-c and there was insignificant weak negative correlation of triglyceride and duration

of disease, and there was insignificant weak positive correlation of LDL-c and duration of disease.

Also the result showed that, there were no correlation between plasma total cholesterol, HDL-c and age of malnourished children, this result agreed with another result, which showed that, there was no correlation between plasma lipid profile levels and age of malnourished children (Akuyam*et al*., 2009). And there were insignificant weak positive correlation between triglyceride, LDL-c levels and age of malnourished children, this result not agree with another study carried by(Akuyam*et al*., 2009). Showed there were no correlation between triglyceride, LDL-c levels and age of malnourished children.

5.2. Conclusion:

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

- The levels of plasma lipid profile (total cholesterol, triglyceride, HDL-c and LDL-c) were significantly decreased in malnourished children.
- There were weak positive correlation between triglyceride, LDL-c levels and age of malnourished children.
- There was insignificant weak negative correlation of triglyceride and duration of disease, and there was insignificant weak positive correlation of LDL-c and duration of disease.

5.3. Recommendations:

From the findings of this study it is recommended that:

- The levels of plasma Total cholesterol, triglyceride, HDL-c and LDL-c in malnourished children should be checked regularly.
- Treatment of malnourished patients together with complete nutritional supplementation is very important.
- Liver function test must be done to avoid liver disease.

REFERENCES

References

- **Akuyam, A., Isah, H.S., and Ogala, W.N.** (2008).Serum Lipid Profile in Malnourished Nigerian Children in Zaria**.** *Niger Postgrad Medical Journal*15**(3):**192-196**.**
- **Akuyam, A., Isah. H.S., and Ogala, W.N.** (2009). Relationship between age and serum Lipid in Malnourished and well fed preschool children in Zaria. *Nigerian Journal of clinical practice*. 15(**3**):192-196**.**
- **Allain,C.C.,Poon,L.S.,Chan,C.S.G.,Richmond,W**and FU PC. (1974). Enzymatic determination of total serum cholesterol. *ClinChem*, 20:470-475.
- **Bhatnagar, D., Soran, H and Durrington, P.N.** (2008). *"Hypercholesterolaemia and its management". BMJ. **337**: 993.*
- **Burstein, M., Scholnick, H.R and Morfin, R.** (1980).Rapid method for the isolation of lipoprptein from human serum by precipitation with polyanions.*Scand J clin lab Invest.*40:583-564.
- **Boulga, A.,Bouchenak, M and Belleville, J.**(2000). Low protein diet prevents tissue lipoprotein lipase activity increase in growing rats. *Br J Nutr;*84:663-71.
- **Calderon, R., Schneider, R. H., Alexander, C. N., Myers, H. F., Nidich, S. I. and Haney, C**. (1999). *"Stress, stress reduction and hypercholesterolemia in African Americans: a review". Ethnicity & Disease. **9**: 451–462.*
- **Carl, A., Edward, R. and David, E**. (2008). *Tietzfundumentals of clinical chemistry*. 6th Ed USA: Andrew Allen:403.
- **Chatterjee, K and Chaudhuri ,J**. (1960). Serum Lipids in Malnutrition of Children. Indian Journal of Pediatrics, 28(**5**):195-202.
- **Chukwuma, B., Uche, R., Oluoha.,Kelechi, A., Uwakwe1., Kelvin, C., Diwe., Irene, A., Merenu., Ifeadike, O.,Chigozie, A., Anthony, C and Iwu**.(2015). Prevalence and Sociodemographic Determinants of Malnutrition among Under-Five Children in Rural Communities in Imo State, Nigeria.*Amer JPH R*; 3**(6)**:199-206.
- **Fossati,P**and **Prenciple,L.(**1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem.*28:2077-2080.
- **Garg, A., Grundy, S.M and Unger, R.H**(1992). "Comparison of effects of high and low carbohydrate diets on plasma lipoproteins and insulin sensitivity in patients with mild NIDDM". Diabetes. 41 *(***10**): 1278–85.
- **Heymsfield, S., Tighe, A and Wang, Z**. (1994*). Anthropometric and Biochemical Methods. In*, Shils, M.E., Olson, J.A and Shike, M. (eds)

Method Nutrition in Health and Disease 8th ed ; I Williams and Wilkins Phil, U.S.A : 812-838.

