Assessment of Acute Malnutrition Using Transthyretin Levels in Blood of Children Under Five Years of Age in Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital, Addis Ababa

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This is to certify that the thesis prepared by Behailu Tsegaye entitled: Assessment of Acute Malnutrition Using Transthyretin Levels in Blood of Children Under Five Years of Age in Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital, Addis Ababa and Submitted in the Partial Fulfillment of the Requirements for the Degree of Master of Science (Medical Biochemistry) compiles with regulation of the university and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this research paper titled on assessment of acute malnutrition using transthyretin levels in blood of children under five years of age in Tikure Anbesa Specialized Hospital and Yekatit 12 Hospital, Addis Ababa is my original work and has not presented for a degree in any other university, and that all sources of materials used for the research have been properly and suitable acknowledged.

Behailu Tsegaye

Signature_____

Date_____

To the Memory of My Mother

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List of Abbreviations

MAM……Moderate acute malnutrition

SAM…….....Severe acute malnutrition

GAM…….Global acute malnutrition

BMI……...Body mass index

PEM……...Protein energy malnutrition

EDHS……Ethiopian Demographic and Health Surveys

MDG1……Millennium Development Goal

TTR……....Transthyretin (pre-albumin)

TBG……...Thyroxine binding globulin

RBP……....Retinol-binding protein

APR……Acute phase response

CRP……C reactive protein

MUST……....Malnutrition Universal Screening Tool

MUAC……Mid upper arm circumference

NS…….. Nutritional status

WHO……..……World health organization

SD……...Standard deviation

SE……Standard error

ROC……....Receiver operative curve

AUC…….... Area under the curve

Operational definitions

Malnutrition: It is the condition that results from an imbalance between dietary intake and requirements. It includes under nutrition, which results from less food intake and hard physical work and over nutrition results from excess food intake and less physical activities (Melkie, 2004).

Anthropometry: Measurement of the variation of physical dimensions and the gross composition of the human body at different age levels and degrees of nutrition by weight-for-age, height-for-age and weight-for-height (WHO, 2009).

Moderate Acute Malnutrition (MAM):- is identified by moderate wasting (WFH < -2 z-score and. -3 z-score for children under 5 years or MUAC < 125 mm and ≥115 mm for children 6-59 months).

Severe Acute Malnutrition (SAM):- is identified by severe wasting (WFH < -3 z-score for children under 5 years or MUAC < 115 mm for children 6-59 months) or the presence of bilateral pitting edema.

True Positive (TP):- The response is Positive, and the Prediction is also positive or Persons who have disease and are test positive**.**

True Negative (TN):- The response is Negative, and the prediction is also negative or Persons who do not have disease and are test negative**.**

False Positive (FP):- A negative response is falsely predicted as Positive or Persons without disease but with positive test**.**

False Negative (FN):- A positive response is falsely predicted as Negative or Person with disease but with negative test.

Sensitivity is defined as the proportion of cases predicted as positive among all positive responses: n (TP) / [(n (TP) + n (FN)]. Ability of the test to be correctly positive among those who are known to have the disease

Specificity is the proportion of cases predicted as negative among all negative responses. n(TN)/[n(TN)+n(FP)]. Ability of the test to be correctly negative among those who are known to be without disease.

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Abstract

Background: *- Malnutrition is one of the leading causes of morbidity and mortality in children under the age of five years in developing countries, including in Ethiopia. The most important forms of malnutrition in Ethiopia is protein energy malnutrition, but there is no reliable laboratory method present to assess acute malnutrition. Transthyretin level of immunochromatography method is one of the newly introduced method for the identification of children with acute malnutrition recently admitted to Hospital and used as valuable laboratory measurements in the identification of patients requiring malnutrition assessment and nutritional support.*

Objective: *- To evaluate acute malnutrition status in children of age less than five years, who attend in Tikur Anbessa Specialized Hospital and Yekatit 12 hospital; by using anthropometric methods, and by measuring albumin and transthyretin level in blood serum samples.*

Methods: *- Hospital based cross sectional study design was applied from August 2014 to December 2014. 51 malnourished and 51 non- malnourished children were recruited for this study. Anthropometric measurements (weight, height, length, MUAC) were performed and serum transthyretin and albumin levels were measured as biochemical parameters in these hospitalized patients.*

Results*: - The mean age of the cases and controls were 1.14 year and 1.48 year, respectively. Comparison of mean between control and study group shows 14.32 cm and 10.45 cm for MUAC, 4.24 g/dl and 3.86 g/dl for albumin and 303.08mg/l and 132.89 mg/l for transthyretin for control group and study group respectively. All above mean values shows significant difference between controls and study groups with p value < 0.0005. Prevalence of malnutrition assessed by weight for height were categorized as 21.6% for moderate malnutrition and 78.4% for severe malnutrition. Using MUAC it could be predicted that 23.5% were moderately malnourished and 76.5% were severely malnourished. But using transthyretin tests, the prevalence of 82.4% for moderate malnutrition and 13.7% for severe malnutrition were obtained. Multivariate analysis revealed positive correlation between albumin and transthyretin level (r = 0.307, P=0 .03), but there was no significant correlation between anthropometrics measurement with biochemical measurements. The sensitivity and specificity of transthyretin using MUAC as reference standard were 91.60 %, and 15.38% (0.0318 Kappa agreement). The sensitivity of transthyretin using weight for height (wasting) as reference standard was 100 %, and specificity was 17.5 %. (0.16 Kappa agreement). Transthyretin validation by using MUAC as reference material was found to be a sensitivity of 81.82 % and specificity of 47.5 % with cut off of transthyretin 120 mg/l.*

Conclusions: *- Transthyretin is a better acute malnutrition marker in the serial nutritional assessment and it is a cost effective, feasible, reliable tool for malnutrition screening, particularly for the purpose of settings where it is difficult to perform a more detailed and comprehensive nutritional assessment.*

Key words: *- Transthyretin, Albumin, Anthropometric parameters, Acute Malnutrition.*

1. 0. INTRODUCTION

Nutrition is a fundamental pillar of human life which provides adequate energy and nutrient to the cells for them to perform their physiological function of growth, reproduction, defense and repairs (WHO, 1999; WHO, 2000). Malnutrition is a deficiency state of both macro and micro nutrients and their over consumption, causing measurable adverse effects on human body structure and function resulting in specific physical and clinical outcomes (Gudina *et al*., 2013). However, in many developing countries, under and over-nutrition occur simultaneously. This phenomenon is referred to as the double burden of malnutrition (Laura, 2011). Child under nutrition remains one of the major public health problems and the leading contributor to child morbidity and mortality in the world (Gudina *et al*., 2013).

Nutrition, infection and the functions of the immune system are interrelated. Malnutrition can predispose an individual to infection and diseases, and make recovery from disease slower. Infections and diseases can lead to malnutrition and nutritional deficiencies by increasing nutrients requirements, utilization, nutrients losses and metabolism as the body tries to generate immune responses against the invading pathogens (Charles *et al.,* 2014). Malnutrition at the early stages of life can lower child resistance to infections, increase child morbidity and mortality, and decrease mental development and cognitive achievement and nutritional status is the best global indicator of wellbeing in children (Solomon *et al.,* 2013). Likewise, good nutrition enhances immunity and the ability to fight infections and diseases (Charles *et al.,* 2014).

Millions of children living in low-income countries suffer from under nutrition, which continues to be a major public health problem in developing countries. It is the most important risk factor for the burden of disease causing about 300, 000 deaths per year directly and indirectly responsible for more than half of all deaths in children (Tsinuel *et al*., 2013; Solomon *et al.,* 2013). Malnutrition is the largest single underlying cause of death worldwide and is associated with over one third of the deaths in young children. It is an underlying cause of the death of 2.6 million children each year, and one-third of the global total of children's (7.6 million child) deaths each year before their fifth birthday through weakening the body's resistance to illness. Malnutrition is a silent killer that are under reported, under addressed and, as a result, under prioritized. Every hour and minute of

every day, 300 and 5 children die because of malnutrition respectively. In the world today, one child in four is stunted due to malnutrition, and in developing countries this figure is as high as one in three and specifically in Africa two out of five children's will suffer from malnutrition (Solomon *et al.,* 2013).

Problems responsible for child under nutrition are numerous. Some are basic problems, like political instability, slow economic growth, and lack of education. Others are underlying causes such as food insecurity and lack of maternal and child care services. The third groups include the highly specific risk factors like frequent infections and inadequate dietary intake (Gudina *et al*., 2013).The improvement of nutrition therefore, is the main prerequisite for the reduction of high infant and under five mortality rates, the assurance of physical growth, social and mental development of children as well as academic achievement (Agozie *et al.,* 2012) not only of current generations but also of the ones to come (Solomon *et al.,* 2013). Globally, under nutrition, which refers to both protein-energy malnutrition and micronutrient deficiency (Camille *et al*., 2014; Se-Eun *et al*., 2012), is the cause of around 3.1 million child deaths annually in low and middle-income countries. Under-nutrition encompasses acute (wasting) and chronic (stunting) forms of malnutrition, and underweight together with deficiencies of other essential micronutrients (Gudina *et al*., 2013).

Protein energy malnutrition is the most frequent form of malnutrition, and it is caused by an imbalance between the supply of protein and energy and the body's demand for them to ensure optimal growth and function (Dechenla *et al*., 2014; Eugenia *et al.*, 2014). Protein-energy malnutrition in children is clinically classified as marasmus (severe thinness), kwashiorkor (bilateral pitting edema), and marasmic kwashiorkor (mixed condition) (Tsinuel *et al*., 2013). Although inadequate protein in the diet, even with an adequate caloric intake, is the major cause of kwashiorkor, consumption of insufficient protein and calories are known to be responsible for marasmus (Andrew *et al.,* 2013).

1.1. Acute malnutrition

Acute malnutrition or wasting is often associated with short-term factors like seasonal variation in food availability (Gudina *et al*., 2013) , acute decrease in food intake often combined with illness,

anorexia, poor appetite, and sometimes medical complications, leading to rapid weight loss or failure to gain weight. Children suffering from this condition have a high mortality risk; however, the situation is reversible with treatment of medical complications and re-feeding in a short period of time (Camille *et al*.,2014).About 10% (55 million children) aged under five is wasted worldwide of which the highest estimate (16%) is reported from South-central Asia. Out of these, 3·5% or 19 million children in developing countries are severely wasted with the highest proportion living in south central and middle Africa . On the other hand, between 36 and 41 million of these children are moderately wasted and about 80% of them live in low income countries. In Ethiopia, the overall prevalence of wasting among under five children currently is 10%, out of which 3% are severely wasted (Gudina *et al*., 2013).

1.1.1. Moderate Acute Malnutrition (MAM)

Globally, approximately 33 million children under five years of age are affected by moderate acute malnutrition (MAM). It is defined as a weight-for-height z-score (WHZ) between -2 and -3 or mid-upper arm circumference (MUAC) ≥ 11.5 cm and < 12.5 cm and no edema (Camille *et al*., 2014, Lindsey *et al.,* 2013). In Ethiopia, moderate malnutrition also accounts for 40% of the deaths of under five children (Gudina *et al*., 2013).

1.1.2. Severe Acute Malnutrition (SAM)

Globally, approximately 19 million children under 5 years are affected by SAM in world. An estimated 0.5 to one million deaths are attributed to SAM (Camille *et al*., 2014). Directly or indirectly, malnutrition contributes to 53% of deaths of children under-five in developing countries (Henock *et al.,* 2013).In Ethiopia; severe malnutrition also accounts for 11% of the deaths of under five year children (Gudina *et al*., 2013). Severe acute malnutrition (SAM), defined as weight-for-height below −3.0 z scores of the median WHO standards in children 6–59 months of age, and mid-upper arm circumference (MUAC) less than 11.5 cm, and the presence of bilateral pitting edema (Roy,2000).