- **Irena, A.H.,Mwambazi, M. and Mulenga,V**.(2011).Diarrhea is a major killer of children with severe acute malnutrition admitted to inpatient set-up in Lusaka, Zambia. *NutrJ*, 10**(1)**:110.
- **Jancen,A.A.J**and **Mannet,W.T.**(1982). Assessment of nutritional status: a comparison of methods. *JTropPaediatr*, 28:38-40.
- **Jobiba, C., Andrew, T., Theresa, B.and Catherine, M.P.F**.(2008). The impact of HIV on mortality during in-patient rehabilitation of severely malnourished children in Malawi.*Trans R SocTrope Med Hyg*; 102: 639-644.
- **kaneta, k . , Choudhury , M . , Hanifi , S and Abbas , A .** (2000).gender inequality and severe malnutrition among children in a Remote Rural Area of Bangladesh .*J health populnutr* , center For Health and population Research; 18**(3):**123-130 .
- **Low,P.S., Saha,N**and **Tay,T.S**.(1996).Ethnic variation of cord plasma aoplipoprotein levels in relation to coronary risk level: a study in three ethnic groups of Singapore. Actapaeditr, 85:1476-82.
- **Marini ,A., Carulli ,G., Azzarà, A., Grassi, B** and**Ambrogi, F** *(*1989). "Serum cholesterol and triglycerides in hematological malignancies". *ActaHaematol*. 81 *(***2)***:* 75–9.)
- **Mcarty, M.F** (2004). "An elevation of triglycerides reflecting decreased triglyceride clearance may not be pathogenic relevance to high-carbohydrate diets*". Medical Hypotheses.* 63 (**6**): 1065–73.
- **Mesham , A.R and Chatterjee , M** .(1999) . Wasting away . The crises of malnutrition in India . Washington DC :The World Bank**.**
- **Micheal,L.B., Edward, P.F and Larry,E.S**. (2010). Clinical chemistry principles procedures correlations; 6th ed. Lippincou Williams and Wilkins :328.
- **Mishra,S.K.,Bastola,S.P and Jha.B**.(2009). Biochemical nutritional indicators in children with protein energy malnutrition attending kanti children hospital, *Kathmandu, Nepal.* 7(**26**):129-34.
- **Moutzouri, E; Elisaf, M** and**Liberopoulos, E.N.** (2011)."Hypocholesterolemia". *Current Vascular Pharmacology*. 9 (**2**): 200-12.
- **Mubarak , A . , Atta-ullah,M and Abid ,H** .(2003) . Acute hypokalemic flaccid paralysis in malnourished children .*Pak Pead J* ; 27**(4)** : 166-171 .
- **Nascimento,H., Costa,E., Rocha,P., Rego, C., Mansilha, H.F.,** and

Quintanilha,A.(2012). Cardiovascular risk factors in portuguese obese children and adolescents: Impact of small reduction in body mass index imposed by lifestyle modifications. *The open Biochemistry Journal*, 6:43-50.

- **Nassar, M.F., Dina, A .A.,Salwa , R.E and Soad ,M.G.** (2010). Markers of bone metabolism in Protein Energy Malnutrition. Intern *J Food NutrPublicHealth*; 3:59-70.
- **Ogunkeye, O.O** and**Ighogboja, I.S**. (1992). Increase in total serum triglyceride and phospholipids inKwashiorkor. *Ann Tropic Peadiator;*12:463-6.
- **Pauline, I. A. (**2008). *Biochemical changes associated with protein energy malnutrition among pregnant women in Enugu metropolis*. university of nigeriensrkka :13-40 .
- **Pejic, R.N, Lee, D.T.** (2006*). "Hypertriglyceridemia". J Am Board Fam Med.* 19 (**3**): 310–6.
- **Schutz ,Y. , Kyle ,U.U and Pilchard , C**.(2002) . Fat-free mass index and fat mass index percentiles in Caucasians aged 18-98 years .*Int J Obese Relate MetabDisorder* ; 26 :953-960..
- **Silva, M.E., Pupo, A.A** and**Ursich, M.J** *(*1987*).* "Effects of a high-carbohydrate diet on blood glucose, insulin and triglyceride levels in normal and obese subjects and in obese subjects with impaired glucose tolerance*".* *Brazilian Journal of Medical and Biological Research.* 20 (**3-4**): 339–50.
- **Soeters, P.B., Reijven, P.L.M., van Bokhorst-de van der Schueren, M.A.E., Halfens, R.J.G., Meijers, J.M.M and van Gemert, W.G.**(2008). A rational approach to nutritional assessment *Clin.Nutr* ; 27: 706-716.
- **Stanfield, P., Brueton , M ., Chan , M ., Parkins , M and Waterston, T.** (1978).*Diseases of Children in the Subtropics and Tropics:* 4th ed. Edward Arnold, Hodder and Stoughton. London : 335 - 366.
- **Teran, J.C.** (1999). Nutrition and liver disease .*CurrGastroenterol Rep;* 1:335-40.
- **Welcome Trust Working Party** . (1970). *50th Centile of Havard Standard*. Lancet, 2: 302.
- **World Health Organization (WHO)**. (1995) .*Physical status*. the use and interpretation of anthropometry. Report of a WHO expert committee. World Health Organization technical report series : 854.
- **World Health Organization (WHO).** (1997). The world Health Report 1997: *conquering suffering, enriching Humanity*. WHO. Geneva.