SAM occurs in one of two forms, marasmus or kwashiorkor. Kwashiorkor refers to a condition commonly thought to occur when carbohydrate is the major dietary energy source and protein is relatively absent from the diet for a prolonged period of time. Hypo-albuminemia is a feature of

kwashiorkor and is part of the clinical symptomology that includes edema, ascites, dermatitis, thin brittle hair, hepatomegaly, and muscle wasting. In contrast, marasmus refers to chronic deprivation of adequate dietary energy to maintain body weight. Severe marasmus is characterized by severe muscle wasting, extreme weight loss and cachexia (Mikkel *et al.,* 2013, Patricia *et al.,* 2004).

1.2. Chronic malnutrition

Chronic malnutrition (Stunting) affects approximately 178 million children under 5 years old in the developing world. Africa and Asia have high stunting rates of 40% and 36% respectively, and more than 90% of the world's stunted children live on these two continents (Laura, 2011). Stunting or chronic malnutrition results from inadequate nutrition, care and health, over long period of time, leading to failure of linear growth and poor development. It does not usually pose an immediate threat to life but it is associated with chronic disease risk in the long-term (Camille *et al*.,2014) and it decreases two standard deviation below the height-for-age index (Se-Eun *et al*.,2012).

1.3. Underweight

Underweight is a general measure that captures the presence of wasting or stunting. It is therefore a composite indicator, reflecting either acute or chronic under nutrition without distinguishing between the two. An estimated 112 million children under-five are underweight. Nearly one in four and 10% of under-fives in the developing world are severely underweight. The prevalence of underweight among children is higher in Asia than in Africa, with rates of 27% compared to 21%. This is mainly due to stunting rather than wasting (Laura, 2011).

1.4. Nutrition Status of children under five years of age in Ethiopia

Ethiopia is the second most populous country in Africa, at nearly 84 million. Approximately 14% are children under five years of age (Dechenla *et al*., 2014; Bemnet *et al*., 2012). Malnutrition is one of the leading causes of morbidity and mortality in children under five years of age in Ethiopia. The country has the second highest rate of malnutrition in Sub-Saharan Africa. Malnutrition in children is one of the most serious public health problem in Ethiopia and the highest in the world (Solomon *et al.,* 2013).

Problems responsible for child under-nutrition are numerous in Ethiopia. Some are basic problems, like widespread poverty, poor infrastructure, high population pressure, slow economic growth, and lack of education. Others are underlying causes such as inadequate access to clean water, poor health and sanitary conditions, food insecurity and lack of maternal and child care services. The third groups include the highly specific risk factors like frequent infections, inadequate dietary intake, limited knowledge of nutritional matters among certain households, and fluctuations in incomes are some of the principal reasons for the high prevalence of malnutrition (Gudina *et al*., 2013; Edris, 2007).

The most important forms of malnutrition in Ethiopia are protein energy malnutrition (PEM), vitamin A deficiency, Iodine deficiency disorders, and Iron deficiency anemia. The problem is more severe among children aged 1-3 years who suffer from Kwashiorkor and Marasmus and underweight (Edris, 2007). Diarrhea and other infectious diseases manifested in the form of fever affect both dietary intake and utilization, which may have a negative effect on improved child nutritional status. The consequences of malnutrition in children also include poor physical development and limited intellectual abilities that diminish their working capacity during adulthood. Local and regional studies in Ethiopia have also shown an increase in malnutrition with increase in age of the child (Girma *et al.,* 2002).

Nutrition outcome data which are taken from the three most recent Ethiopia Demographic and Health Surveys (EDHS) shows that rates have decreased quite a bit in the past decade, most notably with mortality almost halving. However; Ethiopia still needs a concerted effort to accelerate reductions in under-nutrition (Government F. D.E, 2013).

Table 1:- Ethiopia Demographic and Health Surveys (EDHS) (Government F. D.E, 2013).

Indicator	2000	2005	2011	2015 estimate
Infant mortality rate (per 1000 live births[Lb])	-	77	59	-
Children <5 year mortality rate (per1000 Lb)	166	123	88	59
Maternal mortality rate (per 100,000Lb)	-	673	676	-
Children < 5 years underweight	42.1	38	29	23.8
Children <5 year stunted	57.8	52	44	-
Children < 5 years wasted	11	12.4	9.7	-

2.0. LITERATURES REVIEW

2.1. Transthyretin (TTR)

Transthyretin is a 55 kDa, 127 amino acid, found in the plasma and cerebrospinal fluid (Maria *et al.,* 2004, Essam *et al.,* 2005) that is synthesized by the hepatic parenchymal cells or, in fetal life in the yolk sac endothelium. Small amounts of transthyretin are also synthesized by the choroid plexus of the brain, pancreas, and retina, but these probably do not affect the serum protein concentration (Myron *et al.,* 2007).It acts as a transport protein for thyroxine and as a carrier for retinol binding protein (RBP) (Banh,2006). This protein carrying thyroid hormones, as well as retinol (vitamin A) through its interaction with retinol binding protein by forming a tri-molecular complex (Ingenbleek,2002, Maria *et al.,* 2004 and Essam *et al.,* 2005).

Transthyretin was originally named prealbumin by its electrophoretic migration in front of albumin by alkaline electrophoresis (Ingenbleek, 2002). However, it was subsequently shown that transthyretin bind and carry thyroxin (T4), triiodothyronine (T3) and holo-retinol-binding protein (RBP with retinol, or vitamin A) and the name was changed to transthy (roxin) retin(ol) to denote its dual transport function (Wei Zheng, *et al.,*2001, Myron *et al.,*2007).Transthyretin is a tetramer of four identical subunits. Although each of the four monomers has a binding site for RBP (retinol-binding protein), the tetramer binds only one molecule of RBP with high affinity and possibly a second with lower affinity. The binding affinity for holo-retinol-binding protein (apo-RBP ,RBP without retinol) is very low, and the loss of retinol (uptake by tissues) results in the separation and renal excretion of free apo-RBP, accounting for the very short biological half-life of RBP of ;3.5h. Each TTR monomer also has two binding sites for thyroid hormones, but binding of one molecule of T3 or T4 significantly reduces the affinity of the second site. Binding affinity for T3 is lower than that for T4 (Richardson, 2002, Myron *et al.,* 2007).

The hepatic synthesis of transthyretin necessitates a high concentration of the essential amino acid tryptophan, which plays a major role in protein synthesis initiation and is eminently sensitive to recent changes in nutritional status both protein and energy depletion (Bae *et al*., 2011). Transthyretin and retinol-binding protein both manifest rapid turnover rates, and their concentrations quickly decline in response to any change of protein nutritional status and under

stressful conditions. The decrease in Transthyretin is accompanied by concerted decreases in other binding proteins (Ingenbleek, 2002

2.2. Albumin

Albumin is a globular protein of a molecular size of 69,000 Daltons, spherical and highly soluble, consisting of a single polypeptide chain of 585 amino acids. Albumin is very flexible and changes shape readily with binding of ligands and with variations in environmental conditions (Fuhrman et al, 2004). The prognostic value of the visceral protein albumin, has historically been used as a measure of acute and chronic malnutrition and the most frequently used laboratory marker (Barker *et al.,* 2011). However, with a half-life of ~14-20 days, albumin is relatively insensitive to rapid changes in nutritional status taking approximately 14 days to return to normal status (Beck *et al.,* 2002). Optimum albumin status occurs in an environment where nutritional adequacy relating to a sufficient supply of amino acids, hormonal and osmotic balance and the biological energy ATP and/or GTP is appropriate for synthesis (Nicholson *et al.,* 2000).

Albumin is produced exclusively in the liver and is the most abundant visceral protein, despite as little as 5% being synthesized and degraded daily (Marshall, 2008). The rate of synthesis varies with nutritional and disease status. The concentration of serum albumin is the net result of its synthesis, breakdown, volume of distribution, and exchange between intra and extra vascular spaces, as well as losses (Fuhrman et al, 2004). In the human body, albumin has several functions. One of its major functions is maintaining the colloid oncotic pressure, and the regulation of the tissue fluid distribution. Another albumin function is binding and transport of compounds, like drugs and endogenous compounds; bilirubin, haematin, ascorbate, tryptophan, bile acids, copper, zinc, folate, and eicosanoids. It is also used as a carrier protein for thyroid hormones, steroids and fatty acids is a main driver that influences. Additional functions of albumin are its anticoagulant effects, acting as a plasma buffer, having antioxidant properties.

Furthermore, albumin is considered as a negative a cute-phase protein, as it decreases by 25% during physiological stress (Fuhrman et al, 2004; Banh, 2006). The re-apportioning of albumin between intravascular and extravascular space occurs during times of acute and chronic stress on the body as does the catabolism and anabolism of albumin (Barker *et al.,* 2011).

Research has suggested that albumin is a preferable indicator of chronic rather than acute nutrient deficiency (Spiekerman, 1995). The sensitivity of albumin in acute protein malnutrition is more difficult to perceive because of the large half-life and body pool. A shift to low serum albumin concentration has been shown to be an excellent independent predictor of morbidity and hypo-albuminaemia is a valid negative prognostic factor associated with increased mortality. Concluding that albumin is a better predictor of morbidity and mortality than an aberration in nutrition status (Barker *et al.,* 2011).

2.3. Assessment of Malnutrition

It needs considerable efforts to identify patients at risk of malnutrition, with a view to early provision of nutritional support (Shenkin, 2006). Nutritional assessment is the process used to evaluate the nutritional status of the body. Multiple nutritional variables have been used to assess malnutrition and are largely based on an array of anthropometry, biochemical, and clinical and dietary assessments (Laura, 2011). A full nutritional assessment is a complex process, involving detailed assessment of nutritional intake, changes in body composition, signs or symptoms of nutritional deficiency or excess, and laboratory tests, and it should include not only protein-energy status but also vitamins and essential trace elements. Because of this complexity, rapid screening tests have been sought to identify patients who may already be malnourished or are at risk of malnutrition, who can then undergo a more detailed nutritional assessment (Shenkin, 2006).

Malnutrition may be measured in many ways such as, Clinical grading standard, weight-for-height (WFH) index, height-for-age (HFA) index, weight-for-age (WFA) index, body mass index, and skin fold thickness are among those utilized most frequently in the field (Se-Eun *et al*.,2012). Malnutrition Universal Screening Tool (MUST) is one of the methods, but which does time consuming and unreliable, with some indicators have to rely on subjective opinion. In addition, there are the numerous difficulties involved in taking measurements from elderly and bed bound patients who cannot be weighed accurately (Wei Zheng, *et al.,* 2001).

Increasingly, health institutions are aware that focusing on the body mass index for malnutrition monitoring is unreliable. Their limitations and lack of standardization for nutritional assessment have led to interest in laboratory tests for certain biological markers of nutritional status such as

prealbumin, plasma albumin, cholesterol, zinc, iron, vitamin B12 and folic acid, levels of which are significantly lower in malnourished patients. These markers have yet to be routinely used in combination to specifically assess the nutritional status of patients (Wei Zheng, *et al.,* 2001).

2.3.1. Anthropometric Methods of Malnutrition measurement

Anthropometric measurements are well established and widely used as indicators of health and nutritional status in children (Chakraborty *et al.,* 2011). It allows classification of a patient into categories of nutritional status (underweight, stunted and wasting), overweight and obesity according to developed standards or reference data, usually based on large numbers of healthy people from population (Margo, 2012, Khadilkar *et al*., 2007 and WHO, 2010).

It measures the variations in physical growth at different ages and the degree of nutrition. It is a widely used, simple, accurate, rapid, quantitative, inexpensive and non-invasive measure of the general nutritional status of an individual or a population group. These measurements may be performed as part of the physical examination. It is also used as well established and widely used as indicators of health and nutritional status in both children and adults. Despite some limitations, anthropometry remains the most practical tool for the assessment of nutritional status among members of the community in developing countries (Chakraborty *et al.,* 2011).

Mid upper arm circumference (MUAC)

Mid-upper arm circumference (MUAC) is anthropometric measurement used to evaluate adult and children nutritional status that has been found to be particularly effective in determining malnutrition among adults and children in developing countries. MUAC used for the simple screening of nutritional status (Chakraborty *et al.,* 2011). This is useful for screening a large number of children but less useful in long term growth monitoring. The techniques to measure mid-arm circumference include, accurate measurement with a tape and a simple bangle test. Bangle test using plastic bangles of an inner diameter of 3.7 cm (Red Bangle) and 4 cm (Yellow bangle) (Harshal *et al.,* 2012). All MUAC are graduated in millimeters and are color coded (red, yellow and green) to indicate the nutritional status of a child or adult. The color codes and gradations vary depending on the tape type. The bangle was passed up the forearm and the upper arm to decide if the upper mid-arm circumference was below or above 12.5 cm (UNICEF, 2012).

2.3.2. Laboratory Method of Assessment of Malnutrition

Nutritional assessment by using Transthyretin

The association between poor nutrition and illness has long been recognized, there is a lack of reliable, objective, short-term screening methods to evaluate nutritional risk. Determination of the transthyretin level is a cost-effective and objective method of assessing severity of illness in patients who are critically ill or have a chronic disease. Transthyretin is the earliest laboratory indicator of nutritional status and has emerged as the preferred marker for malnutrition because it correlates with patient outcomes in a wide variety of clinical conditions (Beck *et al.,* 2002).

There is a large volume of published studies describing the role of transthyretin in the detection of malnutrition risk (Devakonda *et al*., 2008; Saka, 2011; Johnson *et al.*, 2007). Potter and Luxton (1999) examined the effectiveness of transthyretin levels as a routine diagnostic tool for assessing malnutrition. The study evaluated the effect of malnutrition on emergency department admissions, comparing albumin and transthyretin levels as nutritional markers on hospitalized patients (Potter *et al*., 1999). A similar study by Devoto *et al*., (2006) shows as transthyretin level is a sufficient and reliable screening tool in the evaluation of malnutrition after comparing transthyretin with Detailed Nutritional Assessment (DNA), Subjective Global Assessment (SGA) and the Prognostic Inflammatory and Nutritional Index (PINI) (Devoto *et al.,*2006).

An in-depth research study into the role of transthyretin as a protein marker of nutritional status was first introduced by Ingenbleek in1975, he proposed the index of protein and energy malnutrition characterized by serum transthyretin changes and a faster response to re-feeding. In this year Ingenbleek, assessed malnutrition by-comparing the response of four hepatic proteins: transferrin, albumin and transthyretin-retinol binding protein complex, findings suggested that albumin had low sensitivity, transferrin intermediate and transthyretin -retinol binding protein complex the highest sensitivity (Ingenbleek *et al*., 1975).

Different laboratory techniques allow transthyretin to be measured cost effectively. In the past, most labs analyzed transthyretin levels nephelometrically on specialized instruments. In recent years, this technique has been replaced by more cost-effective immunoturbidimetric technology, which is more suitable for use on a high-volume, automated platform (Wei Zheng, *et al.,* 2001).

Detecting acute changes in nutritional status requires a serum transthyretin level. Because its half-life is just 2 days, its serum level reflects rapid changes in visceral protein status (Cindy, 2006) and not altered by stress or acute inflammation (Myron, *et al.,* 2007). Transthyretin is therefore more sensitive to changes in protein energy status than albumin, and its concentration closely reflects recent dietary intake rather than overall nutritional status (Shenkin, 2006). It is important in the evaluation of nutritional deficiency, nutrition support and it has been deemed as a valid and clinically useful indicator of protein energy malnutrition (Sathishbabu, 2012).

Moreover, transthyretin concentration in plasma, like that of albumin, is affected by changes in trans-capillary escape and its plasma concentration falls in acute phase reactions owing to increased movement into the extravascular compartment (Marshall,2008;Shenkin,2006). Its plasma concentration falls in protein-energy malnutrition and increases with re-feeding. A low transthyretin concentration can therefore be regarded primarily as a signal identifying patient at-risk who requires careful assessment and monitoring and for whom nutritional support may be needed as part of the treatment plan (Shenkin, 2006). However, like albumin, thus, a low concentration may reflect a poor recent nutritional intake or be a result of severe illness, though both of these are risk factors for the development of malnutrition. Different studies have shown that admission transthyretin concentrations are good predictors of risk of malnutrition (Marshall, 2008). Associated with its half-life of two days, transthyretin responds more quickly to a change in nutritional status than albumin (Ingenbleek, 2002).

2.5.2. Nutritional assessment by using Albumin

Studies have shown that among various biochemical markers, serum albumin is widely used to assess malnutrition and the level of albumin in the serum reflects the visceral protein status. But however; there are many factors which can influence the synthesis, distribution, degradation of albumin in the body. Even though albumin is well known biomarker for assessment of malnutrition because of longer half-life it cannot be a sensitive indicator for nutritional therapy (Sathishbabu, 2012).This longer half-life makes the albumin level changes slowly in response to reduced protein intake and make a good indicator of chronic malnutrition, it means it's not a good tool for identifying patients at risk for protein-calorie malnutrition or for monitoring the short-term effects of nutritional therapy (Cindy, 2006;Banh,2006).

Although albumin synthesis decreases rapidly in response to a decrease in food intake, so too does its rate of catabolism; these factors, together with the size of the body's albumin pool and its low rate of turnover (plasma half-life approximately 20 days) result in there being little change in its plasma concentration in simple starvation. Even with long-term protein under nutrition accompanied by loss of lean body weight and impaired muscle function, plasma albumin concentrations may remain within the normal reference range (Marshall, 2008).

The main factor affecting plasma albumin concentration in patients is the rate of trans-capillary escape into the interstitial fluid. This trans-capillary escape of albumin is markedly increased in disease (as part of the systemic inflammatory response syndrome (SIRS)), leading to decreased plasma albumin concentrations. It is inevitable that postoperative patients and patients with severe infection will have low plasma albumin concentrations. The more severe the disease, the lower the albumin, and therefore the lower the albumin, the worse the prognosis (Shenkin, 2006). The fact that low serum albumin concentrations correlate well with poor outcome in a variety of conditions and clinical settings reflects the effects of systemic illness, not nutritional status. Measurements of albumin concentration cannot therefore be recommended for nutritional assessment (Marshall, 2008).

2.4. Statement of the problem

The nutritional status of children has an impact on their health and development. Therefore, the physical, mental, and social and nutritional status of children, as well as other characteristics related to malnutrition should be evaluated periodically to monitor malnutrition, thereby enabling appropriate measures that can improve nutrition assessment.

Malnutrition is previously measured via anthropometric methods such as body mass index (BMI), MUAC, age, height and weight. Increasingly, health services are recognizing that anthropometric methods alone for monitoring of malnutrition is unreliable, and that the use of these measurements in conjunction with specific malnutrition assessment laboratory tools provide a better indication of malnourishment. Limitations of anthropometric methods and lack of standardization for nutritional assessment have led to interest in laboratory tests for certain biological markers of nutritional status such as transthyretin, plasma albumin, transferrin and retinol binding protein levels of which are significantly lower in malnourished patients.

Currently there is no fast and reliable laboratory method to assess acute malnutrition. Hence we want to introduce this new immunochromatography transthyretin assay method as a reliable, accurate, fast and much more cost-effective, than methods used previously to assess acute malnutrition in children of under-five years age group. Associated with its half-life of two days, transthyretin responds more quickly to a change in nutritional status than albumin. The purpose of this research is to evaluate and validate how transthyretin is reliable marker than anthropometric methods and albumin, in fast testing of acute malnutrition in hospitalized children of less than five years old.

2.5. Significance of study

There are many studies done in Ethiopia on malnutrition of children age of less than five years old, but most of these researches were done by anthropometric methods and by measuring albumin and other proteins in blood samples. This research was performed by measuring transthyretin level in blood as a reliable diagnostic tool and validate test method as a fast indicator of acute malnutrition. The method is noninvasive and can be used as valuable laboratory based investigation for the assessment of nutritional status in children. The method involved is simple, handy and more accurate test kit which utilizing a small strip of immunochromatography paper and a reader with the size of a small calculator.

This method is new and has never been used in Ethiopia and our research hopefully gives a better picture of nutritional status of hospitalized children in Addis Ababa and its surrounding. Not too many laboratory based studies were conducted previously in our country and our findings could add more information and data for nutritionists, clinicians, stake holders and families of malnourished children. This laboratory based investigation, as a sensitive and accurate test method, can be used in the future, side by side the anthropometric methods as reliable way of assessment of acute malnutrition in children. Our country does not have a set standard for transthyretin or albumin levels that can be used as a reference. If this method is validated then, we can think of working in larger population studies so that we can statistically calculate the national standard; which can be used as a reference.

2.6. Hypothesis

Laboratory testing of transthyretin level in blood of children less than five years could give a better and reliable picture of acute malnutrition and hence could serve as a dependable marker along with anthropometric measurements.

3.0. OBJECTIVE

3.1. General Objective

To evaluate acute malnutrition status in children of age less than five years, who attend Tikur Anbessa Specialized Hospital and Yekatit 12 hospital; by using anthropometric methods, and by measuring albumin and transthyretin level in blood serum samples.

3.2. Specific Objective

- ✓ To assess the prevalence of acute malnutrition in children of under-five years of age by using transthyretin measurement in blood.
- ✓ To evaluate the valuable advantage of short lifespan of transthyretin in the measurement of protein energy malnutrition.
- ✓ To determine malnutrition in children of under five years of age using anthropometric methods.
- ✓ To compare and contrast the results obtained through determination of transthyretin level and anthropometric methods.
- ✓ To validate transthyretin measurement as valuable laboratory based method for the assessment of nutritional status in children.

4.0. MATERIALS AND METHODS

4.1. Study design

Hospital based cross sectional study design was conducted to assess acute malnutrition in children age of less than five year.

4.2. Source and Study population

4.2.1. Source Population

The source of population was all hospitalized children age of less than five years who are suspected of under malnutrition and attending Tikur Anbessa Hospital and Yekatiti 12 Hospital in Addis Ababa.

4.2.2. Study population

The study population consisted 51 malnourished children and 51 normal children of a total 102 children who had not started medication and treatments were considered in the study.

4.3. Study Subjects

Study subjects were children, age of less than five year, who are affected by acute malnutrition, those who have attended in the study periods, who fulfilled the inclusion criteria and who gave their informed consent and assent were included in the study.

4.4. Study Area and period

The study was conducted in Tikur Anbessa specialized Hospital and Yekatit 12 Hospital in Addis Ababa from August – December 2014. In this period patient card review, blood sample collection and analysis was conducted for observation of health status of children less than five years age.

4.5. Exclusion and inclusion criteria

4.5.1. Inclusion criteria

Children of age less than five years (both sexes) who are affected by acute malnutrition attending the hospital and well-fed children under five year who attend Tikur Anbessa hospital to take meningitis vaccination were taken as control group in the study.

4.5.2. Exclusion criteria

Factors which affect transthyretin levels in serum were excluded. This includes factors which increase, the level of transthyretin such as severe renal failure ,liver failure and factors which decrease the level of transthyretin such as Post-surgery**,** Dialysis**,** Hyperthyroidism**,** Significant hyperglycemia was excluded from the study.

4.6. Sampling technique and Sample Size determination

Simple random sampling techniques was applied in the study period and the sample sizes were estimated by using a single proportion formula and calculated as follows. Since the prevalence of acute malnutrition for under five year children at 2011 was 9.7% out of 1000 children and totally in Ethiopia 4 million children were estimated age of less than 5.

$$\text{Prevalence rate of malnutrition is} = \frac{\text{Number of children with malnutrition}}{\text{Total number of children in population}}$$

$$\frac{97*4000000/1000}{4000000} = 0.097 \text{ so the prevalence of malnutrition is } 0.09$$

Malnutrition < 5 year old is (P = 0.09, q = 1- 0.09 = 0.91 at 95 CI by assuming a margin of error 5% = 0.05).