APPENDICES

Questionnaire

Appendix (I)

Sudan University of Science and Technology
Collage of graduate studies

Evaluation of plasma Lipid profile among Sudanese Children with Malnutrition in Khartoum state

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

Investigation requirement:

Plasma total cholesterol concentration=…… mg/dl Plasma triglyceride concentration= …… mg/dl
Plasma HDL-c concentration= ……... mg/dl
Plasma LDL-c concentration= …… mg/dl

COD 11505 1 x 50 mL	COD 11506 1 x 200 mL	COD 11506 1 x 500 mL	COD 11539 1 x 1 L
STORE AT 2-8°C			
Reagents for measurement of cholesterol concentration Only for *in vitro* use in the clinical laboratory			

Appendix II

CHOLESTEROL

BioSystems
REAGENTS & INSTRUMENTS

CE

CHOLESTEROL
CHOLESTEROL OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholestenol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2} O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\ H_2O_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

CONTENTS

	COD 11505	COD 11506	COD 11506	COD 11539
A. Reagent S. Standard	1 x 50 mL 1 x 5 mL	1 x 200 mL 1 x 5 mL	1 x 500 mL 1 x 5 mL	1 x 1 L 1 x 5 mL

COMPOSITION

A. Reagent. Pipes 35 mmol/L, sodium cholate 0.5 mmol/L, phenol 28 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

S. Cholesterol Standard. Cholesterol 200 mg/dL (5,18 mmol/L). Aqueous primary standard.

STORAGE

Store at 2-8°C.

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

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum or plasma collected by standard procedures.

Cholesterol is stable for 7 days at 2-8°C. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1)

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

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
4. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

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

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

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

$\frac{A_{Sample}}{A_{Standard}}$	x 200 = mg/dL cholesterol x 5.18 = mmol/L cholesterol

REFERENCE VALUES

The following uniform cut-off points have been established by the US National Cholesterol Education Program and have also been adopted in many other countries for the evaluation of coronary artery disease risk[3].

Up to 200 mg/dL = 5.2 mmol/L 200-239 mg/dL = 5.2-6.21 mmol/L > 240 mg/dL = > 6.24 mmol/L	Desirable Borderline High High

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.3 mg/dL = 0.008 mmol/L.
- Linearity limit: 1000 mg/dL = 26 mmol/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

Mean Concentration	CV	n
121 mg/dL = 3.13 mmol/L	1.1 %	20
257 mg/dL = 6.66 mmol/L	0.9 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
121 mg/dL = 3.13 mmol/L	1.9 %	25
257 mg/dL = 6.66 mmol/L	1.0 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides 10 g/L) does not interfere. Bilirubin (>10 mg/dL) and hemoglobin (>5 g/L) may affect the results. Other drugs and substances may interfere[4].

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

DIAGNOSTIC CHARACTERISTICS

Cholesterol is a steroid of high molecular weight and possesses the cyclopentanophenanthrene skeleton. Dietary cholesterol is partially absorbed and it is also synthesized by the liver and other tissues. Cholesterol is transported in plasma by lipoproteins. It is excreted unchanged into bile or after transformation to bile acids.

Increased total cholesterol values are associated with a progressively escalating risk of atherosclerosis and coronary artery disease[3,5].

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

NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Allain CC, Poon LS, Chan CSG, Richmond W and Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470-475.
2. Meraldini F, Principe L, Bardelli F, Giannini G and Tarli P. The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. Clin Chem 1978; 24: 2161-2165.
3. National Cholesterol Education Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: National Heart, Lung, and Blood Institute; 2001.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.

COD 11828	COD 11528	COD 11529
1 x 50 mL	4 x 50 mL	2 x 250 mL
STORE AT 2-8°C		
Reagents for measurement of triglycerides concentration Only for *in vitro* use in the clinical laboratory		

TRIGLYCERIDES

Appendix III

BioSystems
REAGENTS & INSTRUMENTS

CE

TRIGLYCERIDES
GLYCEROL PHOSPHATE OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Triglycerides in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2].

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{glycerol kinase}} \text{Glycerol} - 3 - P + ADP$$

$$\text{Glycerol} - 3 - P + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihidroxyacetone} - P + H_2O_2$$

$$2\,H_2O_2 + 4 - \text{Aminoantipyrine} + 4 - \text{Chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS

	COD 11828	COD 11528	COD 11529
A. Reagent	1 x 50 mL	4 x 50 mL	2 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent: Pipes 45 mmol/L, magnesium chloride 5 mmol/L, 4-chlorophenol 6 mmol/L, lipase > 100 U/mL, glycerol kinase > 1.5 U/mL, glycerol-3-phosphate oxidase > 4 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.0.

S. Triglycerides Standard: Glycerol equivalent to 200 mg/dL (2.26 mmol/L) triolein. Aqueous primary standard.

STORAGE

Store at 2-8°C.

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

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum or plasma collected by standard procedures.

Triglycerides in serum or plasma are stable for 5 days at 2-8°C. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1)

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

3. Mix thoroughly and incubate the tubes for 15 minutes at room temperature (18-25°C) or for 5 minutes at 37°C.
4. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

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

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

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

$\frac{A_{Sample}}{A_{Standard}}$	x 200 = mg/dL triglycerides x 2.26 = mmol/L triglycerides

REFERENCE VALUES

The following uniform cut-off points have been established by the US National Institutes of Health and have also been adopted in many other countries for the evaluation of risk[3].

Up to 150 mg/dL = 1.7 mmol/L	Normal
150-199 mg/dL = 1.70-2.25 mmol/L	Borderline-high
200-499 mg/dL = 2.26-5.64 mmol/L	High
> 500 mg/dL = > 5.65 mmol/L	Very high

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.6 mg/dL = 0.018 mmol/L
- Linearity limit: 600 mg/dL = 6.78 mmol/L. For higher values dilute sample 1/ 4 with distilled water and repeat measurement.
- Repeatability (within run):

Mean Concentration	CV	n
100 mg/dL = 1.13 mmol/L	1.7 %	20
245 mg/dL = 2.77 mmol/L	0.7 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
100 mg/dL = 1.13 mmol/L	2.6 %	25
245 mg/dL = 2.77 mmol/L	1.7 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (10 g/L) does not interfere. Bilirubin (2.5 mg/dL) may interfere. Other drugs and substances may interfere[4].

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

DIAGNOSTIC CHARACTERISTICS

Triglycerides are esters of glycerol and fatty acids coming from the diet or obtained by synthesis mainly in the liver. Triglycerides are transported in plasma by lipoproteins and used by adipose tissue, muscle and other. Their primary function is to provide energy to the cell.

Elevated serum triglycerides leves can be caused by liver disease, diabetes mellitus, nephrosis, hypothyroidism, alcoholism, familial hyperlipoproteinemia IV and V, and other[3,5].

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

NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Bucolo G and David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 1973; 19: 476-482.
2. Fossati P and Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28: 2077-2080.
3. National Cholesterol Education Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: National Heart, Lung, and Blood Institute; 2001.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.