$n == Z^2pq / d^2$

n = Sample size p = Proportion of malnutrition 0.097

d = Margin of error =0.05 q = 1-p =1- 0.097 =0.903

Z = 1.96 at 95% Confidence Interval (CI)

$$n == \frac{(1.96)^2 \times 0.097X\ 0.91}{0.05 \times 0.05} = \frac{0.339098032}{0.0025} = 135.639=136$$

By considering 10% (12.5) for non-response rate a total sample size of 147 were proposed.

4.7. Variables

4.7.1. Dependent variables

Malnutrition indicated by transthyretin level, albumin level, MUAC, wasting, stunting and an underweight status in children less than five year age.

4.7.2. Independent variables

Age of child, child sex, height, weight.

4.8. Data Analysis

Collected quantitative data was coded, entered to computer, processed, edited, and analyzed using EPI-INFO and SPSS (21^{th} version) and expressed at 95% confidence interval and the p-value were considered significant at $p < 0.05$. Then data computed using appropriate statistical methods (mean, standard deviation, p-value, F test statistic value and one-way repeated measures ANOVA) and the results were presented using tables and figures. Clinical and laboratory data were expressed as the mean ± standard error of mean (SE). Differences in the means between the studies group and control group were evaluated by un-paired student's t-test and chi (χ^2) tests.

Correlations were evaluated by the Pearson correlation test. Graph Pad Prism version 5.00 was used for statistical analyses and for graph drawing. The relationship between anthropometric and biochemical measures were analyzed by using SPSS for windows version 21. The discriminative abilities of anthropometric and biochemical markers for identifying malnutrition cases were computed by means of ROC area under curve analysis. Sensitivity and specificity cut-off points for the various anthropometric and biochemical markers were also determined. This analysis was done using Medcalc version 12.1.4.0, www.medcalc.org. EPI-INFO programme was used to categorize malnutrition based on their Z-scores by using WHO as standard.

4.9. Data Quality Assurance

The data quality was started with appropriate sample collection. The sample were taken in aseptic techniques and collected with considering proper procedure. The kit was made free from contamination and kit was checked for consistency. Collected results were checked for

completeness on daily basis by the immediate supervisor. Due attention was given during data insertion to software on computer. The completed result was rechecked repeatedly to maintain the quality of data.

4.10. Ethical approval

Ethical clearance was obtained from Research and Ethical Committee of the Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University after fully review conducted with meeting No. DRERC 06/14 attended by the main researcher committee and give approval with protocol number of M.Sc. Thesis 05/14. Additionally; Ethical clearance was obtained from Pediatrics and Child Health Department of Black Lion Hospital by letter number PD/CH/327/2006. A consent form was prepared with detailed explanation of objectives, risks, and benefits to the study subject and the confidentiality of responses were given to participants. Data were collected after obtaining informed consent and agreement from the parent of children. Sample collection was performed by trained health professionals following ethical steps and procedures.

4.11. Method of Data collection and Analysis

4.11.1. Anthropometric Methods of Malnutrition Measurement

Anthropometric measurements were carried out according to the WHO recommendations, and data was collected by the principal investigator and by well trained Nurses. Data collection form was designed to record sex, age, weight, height, arm circumference and medical history of each child. Portable mechanical analog scales were used to measure height and weight, respectively. Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg. Each child was weighed with minimum clothing and no foot wear. Using the anthropometric measures, children were classified as underweight, stunted or wasted using the WHO international growth standards as reference.

4.11.2. Weight for age, Weight for height and Height for age

Anthropometric measurement classify: - underweight (Weight for age), Wasting (Weight for height), Stunting (Height for age) by measuring height and compare weight with age for differentiating between acute and chronic malnutrition (Margo, 2012; Harshal *et al.,* 2012).

Weight for Age

The advantage of this index is that it may reflect both past (chronic) and/or present (acute) under nutrition (although it is unable to distinguish between the two).Underweight, based on weight-for-age, is a composite measure of stunting and wasting (Harshal *et al.,* 2012).

Height for Age

This index is an indicator of past under nutrition or chronic malnutrition. It cannot measure short term changes in malnutrition. It compares the child's height with the expected height for the age. Stunting is an indicator of past growth failure (Harshal *et al.,* 2012).

Weight for Height

Weight for Height this compares a child's weight with the expected weight of the same height. It is useful for differentiating between acute and chronic malnutrition (Margo, 2012).The Z-scores for different nutritional indices, weight-for-age, height-for-age and weight-for-height were calculated in reference to WHO standards by using EPI-INFO programme.

Table 2 :- Reference value for Z-score (Nguyen *et al.,* 2008).

Classification	Reference value	Status	Moderate malnutrition	Severe malnutrition
Weight for Age	WAZ<- 3	underweight	z-score -3 and < -2	< -3 z-score
Height for Age	HAZ< - 3	Stunting	z-score -3 and < -2	< -3 z-score
Weight for Height	WHZ<- 3	wasting	z-score -3 and < -2	< -3 z-score

4.11.3. Mid upper arm circumference (MUAC)

The mid upper arm circumference is an indicator of muscle growth. It is age independent, relatively easy to measure and a good predictor of immediate risk of death. It is used for rapid screening of acute malnutrition in children from 1-5 years of age. MUAC can be used for acute under-nutrition

screening in emergency situations and for estimating prevalence of under-nutrition at the population level. MUAC was measured using a flexible non-stretched tape to the nearest 1 mm. The left arms of children were measured. A point was marked midway between the acromion (shoulder) and the olecranon (elbow) on the vertical axis of the upper arm with the arm bent at a right angle and between the lateral and medial surface of the arm. The tape was passed around the arm at the level marked and is tightened to touches without compressed the skin.

Table 3: - Cut – off points MUAC (Roy, 2000).

Color	Range	Status
Read	0-11.5 cm	Severe Malnutrition
yellow	11.5 cm- 12.5cm	Moderate Malnutrition
Green	From 12.5cm	Normal

4.11.4. Laboratory Testing Methods

Immunochromatography method of Transthyretin assay

Dye-labelled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and either antibody specific for the labelled antibody, or antigen, is bound at the control line. Blood and buffer, which have been placed on strip or in the well, are mixed with labelled antibody and are drawn up strip across the lines of bound antibody. If antigen is present, some labelled antibody will be trapped on the test line. Excess-labelled antibody is trapped on the control line (Mori, 2012).

When a sample liquid containing an antigen is dropped onto the sample drop section, the labeled antibody in the conjugate pad is specifically bound to the antigen. The sample liquid is spread horizontally by the capillary force of the porous carrier and absorbed by the absorber. The detection line on the porous carrier is coated with an antibody that reacts specifically with a virus. The label is fixed to the detection line with the virus sandwiched between the antibody and the labeled

antibody. That colors the detection line and indicates that the result is positive. Common substances used as the label include gold particles and dyed latex particles (Mori, 2012).

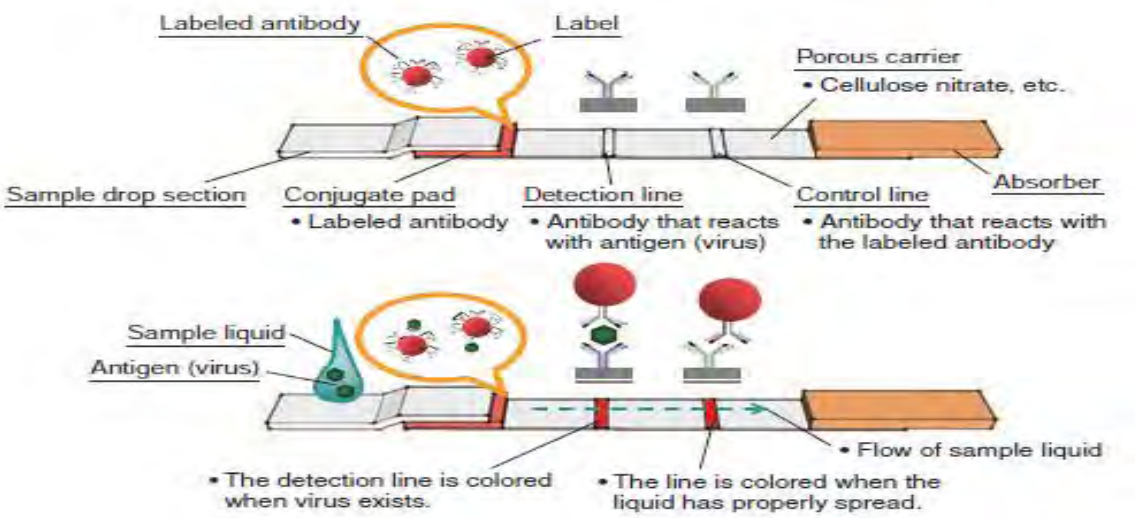


Figure 1:- Principle of immunochromatography detection kit (Mori, 2012).

Laboratory assay procedure for TTR

The Collected 10µl of blood was added to 5ml of diluents with invert gently 10 times to mix the sample properly. Then 100µl of buffer and 10µl of diluted sample was added to reaction well. The mixture was stirred gently with the pipette and the strip was put in the reaction well for 15 minutes. Finally after 15 minutes the strip was putted to TTR kit and measurement ware recorded (NIPRO pharmaceutical, 2014).

Table 4:- reference value of Transthyretin (Shenkin, 2006).

Protein	Status	Reference range
Transthyretin	Normal	>170
	Moderate malnutrition	100-170mg/l
	Severe malnutrition	<100mg/l

Albumin determination: Bromocresol Green (BCG) Methods

BCG is an indicator, which is yellow between pH 3.5 - 4.3. When bind to albumin the color of the indicator changes from yellow to blue- green. The absorbance of the color produced was measured in a spectrophotometer at 632nm wave lengths.

Albumin + BCG $\xrightarrow{\text{pH 4.3}}$ Albumin-BCG complex (blue –green) (Working manual, 2014).

Sample collection and Storage

To get preferred Serum appropriate care was taken to avoid a tourniquet in specimen collection, because haeme- concentration due to venous stasis increases the apparent concentration of albumin and other plasma proteins. After 2 hours of collection of sample serum was Separated and stored at -20 ^{O}C in a tightly stoppered container.

Procedure

4.0ml working dye solution was pipetted into test tubes then 20µl of standard, control, test sample were added separately for each measurement, mixed properly and absorbance was measured immediately within 30 seconds at 632nm after setting the instrument to zero absorbance with the working dye solution (Working manual, 2014).

Table 5 :- Reference value of Albumin (Banh, 2006).

Protein	Status	Reference range
Albumin	Normal	38-50 g/L
	Malnutrition	< 38g/L

5.0. RESULTS

In this study 51 controls and 51 malnourished children which meet inclusion and exclusion criteria were enrolled. Assessment of malnutrition among the study and control groups was performed using anthropometric techniques and laboratory based tests using standard kits. Mid upper arm circumference (MUAC) of enrolled individuals was performed using standard tapes for controls and study group. The age group ranged between one month and five years. There was a good matching with regards to age, gender and height between the control and study groups. The demographic data of the two groups is shown in Table 6 below. There were significant differences between the mean MUAC value in the control group (14.32 ± 0.20 cm) and malnourished group (10.45 ± 0.20 cm) with significantly lower p-value < 0.0001.

Serum albumin level was measured for controls and study subjects and the results showed that there was a significant difference between the two groups. The study group had lower mean average albumin level (3.86 ± 0.07 g/dl) than the control group (4.24 ± 0.053g/dl) with a p-value <0.0001. Serum transthyretin level was also measured using a newly designed kit. The study group mean average value of serum transthyretin level was (132.89 ± 12.3 mg/l) and the control mean value was (303.08 ± 10.01 mg/l) with a p-value < 0.0001. This shows that the transthyretin level had really gone down for the malnourished children than control group.

Table 6: - Comparison of mean anthropometric and biochemical measurement.

Parameter	Control (n=51) Mean ± SE	Malnourished(n=51) Mean ± SE	p-value
Age (year)	1.48 ± 0.18	1.145±0.13	0.13
Height (cm)	68.55 ± 2.93	61.55 ± 1.09	0.061
Weight (kg)	9.26 ± 0.59	4.5 ± 0.34	< 0.0001
MUAC (cm)	14.32 ± 0.20	10.45 ± 0.20	< 0.0001
Albumin (g/dl)	4.24 ± 0.053	3.86 ±0.07	<0.0001
Transthyretin(mg/l)	303.08 ± 10.01	132.89±12.3	< 0.0001

Anthropometric and laboratory measurements were also performed to see the prevalence of malnutrition between control groups and study subjects by measuring the value of height, age weight, MUAC, transthyretin and albumin to categorize them into malnourished or normal children. The results obtained are shown in Table 7 below. All the study children were either severely or moderately malnourished. MUAC findings showed that 39 (38.2%) children were severely malnourished and 12 (11.8 %) children were moderately malnourished by using the WHO MUAC cut-off values (> 12.5cm normal, 12.5cm- 11.5cm moderate malnutrition and <11.5cm severe malnutrition). All controls were normal and did not fall into any extent of malnutrition.

To evaluate the prevalence of malnutrition by anthropometric technique using the WHO growth standards, < -3 SD for severe malnutrition, -3SD to -2SD for moderate malnutrition and > -2SD for normal were used as cut point values. Regarding wasting (Weight for Height) among malnourished children 40 (78.5 %) were severe malnourished and 11 (21.5 %) were moderate malnourished but the controls were fall all in the normal range. The prevalence of underweight (Weight for Age) was 43 (84.3 %) Severe malnutrition, 5 (9.9 %) moderate malnourished and 3(5.8 %) fall under normal. The prevalence of stunting (Height for Age) was 30 (58.84 %) for severe malnutrition and 15 (29.5 %) for moderately malnourished and 6 (11.7 %) were fall under normal as shown in Table 7. All of Anthropometric measurements was assumed statistically significant and its P-value is < 0.0001. The results for anthropometric measurements showed that all controls were in the normal range but the study groups were either severely or moderately malnourished.

Biochemical indicator of malnutrition such as transthyretin and albumin were used to evaluate the prevalence and to categorize control group and study group into moderate, severe and normal malnutrition. Cut-off point value of <100 mg/L for transthyretin indicated severe risk of protein energy malnutrition, 100–170 mg/L moderate risk protein energy malnutrition and >170 mg/L was considered as normal. Based on this cut-point, values obtained for the study group and control group were assessed and compared. Among the malnourished children, 7 (13.7 %) were severely malnourished, 44 (86.3 %) were moderately malnourished. Additionally 2 (3.9 %) of control group was categorized under normal value.

Table 7:- Categorization of malnutrition between studies group and control group using anthropometric and biochemical markers.

Variable	Total (102) N (%)	Control (51) N (%)	malnutrition (51) N (%)	p-value
Male	63(61.8)	32 (62.7)	31 (60.7)	
Gender				1.0
Female	39 (38.2)	19 (37.3)	20 (39.3)	
Height for Age				
Severely malnourished	30 (29.4)	0(0)	30(58.8)	<0.0001
Moderately malnourished	15(14.7)	0(0)	15(29.5)	
Normal	57(55.9)	51(89.5)	6(11.7)	
Weight for Height				
Severely malnourished	40(39.2)	0(0)	40(78.5)	<0.0001
Moderately malnourished	11(10.8)	0(0)	11(21.5)	
Normal	51(50)	51(100)	0(0)	
Weight for Age				
Severely malnourished	43(42.15)	0(0)	43(84.3)	<0.0001
Moderately malnourished	5(4.9)	0(0)	5(9.9)	
Normal	54(52.95)	51(94.4)	3(5.8)	
MUAC				
Severely malnourished	39(38.2)	0(0)	39(76.5)	<0.0001
Moderately malnourished	12(11.8)	0(0)	12(23.5)	
Normal	51(50)	51(100)	0(0)	
Transthyretin (Prealbumin)				
Severely malnourished	7(6.9)	0(0)	7 (13.7)	<0.0001
Moderately malnourished	46 (45.1)	2(3.9)	44 (86.3)	
Normal	49(48.0)	49(96.1)	0(0)	
Albumin				
Normal	77(75.5)	45(88.2)	32(62.7)	<0.0001
Malnutrition	25(24.5)	6 (11.8)	19(37.3)	

In another test using albumin to assess the prevalence of malnutrition with cut-off point of 3.8-5.0 g/dl is consider normal and < 3.8 g/dl were malnutrition, based on this cut-off point 77 (75.5%) was considered normal ,out of this 45 (88.2 %) from control group and 32 (62.7%) was from malnourished children group. Additionally; 25 (24.5 %) were malnutrition children, out of this 6 (11.8 %) from control group and 19 (37.3 %) were from malnutrition group. Both biochemical laboratory indicators such as transthyretin and albumin were certain as it is statically significant with giving P-value < 0.0001 that illustrated in table 7.

Evaluation of nutritional status in different category by biochemical measurement and anthropometric measurement is illustrated in percentage in table 8 below. It appeared that anthropometric measurements, in general classify most samples as severely malnourished. Based on anthropometric measurement 84.3 %, 78.4 %, 76.5 % and 58.9 % were classified as severely malnourished by weight for age, weight for height, MUAC and height for age respectively. From these result a very good indicator for severe malnutrition is weight for age and the least indicator is MUAC. About 29.4 %, 23.5 %, 21.6 % and 9.8 % were identified as moderately malnourished by height for age, MUAC, weight for height and weight for age respectively. In addition about 11.7 % and 5.9 % were classified as normal by height for age and weight for age respectively.

Through transthyretin measurement it was found that 82.4 % were moderately malnourished, 13.7 % were severely malnourished and 3.9 % were normal. Categorization by albumin measurement indicated that 62.7 % were normal and 37.3 % were categorized as severely malnourished. From these results, it can be observed that transthyretin measurements, better identifies moderately malnourished children than albumin, but albumin can identify the severely malnourished kids better than transthyretin.

Table 8: - Prevalence of malnutrition assessed by different methods in 51 malnourished children

	Normal %	Moderately malnourished %	Severely malnourished %
Height for Age	11.7	29.4	58.9
Weight for Height	0	21.6	78.4
Weight for Age	5.9	9.8	84.3
MUAC	0	23.5	76.5
Transthyretin	3.9	82.4	13.7
Albumin	62.7	0	37.2

Pearson correlation for different anthropometric and biochemical measurements for the studies groups and control groups were done and are shown in Table 9. Transthyretin was positively correlated with nutritional indicators of serum albumin to (r = 0.307, P=0 .03) in study group. However, there is no positive correlation for control group. Transthyretin level showed negative correlation with height and weight for both control and study group, but it was not statically significant. Albumin also showed a positive correlation with height and weight and negatively correlated with age, but the reverse was true for control group.

MUAC also had a significant positive correlation with age (r = 0.326, P=0.019), height (r = 0.405, P=0 .003) and weight ($r = 0.430$, P=0.002) respectively. Additionally; it was observed that MUAC value of the control group correlated positively with transthyretin and albumin; however it is not statically significant. There was no correlation between biochemical measurement and anthropometric measurements with p value is > 0.05 for both control group and malnourished children.

Table 9:- Gender adjusted Pearson correlation co-efficient between anthropometric and biochemical indices for malnutrition children.

MUAC correlation between study and control groups.

	age	height	weight	TTR	Albumin	age control	weight control	height control	TTR control	Albumin control
R	0.32*	0.405**	0.43**	0.04	0.085	-0.08	-0.032	-0.105	-0.171	0.022
P	0.019	0.003	0.002	0.73	0.553	0.593	0.826	0.463	0.877	0.23

Transthyretin level correlation between study and control groups.

	age	height	weight	MUAC	Albumin	age control	weight control	height control	MUAC control	Albumin control
R	-0.05	-0.07	-0.079	0.048	0.307*	0.026	-0.085	-0.013	0.133	0.183
P	0.73	0.59	0.632	0.737	0.03	0.854	0.554	0.958	0.351	0.200

Albumin level correlation between study and control groups.

	age	height	weight	MUAC	TTR	age control	weight control	height control	MUAC control	Albumin control
R	-0.05	0.038	0.164	0.085	0.307*	0.055	-0.051	-0.068	0.052	0.013
P	0.73	0.79	0.84	0.55	0.03	0.702	0.724	0.635	0.718	0.188

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

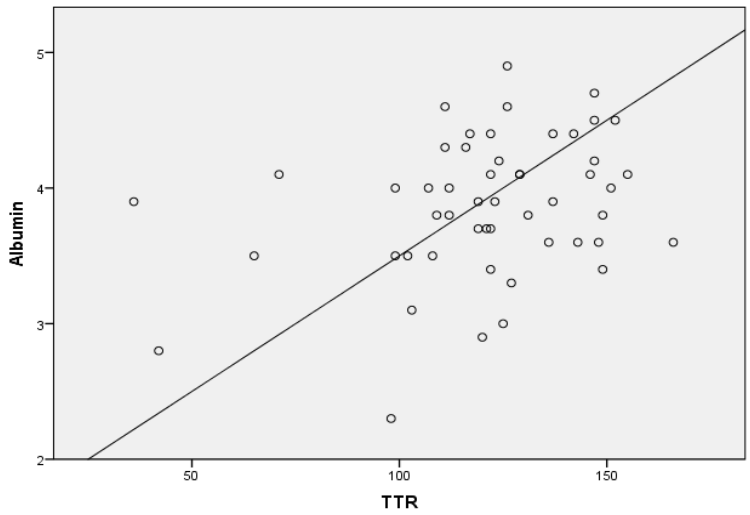


Figure 2:-Scatter plot showing association between TTR and albumin.

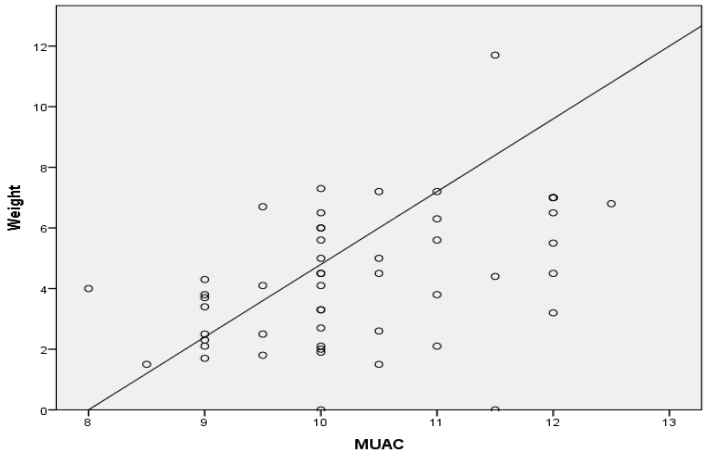


Figure 3:- Scatter plot showing association between MUAC and Weight for study group.

The sensitivity and specificity of transthyretin using MUAC as reference standard were 91.60 %, and 15.38 %. The extent of agreement between MUAC and transthyretin was improved on chance agreement at kappa value of 0.0318 (Table 10). The sensitivity of transthyretin using weight for height (wasting) as reference standard was 100 %, and specificity was 17.5 %. The extent of agreement between transthyretin and weight for height improves on chance agreement at kappa value of 0.16 (Table 11). The sensitivity of transthyretin using height for age (Stunting) as reference standard were 10 %, and specificity was 80.95 %. The extent to which the agreement between MUAC and Height for age improves on chance agreement was - 0.403 (Kappa agreement) (Table 12). The sensitivity of albumin using MUAC as reference standard was 38.46 %, and

specificity was 66.66 %. The extent to which the agreement between albumin and MUAC improves on chance agreement was 0.035 (Kappa agreement) (Table 13).

Table 10:-Validity of transthyretin using MUAC as reference standard.

	Positive	Negative	Total
Positive	11	33	44
Negative	1	6	7
Total	12	39	51

Sensitivity = 91.60 % and specificity = 15.38 %, Kappa = 0.0318 (negligible agreement).