Appendix IV

COD 11648 50 mL
STORE AT 2-8°C
Reagents for measurement of HDL cholesterol concentration Only for in vitro use in the clinical laboratory

CHOLESTEROL HDL PRECIPITATING REAGENT

BioSystems
REAGENTS & INSTRUMENTS

CE

CHOLESTEROL HDL
PRECIPITATING REAGENT

PRINCIPLE OF THE METHOD

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1,2]:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS AND COMPOSITION

A. Reagent: 1 x 50 mL. Phosphotungstate 0.4 mmol/L, magnesium chloride 20 mmol/L.

S. HDL Cholesterol Standard: 1 x 5 mL. Cholesterol 15 mg/dL. Aqueous primary standard.

STORAGE

Store at 2-8°C.

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

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity.
- Standard: Presence of particulate material, turbidity.

ADDITIONAL REAGENTS

These auxiliary reagents are to be used together with the Cholesterol Reagent contained in any of the BioSystems Cholesterol kits (cod. 11805, 11505, 11506, 11539).

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Desktop centrifuge.
- Thermostatic water bath at 37°C.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum or plasma collected by standard procedures.

HDL cholesterol in serum or plasma is stable for 7 days at 2-8°C. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

Precipitation

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

Sample	0.2 mL
Reagent (A) (Cholesterol HDL kit)	0.5 mL

2. Mix thoroughly and let stand for 10 minutes at room temperature.
3. Centrifuge at a minimum of 4000 r.p.m. for 10 minutes.
4. Carefully collect the supernatant (Note 2).

Colorimetry

5. Bring the Reagent (Cholesterol kit) to room temperature.
6. Pipette into labelled test tubes: (Note 3)

	Blank	Standard	Sample
Distilled water	100 µL	—	—
HDL Cholesterol Standard (S)	—	100 µL	—
Sample supernatant	—	—	100 µL
Reagent (A) (Cholesterol kit)	1.0 mL	1.0 mL	1.0 mL

7. Mix thoroughly and incubate the tubes for 30 minutes at room temperature (18-25°C) or for 10 minutes at 37°C.
8. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

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

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the HDL Cholesterol Standard provided has been used to calibrate (Note 4):

$\frac{A_{Sample}}{A_{Standard}}$	x 52.5 = mg/dL HDL cholesterol x 1.36 = mmol/L HDL cholesterol

REFERENCE VALUES

HDL cholesterol concentrations vary considerably with age and sex. The following cut-off point has been recommended for identifying individuals at high risk of coronary artery disease[3]:

Up to 35 mg/dL = 0.91 mmol/L	High
> 50 mg/dL = > 1.56 mmol/L	Low

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005 and 18009) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 3.0 mg/dL = 0.078 mmol/L.
- Linearity limit: 150 mg/dL = 3.9 mmol/L.
- Repeatibility (within run):

Mean Concentration	CV	n
20 mg/dL = 0.78 mmol/L	3.3 %	20
55 mg/dL = 1.42 mmol/L	2.0 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
30 mg/dL = 0.78 mmol/L	4.2 %	20
55 mg/dL = 1.42 mmol/L	3.2 %	20

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

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

DIAGNOSTIC CHARACTERISTICS

HDL play an important part in the removal of cholesterol from tissues and its transportation to the liver for removal as bile acids.

Decreased plasma HDL-cholesterol concentrations are positively correlated with the incidence of atherosclerotic diseases, basis of myocardial infarction and cerebrovascular accidents[3,5].

There are several disease states or environmental influences associated with reduced levels of HDL: acute or chronic hepatocellular diseases, intravenous hyperalimentation, severe malnutrition, diabetes, chronic anemia, myeloproliferative disorders, Tangier disease, anolphalipopro-leinemia, acute stress, some drugs and smoking[3,5].

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

NOTES

1. Sample and Reagent A volumes may be varied as long as the same ratio is maintained.
2. Supernatant must be clear. When supernatant is turbid or the pellet floats, add again 0.5 mL of Reagent A, mix thoroughly and centrifuge. Multiply the obtained concentration by 1.7 (dilution).
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
4. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Grove TH. Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin Chem 1979; 25: 560-564.
2. Burstein M, Scholnick HR and Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Scand J Clin Lab Invest 1980; 40: 583-595.
3. National Cholesterol Education Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: National Heart, Lung, and Blood Institute; 2001.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co. 2005.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press 2001.

BioSystems S.A. Costa Brava 30, 08030 Barcelona (Spain)

Quality System certified according to