Table 11:- Validity of transthyretin using weight for height as reference standard.

	Positive	Negative	Total
Positive	11	33	44
Negative	0	7	7
Total	11	40	51

Sensitivity = 100 % and specificity=17.5 %, Kappa = 0.16 (negligible agreement).

Table 12:- Validity of transthyretin using height for age as reference standard.

	Positive	Negative	Total
Positive	3	4	7
Negative	27	17	44
Total	30	21	51

Sensitivity=10 % and specificity=80.95 %, Kappa= - 0.403 (poor agreement).

Table 13:- Sensitivity and specificity of albumin using MUAC as reference standard

	Positive	Negative	Total
Positive	15	4	19
Negative	24	8	32
Total	39	12	51

Sensitivity=38.46 and specificity=66.66 %, Kappa=0.035 (negligible agreement).

A receiver operating curve was plotted to determine the optimal cut off, sensitivity and specificity for the transthyretin, and was found to be a sensitivity of 81.82 % and specificity of 47.5 % with

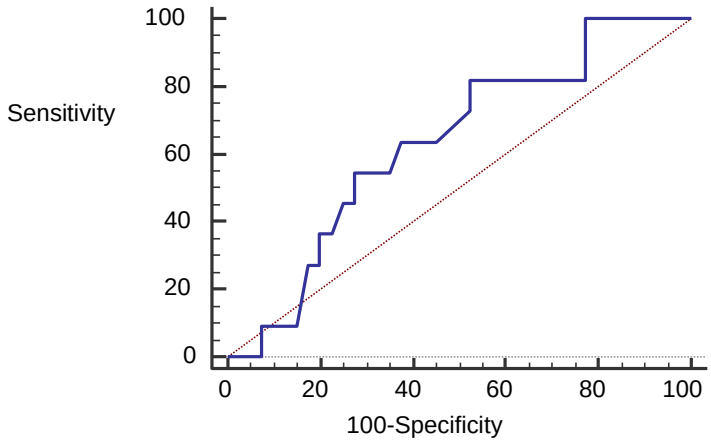
ROC value of 0.633 in significance of 0.1389. At these optimal reliability values, the cut off for transthyretin was 120 mg/l with area under the ROC curve (AUC) 0.633 (Table 14) (Figure 4). Additionally; table 14 showing the validity of transthyretin using MUAC at different cut-offs were <120 mg/l cut off value of transthyretin for severe malnutrition, 120 mg/l -166 mg/l for moderate malnutrition and associated criterion cut off > 166 mg/l for normal value of transthyretin proposed for assessing malnutrition in transthyretin level (Table 15).

A receiver operating curve was used to get optimal cut off, sensitivity and specificity for the transthyretin using weight for height as reference standard and was found to be a sensitivity of 45.45 % and specificity of 87.5 % with significance of 0.0768 (Table 14). At these optimal reliability values, the cut-off for transthyretin was 146mg/l with area under the ROC curve (AUC) 0.670. In validity of transthyretin using weight for height at different cut-offs were <146 mg/l cut off value of transthyretin for severe malnutrition, 146mg/l -155 mg/l for moderate malnutrition and cut off > 155 mg/l for normal value of transthyretin proposed for assessing malnutrition in transthyretin level (Table 16) (Figure 5).

Height for age using as standard in validation of transthyretin, sensitivity 61.54 % and 63.16 % specificity were observed with significance of 0.1671(table 14). At these optimal reliability values, the cut off for transthyretin was 125 mg/l with area under the ROC curve (AUC) 0.631. Validity of transthyretin at different cut-offs were <125 mg/l cut off value of transthyretin for severe malnutrition, 125 mg/l -152 mg/l for moderate malnutrition and cut off > 152 mg/l for normal value of transthyretin proposed for assessing malnutrition in transthyretin level (Table 17) (Figure 6).

Table 14 :- Transthyretin validation by using different reference material.

Standard Reference	ROC value	p-Value	Optimal cut-off	Sensitivity	Specificity
MUAC	0.633	0.1389	120	81.82	47.5
Weight for height	0.670	0.0768	146	45.45	87.5
Height for age	0.631	0.1671	125	61.54	63.16

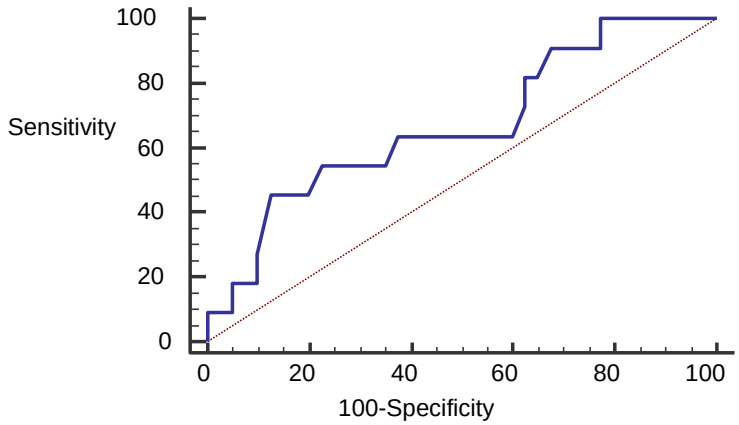


Optimum cutoff 120 mg/l of transthyretin can detect malnutrition state in children with significant AUC of 0.670 sensitivity of 81.2 % specificity 47.2 %.

Figure 4:- ROC curve of transthyretin using MUAC as reference value.

Table 15:- Validity of transthyretin using MUAC as standard reference at different cut-offs value.

Cut-off	≥36	>103	>120	>125	>126	>131	>136	>142	>146	>149	>166
Sensitivity	100	100	81.8	63.6	54.55	45.5	45.5	36.4	27.3	9.09	0
Specificity	0	22.5	47.5	62.5	65	72.5	75	80	82.5	92.5	100



Optimum cutoff 146 mg/l of transthyretin can detect malnutrition state in children with significant AUC of 0.633 sensitivity of 45.45 % specificity 87.5 %.

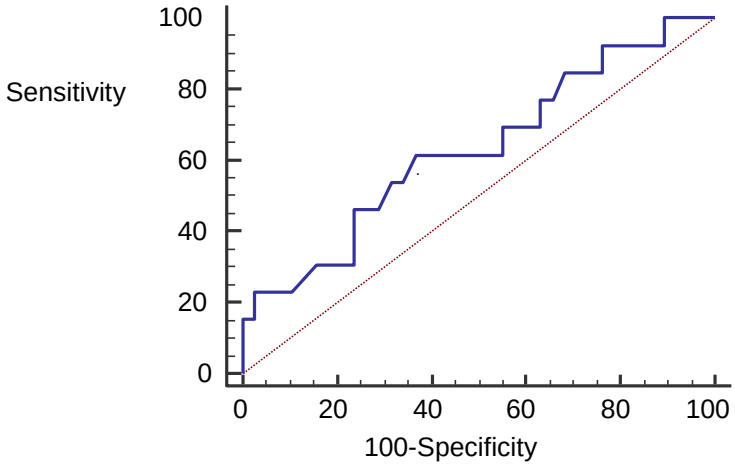
Figure 5:- ROC curve of transthyretin using weight for height as reference value.

Table 16:- Validity of transthyretin using weight for height as standard reference at different cut-offs value.

Cut-off	≥36	>103	>111	>119	>125	>136	>146	>149	>151	>155	>166
Sensitivity	100	100	90.9	63.6	63.6	54.6	45.5	18.2	9.09	9.09	0
specificity	0	22.5	32.5	40	62.5	77.5	87.5	95	95	100	100

Table 17:- Validity of transthyretin using height for age as standard reference at different cut-offs value

Cut off	≥36	>98	>117	>120	>125	>136	>146	>149	>151	>166
Sensitivity	100	92.31	69.23	69.23	61.54	46.15	30.77	23.08	15.38	0
Specificity	0	10.53	36.84	44.74	63.16	76.32	84.21	97.37	97.37	100



Optimum cutoff 125 mg/l of transthyretin can detect malnutrition state in children with significant AUC of 0.631 sensitivity of 61.54 % specificity 63.16 %.

Figure 6:- ROC curve of transthyretin using height for age as reference value.

6.0. DISCUSSION

Malnutrition is a complex problem which results from a long chain of interrelated events and it continues to be a major health burden and affects mostly infants and young children in developing countries (Mahaman *et al*., 2014; Muller *et al*., 2005). It causes alteration in the body composition which results in a reduced body cell mass, organ dysfunctions, and an abnormal serum chemistry value (Orluwene *et al*., 2013).

In different occasions, many nutritional screening tools have been developed, tested and implemented in clinical practice, several combining laboratory tests and patient information. In many malnutrition assessments anthropometry and biochemical methods have been used together in clinical trials. Anthropometry which involves the external measurement of morphological traits of human beings is an important tool for nutritional assessment, and the techniques should allow increased precision of measurement (Harshal *et al.,* 2012). However; biochemical markers have always been an attractive option because they are easier to introduce and standardize in clinical practice (Orluwene *et al*., 2013).

In this study, we have assessed acute malnutrition by using anthropometry and biochemical malnutrition markers and also an attempt was made to identify the most valid method to assess acute malnutrition in hospitalized children. The use of anthropometric parameters in this assessment is a relatively simple method that is readily available, inexpensive and has been shown to be an inaccurate indicator of nutritional status since it need a precise means of comparison in order to be valid, which is often difficult to achieve in a population of critical malnourished children (Orluwene *et al*., 2013; Martore *et al*., 2000).

MUAC is an indicators for the amount of fat and muscle in the upper arm and thickness of subcutaneous fat respectively and is used in determining malnutrition levels in children (Martore *et al*., 2000). In our study, the significance of MUAC also indicated that children with malnutrition had low body fat and muscle and subcutaneous fat when it was compared with the control group. The difference in physical appearance between study group and control group that were identified by MUAC measurement, suggest that it is possible to identify the malnutrition in children easily without invasive procedures like blood drawing, and autopsy methods.

The liver is the main site of synthesis for most plasma proteins so that low concentration of transthyretin and albumin in our studies reflects impairment in the hepatic synthesis of plasma proteins. In malnourished children this decrease in protein synthesis is due to the limiting supply of substrate. However, plasma concentrations of proteins are dependent not only on rates of synthesis but also utilization, intravascular-extravascular transfer, catabolism, excretion, and hydration. Consequently, the overall balance between these physiological processes determines whether plasma concentration of a specific protein reflects the overall decrease in protein turnover associated to protein calorie malnutrition (Gerald *et al.,* 1978).

Transthyretin levels go up with increases in protein and calorie intake and to decrease when protein intake is inadequate. Plasma transthyretin concentration has been shown to significantly decrease only three days after inadequate nutrient intake, and to increase one mg/day when the nutrient needs are satisfied (Glenn *et al*., 2000; Seung *et al*., 2005; Qionghong *et al*., 2011 and Orluwene *et al*., 2013). In this study, there were also significant differences between the mean transthyretin value in the control group and study group. Although, it has been shown that the mean value of transthyretin in malnourished children is less than the average value of normal children, this results generally agree with those of Rostami *et al.* (1999) who also showed that mean transthyretin concentrations were low in malnourished children than control group.

This could reflect that malnutrition reduces the hepatic synthesis of visceral proteins, and increases its catabolism. This led us to investigate transthyretin as a potential marker of nutritional status (Mark S., 2008). Additionally; it indicates that protein intake is inadequate in malnourished children. A low transthyretin concentration can therefore, be regarded primarily as a signal identifying the at-risk patient who requires careful assessment and monitoring and for whom nutritional support may be needed as part of the treatment plan.

It is therefore imperative that, the less amount of transthyretin in blood of malnourished children makes transthyretin as more sensitive indicator of nutritional status and considered as a sign of increased malnutrition risk in hospitalized patients which requires further nutritional assessment. The results obtained in the present work is supported by Glenn *et al.* (2000) which the mean serum value of transthyretin was 126.6 mg/l in malnutrition group when compared to the mean value of

132.89 mg/l in this study. However; this decrease amount of transthyretin may be due to the effect of inflammation. This was suggested by Dana *et al*. (2007), Glenn *et al*. (2000) and Qionghong *et al*. (2011) studies which confirmed that during inflammation there is a statistically significant decrease in serum concentration of transthyretin by the transfer of plasma proteins to the reactants of the acute phase, such as C-reactive protein (CRP). With higher CRP there is a statistically highly significant influence on the transthyretin level and the transthyretin level decrease during inflammation in the acute phase. However, Orluwene *et al.* (2013) also proved the less amount of transthyretin as reliable indicator of malnutrition in cases involving inflammation.

Albumin synthesis decreases rapidly in response to a decrease in food intake, so too does its rate of catabolism; these factors, together with the size of the body's albumin pool and its low rate of turnover result in there being little change in its plasma concentration during starvation. Even with long term protein under nutrition, accompanied by loss of lean body weight and impaired muscle function, plasma albumin concentrations may remain within the normal reference range. It is relatively insensitive to rapid changes in nutritional status taking approximately 14 days to return to normal status (Marshall, 2008; Beck *et al.,* 2002).

Our measurement gave a value of 4.24 g/dl and 3.86 g/dl of albumin for control and malnourished children which shows relatively the same value of 4.58g/dl and 3.4 g/dl respectively for control and malnourished children by using anthropometric and biochemical assessment among under-five year old of Nigerian children (Adegbusi *et al.,* 2011). This study is in line with the observations seen in other studies which proposed that serum albumin is low in malnourished children in comparison to normal healthy controls (Ibrahim *et al*. 1994; Ingenbleek *et al.* 1975 and Sathishbabu *et al.*2012).

A low serum albumin concentration is a common finding in protein energy malnutrition; as a result it is commonly employed as a measure of the child‘s nutritional status. The practical significance of serum albumin analyses in the assessment of nutritional status in our research has been reappraised the figure of chronic malnutrition. The most consistently confirmed biochemical parameter in children with severe protein energy malnutrition measurement has been a low serum albumin concentration (Weinsier *et al*.1979). Our finding also showed a statistically significant

difference in serum albumin concentrations between malnourished children and the controls. However, albumin can't be used as a marker to assess acute phase malnutrition since it has a relatively long half-life and because its concentration is also affected by inflammation.

In our study, the result (table 7), shows prevalence of malnutrition which was assessed by anthropometric and biochemical measurements. Almost all methods, except albumin give positive result and shows agreement each other in general malnutrition assessment and identification. However; categorizing children as sever, moderate and normal based on their nutrition status shows that all anthropometric method relatively agree each other but, anthropometric method doesn't agree with biochemical measurements. When evaluate the performance of weight for height, MUAC and transthyretin in assessment of acute malnutrition gives different prevalence, this may indicate as each method is not accurate that used as standard method. Even though it appears to be inaccurate, it is possible to identify the method that is more reliable to use for day to day acute malnutrition assessment.

Anthropometry is a simple method that could be used by health workers day to day for identifying under five children with malnutrition (Harshal *et al.,* 2012). It has limitations as it is based on assumption that different populations given the same diet, will have similar height and weight outcome, irrespective of race, ethnicity and geography (Girish *et al*., 2014). In the present study, out of fifty one malnourished children most of them can be categorized as under sever malnourished than moderate malnourished. This result clarifies that, MUAC and weight for height is not much sensitive for moderate malnutrition.

Using of this anthropometric measurement methods for routine clinical purpose is inaccurate, since it cannot diagnose acute malnutrition who have not taken enough nutrition recently. These methods are best effective to identify severe malnutrition, in subjects who are under long term deprivation of food. This could be explained by Orluwene *et al.* (2013) by the fact that as anthropometric measurement such as MUAC an early method of nutritional assessment, has been shown to be an inaccurate indicator of nutritional status. Although, the WHO study work involved several countries support our result, it should be noted that MUAC is not an ideal tool in monitoring acute malnutrition in children. This is due to the fact that the performance of MUAC measurements, in

terms of sensitivity and specificity, was very low in determination of acute malnutrition (Pius *et al*., 2014).

From biochemical malnutrition markers albumin and transthyretin are the noticeable one. Albumin is a traditional marker of nutritional status, but its large body pool, with a half-life of 20 days, and also as 60% of total body albumin is extravascular, the response to protein deficiency is slow, thereby limiting its utility as a marker of protein energy malnutrition (Seung *et al*., 2005; Qionghong *et al*., 2011 and Orluwene *et al*., 2013). The main factor affecting plasma albumin concentration in patients is the rate of transcapillary escape into the interstitial fluid. This transcapillary escape of albumin is markedly increased in inflammatory disease, leading to decreased plasma albumin concentrations. The more severe the disease, the lower the albumin, and therefore the worse the prognosis (Shenkin, 2006; Benjamin, 1989).

Albumin's long half-life leads to change its level slowly in response to reduced nutrition intake. While that makes albumin a good indicator of chronic malnutrition, it means it's not a good tool for identifying patients at risk for protein calorie malnutrition or for monitoring the short-term effects of nutritional therapy. This study is in line with the observations seen in other studies which proposed that serum albumin are low in severely malnourished children and serum albumin estimation is not a sensitive marker of protein energy malnutrition (Coward,1981; Dahan,1985 and Reeds,1976).

Our result also shows that most malnourished children are categorized as normal via albumin measurement and the moderately malnourished are regarded as severely malnourished. This is because albumin is insensitive to acute nutritional shortage. Therefore, using albumin as a reference may lead to erroneous conclusion. It also cannot distinguish between severe and chronic malnutrition as a laboratory based method or as a marker.

Transthyretin is a better nutritional marker because it has two days of a short half-life, relatively small body pool, and a rapid rate of synthesis that responds to protein intake. Plasma transthyretin concentration has been shown to significantly decrease only three days after inadequate nutrient intake, and to increase one mg/day when the nutrient needs are satisfied intake (Seung *et al*., 2005;

Qionghong *et al*., 2011; Chaowanee *et al*., 2011 and Orluwene *et al*., 2013). Alteration in protein and calorie intake can be assessed by measuring transthyretin in the blood, since transthyretin has been possesses the shortest biological half-life and its serum level reflects rapid changes in visceral protein status. Thus transthyretin is the most suitable protein marker for evaluation of acute malnutrition as well as monitoring of nutritional state (Dana *et al*., 2007; Chaowanee *et al*., 2011).

The results of the present work are comparable with the research and result of Potter *et al.* (1999) which examined the effectiveness of transthyretin as a routine diagnostic test for protein calorie malnutrition (PCM) in emergency admissions. They found that 24% of the 147 patients had moderate protein calorie malnutrition and 12% severe protein calorie malnutrition. Overall, our results indicated that 44% are moderate malnutrition and 7% as severe malnutrition. This indicated that our study subjects had higher moderate malnutrition than severe malnutrition.

This work supports our contention that the prevalence of acute malnutrition as assessed by transthyretin level and which may give evidence that immunochromatography transthyretin assay method is more reliable in detecting acute malnutrition. Moreover, our result show that transthyretin is a sensitive acute malnutrition marker by detecting many of malnourished children as moderate malnourished. In this study, transthyretin assay showed two cases of moderate malnutrition instead of normal. This indicates that transthyretin can better detect moderate malnutrition that is misdiagnosed by MUAC.

Malnutrition is costly in multiple ways. Hospitalized patients who are undernourished can develop clinical complications with a risk of increased morbidity and mortality. Increased length of hospital stay is required by patients admitted with malnutrition, and this has been 4-12 days longer in malnourished compared to well-nourished patients (Mears, 2007; Saka *et al*., 2011). The present work introduces the new immunochromatographic transthyretin assay method as an accurate, fast and much more cost-effective method to assess acute malnutrition. This method can also be used as acute malnutrition indicator to reduce length of hospital stay and for early nutritional supplementation planning. The cost for one immunochromatography transthyretin strip is $5(100 birr). The cost when compared with current cost to assess malnutrition in Addis Ababa hospitals seems a little expensive, but when we compare its aid in decreased length of hospital stay, it is

much economical. The results also validated the transthyretin method as valuable laboratory based method to assess acute nutrition status among children attending pediatric clinics.

The Prevalence of malnutrition assessed by different methods in this study population is shown in table 8. The table shows the prevalence of acute malnutrition which is usually caused by a relatively recent food shortage and it was higher compared to the prevalence of stunting or underweight indicating chronic malnutrition. Prevalence of malnutrition assessed by transthyretin level among 51 malnourished children shows 86.3% moderate malnourished and 13.7% sever malnourished. The result obtained indicates that transthyretin is the best indicator of moderate malnutrition and had a good sensitivity and is also effective for detecting acute malnutrition. This result also supports the sensitivity of transthyretin for identification of moderate malnutrition similar to results obtained in a different study by Gianluigi *et al*. (2006) where 42 % moderate malnourishment and 17% severe malnutrition were observed.

Transthyretin has been shown to decrease in the face of chronic inflammation, limiting its specificity as a marker of nutritional intake (Glenn *et al*., 2000; Alan, 2006).We have found that transthyretin level is low in children with acute malnutrition in response to its sensitivity to inflammatory stress which is common in children in these ages. The present work shows higher numbers which is indicative of false positive results. This could be due to chronic inflammation in these patients. No comparison is made because we have not measured the C-reactive protein to indicate inflammation in these patients. Therefore no attempt is made to rule out the possibility of inflammation in these patients as low transthyretin levels can be regarded primarily as a signal in identifying the at-risk patients who require nutritional support.

MUAC seems to be a potential anthropometric indicator of child nutrition. Measuring arm circumference is simple, and requires little time. The prevalence of moderate malnutrition assessed by MUAC shows 23.5 % in our studies, this high compared with the findings of Adegbusi *et al.* (2011) where 16 % moderate malnutrition was observed in malnourished children. This high prevalence in our study indicates that the children admitted in the two hospital clinics were malnourished due to lack of proper feed and clearly shows that the children require proper nutritional supplement. Nutritional assessment using MUAC requires skilled personnel and

measurement shall be taken with care. Wrong positioning of the tape and careless measurement could lead to erroneous results. Therefore, assessment of serum transthyretin level in addition to anthropometric methods provides a relatively definitive identification of malnutrition among hospitalized children.

The clinical findings false negatives have to be reported by MUAC but, transthyretin shows cases positive and vise verse. To suggest MUAC as good as or better than transthyretin for malnutrition assessment is not possible since there is no any accurate gold standard method that can be used as reference for comparison purpose. However; transthyretin has relatively short half-life and is sensitivity to acute nutrition makes it useful clinically and can be used routinely in hospitals for assessment of moderate malnutrition. Likewise, there was high prevalence 21.6 % of wasting observed in our study when compared to prevalence of 11% wasted malnutrition in a study made in Nigeria (Adegbusi *et al.,* 2011).

Analysis using Pearson correlation as shown in table 9 indicated that there was positive correlation between albumin and transthyretin. These visceral proteins are synthesized in liver cells, so when there is any capacity increase of liver function reserve, their synthesis will also increase. Different studies showed that transthyretin was directly correlated and significantly interrelated with serum albumin and the level of albumin increases as the level of transthyretin increases (Glenn *et al*., 2000; Mervin *et al*., 1987; Ayaka *etal*., 2015 and Sathishbabu *et al*., 2012). Additionally, the correlations between albumin, and transthyretin may be weakened by the disproportionate response of these variables to the different degrees of stress and inflammation in these children.

There was no correlation between albumin and transthyretin measurement level with anthropometric measurements with p value > 0.05. This result also supported by Yovita *et al*. (2004) who showed there was no correlation between anthropometric and biochemical parameters in malnutrition assessment and they don't have effect on each other. Even this correlation was not statistically significant, but there was negative tendency between transthyretin levels with anthropometric measurements of height, weight and age. Age had a negative correlation with transthyretin level, similar to previous studies (Rico *et al*,. 1995; Rambod *et al*,. 2000; Ayaka *et al*,. 2015), suggesting aging is associated with increased risk for a poor diet. These indicate that

the higher the age, height, weight the lower level of transthyretin level. When the age increase, with increase in height and weight, the level of transthyretin decreases respectively. Different studies have shown that transthyretin levels decrease significantly as age advances and it is inversely correlated with age, but it is significantly lesser in amount, when compared to albumin (Glenn *et al*., 2000; Sathishbabu *et al*., 2012).

Even though; not statistically significant, there was positive tendency of correlation between transthyretin and MUAC. This shows since MUAC indicate the amount of fat and muscle in the upper arm and thickness of subcutaneous fat respectively, the level of transthyretin production increase as the children's body fat increased. Indirectly this happens when subcutaneous fat is degraded by different metabolic events, with diseases or other causes, depletion of proteins due to higher utilization is a possibility. The consequence of this is lower energy production, leading to less production of transthyretin, and less possible detection in the liver cells. The decreased level of transthyretin can be used to identify the level of protein energy malnutrition in hospitalized children.

Likewise, there was significantly positive affinity of correlation between MUAC with age, height and weight. This result reflects that when age increases in development of children with increasing fat and muscle in upper hand, consequently the value of MUAC increases side by side with increasing in height and weight. This result was reliable when comparing with Dairo *et al* (2012) studies of MUAC validation for the assessment of wasting among children age less than five. His result showed that the value of MUAC increased and reached higher peaks as the age reaches to five. The result gets reversed when MUAC of study group was compared with control group and showed negative correlation with age, height, weight and transthyretin level of control group. This indicates the rising of age, height and weight doesn't make effect on MUAC value. Additionally; when the value of transthyretin increases the value of MUAC decreases respectively.

Our result shows that sensitivity and specificity are not certain because there is no clear gold standard method for malnutrition assessment. Due to this reason our study attempted to use different malnutrition assessment method as reference method to compare each other for validation of transthyretin. Transthyretin was found to be the best indicator of moderate malnutrition and had

a good sensitivity/specificity profile using weight for height as reference material when compared with other methods and it had agreement with the literature (Orluwene *et al*., 2013; Gianluigi *et al*., 2006). However; it has negligible agreement between weight for height and transthyretin in assessment of moderate malnutrition.

Most of the cut-off value used for the various anthropometric and biochemical indices were inferred from studies done elsewhere and even in some cases these cut-offs were generated from healthy adults and children. This suggests the need for specific cut-offs for determining malnutrition in children using serum transthyretin level measurement. As shown in our study, an optimal cut- off of 120 mg/l value for transthyretin yielded a sensitivity of 81.82% and specificity of 47.5 % for the prediction of malnutrition. This might be a better cut-off as it had a higher sensitivity and therefore detect majority of those with malnutrition and yield better results for using clinical assessment purpose. The 120 mg/l to 166 mg/l cut off for moderate malnutrition,<120 mg/l for severe malnutrition and >166 mg/l for normal value of transthyretin were recommended for adoption in Ethiopia and further studies on the reliability and desirability of this new cut off is suggested. Our result showed high sensitivity and gave better cut off to detect malnutrition as compared to Sathishbabu *et al* (2012) who showed ROC curve with optimum cutoff 295mg/l of transthyretin to detect malnutrition with significant AUC of 0.705, sensitivity of 72%, and specificity of 60% .

7.0. STRENGTHS AND LIMITATIONS OF THE STUDY

7.1. Strength

To our knowledge, this study was the first study in Ethiopian which attempted to evaluate whether transthyretin screening increases the detection ability of malnourished children and classify them as moderate or severe malnutrition via the new immunochromatographic transthyretin assay method. Results from this study were compared to current evidence based practice guidelines for the management of patients at nutritional risk as well as malnutrition screening studies performed nationally and internationally.

The data provided strong evidence that is accessible for use by dietetic managers across Ethiopia that could potentially provide useful information on malnutrition risk screening protocols at the time of admission. Furthermore it is hoped that through the results of this study, the evidence has shown that an increased number of malnourished children were identified at risk of acute malnutrition and that transthyretin is fast, reliable and useful tool in acute malnutrition screening in contrast to other malnutrition screening tools. This method can be adopted by future researchers for further elaboration of the effectiveness of the method. The results are obviously relevant for the development and improvement of malnutrition screening protocols, it is important to acknowledge several limitations that could be addressed in future studies.

7.2. Limitation

The study doesn't include all sample size (#147), it included only 102 children. Some of the test strips expired before completion of sample collection hence, we couldn't collect enough sample. Additionally; since there is not standard set point in Ethiopia for malnutrition assessment, this research can only be done by comparison of well-fed children with malnourished Children. Moreover, when interpreting the findings from this research, a low transthyretin and albumin levels may have been affected by an inflammatory process. This study did not measure any inflammatory markers such as a C-reactive protein, which may be used to evaluate the influence of inflammation on transthyretin and albumin levels. The study doesn't include other hospitals in Addis Ababa and doesn't give a good picture regarding chronic malnutrition within Addis. Other biological nutrition markers such as transferrin, retinol binding protein and other marker are not assessed in this study.

8.0. CONCLUSION

Malnutrition is a problem in hospitalized children in Ethiopia, whose age is under five years and can predispose an individual to infection and diseases, and make recovery from disease slower. Malnutrition at the early stages of life can lower child resistance to disease, increase child morbidity and mortality, and decrease mental development and cognitive achievement. Enrichment of nutrition through supplementation and marking a protocol guideline on transthyretin levels in malnourished hospitalized children. This can minimize malnutrition and the complications arising out of malnutrition in hospitalized children.

Using transthyretin immunochromatographic assay method for the identification of children with malnutrition newly admitted to Hospital is a cost effective, feasible, reliable tool for malnutrition screening particularly for the purpose of settings where it is difficult to perform a more detailed and comprehensive nutritional assessment. Comprehensive nutritional assessment can also be made on this basis for" malnourished" children as well as at the "risk of malnutrition" and this protocol can be introduced in hospitals in Ethiopia.

The value of laboratory measurements in the identification of patients requiring malnutrition assessment and nutritional support is limited in Ethiopia. It is not appropriate to consider nutritional support and treatments on the basis of biochemical laboratory result and anthropometric value alone. It should be the diagnosis of malnutrition primarily based on the clinical history, anthropometric and laboratory examination data must be used to get effective reliable result. Since both measurement have their own limitations and the level is affected by different disease, it is advisable to use both measurements to get feasible assessment strategy and identification of reasons for malnutrition.

Transthyretin has been proposed as a laboratory marker that can identify malnutrition risk even when clinical signs are not immediately evident. Since it has relatively small body pool, a rapid rate of synthesis that responds to protein intake and possesses the shortest biological half-life and thus it is most suitable for evaluation of acute malnutrition as well as monitoring of nutritional state. A cut-off of >120 mg/l for transthyretin is recommended for hospitalized screening purpose

for malnutrition in children in Addis Ababa and similar areas. Further studies with larger sample sizes are recommended to confirm this finding.

In conclusion, using a biochemical marker such as transthyretin for the diagnosis of malnutrition allows many children to be evaluated for the risk acute malnutrition regardless of underlying diagnosis for nutritional status. Therefore, it is hoped that Ethiopian hospitals may consider the implementation of transthyretin in conjunction with anthropometric measurement. It has the potential of early identification of patients at risk of malnutrition that elude detection and provide a good picture regarding the assessment of acute malnutrition.

9.0. RECOMMENDATIONS

A limitation found in reviewing the studies was the lack of a universally accepted gold standard for evaluating nutritional status. Having an accepted universal nutritional assessment validation tool to categorize patients into varying degrees of nutritional status will facilitate the comparison of study results across different study populations. Study of the prevalence of malnutrition in a larger community setting will make the impact and attendant problems to be appreciated better. It had a relatively small sample size for both the malnourished group and control group. We would like to recommend for future investigators the use of larger size of study samples, which will enable to explore the problem in depth and to set a standard for Ethiopia.

Using a transthyretin level for the identification of children with malnutrition newly admitted to hospital is a cost effective, and accurate tool for malnutrition screening. Future prospective research is necessary to further validate the findings of this research and show a cost benefit of using a transthyretin level for screening and treatment of malnutrition. Additionally; there is need to validate and cross cheek the efficiency of transthyretin in assessment of acute malnutrition with other nutrition-marker protein like transferrin, retinol binding protein and C-reactive protein.

Federal Ministry of Health (FMOH) of Ethiopia in collaboration with Food, Medicine and Health Care Administration and Control Authority Ethiopia (FMHACA) should strengthen the regular monitoring of acute malnutrition by using transthyretin as one nutrition marker in all Hospitals in Ethiopia to decrease malnutrition effect by detecting malnutrition earlier that be used to give support for children before complications set in, to decrease hospital stay and to minimize the treatment costs.

It is proposed and recommended that in future workers that it is necessary to use side to side or together the anthropometric assessment value with biochemical measurement value for a better nutritional diagnosis of these children and certainly, the testing for transthyretin and the MUAC will contribute to a much more precise nutritional analysis.

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APPENDIX

Annex I: Consent form in English

Purpose

Malnutrition is the main problem of developing countries in the world, especially in Ethiopia. The most important forms of malnutrition in Ethiopia are protein energy malnutrition, but still there is not reliable laboratory method is present to assessment of acute malnutrition. Transthyretin is the protein in serum which has half-life of two days, which used to examine acute malnutrition and it is more sensitive to changes in protein-energy status than albumin, and its concentration closely reflects recent dietary intake rather than overall nutritional status. The aim of this studying is to investigate the magnitude of acute malnutrition and to validate Transthyretin measurement as valuable laboratory based investigation for the assessment of nutritional status in children.

Participation

Without your participation, voluntarism and active part of the study the feasibility of the research are under question, so we asking you and all other to voluntary participant in this study. What we expect from you as parent (guardian) is to be willing to allow your child to participate by give blood to us for examine nutritional status. The examination involves laboratory procedure with collection of 3ml blood from hand. Sample will be collected using sterile and disposable needles and test tubes.

Risks

Taking 3ml of blood has a very low risk to your child, but minor needle pain may last for seconds. If there comes any discomfort, we shall offer you necessary medical treatment freely.

Benefits

Any status of your child from examination will be communicated with your physician and facilitate appropriate measures need to be taken accordingly.

Confidentiality

Any information collected from you will be kept confidential. Your child identity will not be disclosed in any situation or study result as we use different code number instead of your name.

Sharing the Result

After analysis of the data, we will present the result of the study to the responsible bodies. The report will not bear any information about your children; because we use code to disseminate the results to concerned bodies and for the purpose of publication.

Right to Refuse

Since your child participation in this study is entirely on voluntary of you since you are parent who take responsibility to your kid, you have right as parent (guardian) to refuse to accept this request. Your refusal will not affect any part of your child treatment in hospital concerning our study.

Contact Address

If you have any question or concern, you can contact Behailu Tsegaye at any time using the following address

Behailu Tsegaye Addis Ababa University, Faculty of Medicine, Department of Medical Biochemistry.

Tel: 0937914134 ,Email: bhty99@yahoo.com

Addis Ababa, Ethiopia

Consent Form

I, the under signed, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind.

I……. do hereby give consent to Dr. /Mr. /Mrs. /Miss……..to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal is explained to me in the appropriate language I understand.

Unique number of the Participant ……Signature ……

Name of the Investigator……..... Signature…….

Date: …….

Assurance of the Principal Investigator

I, who undersigned, agree to accept responsibility for the scientific ethical and technical conduct of the research project and for the provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this publication.

Name of the student: _____Date …… Signature…….

Assurance of Advisors

1. Dr. Solomon Genet. Date …… Signature……
2. Dr. Ameha Mekasha , Department of pediatrics.Date ……Signature……

Annex 2 : Consent form in Amharic

<u>የጥናቱ አላማ</u>

ያልተመጣጠነ ምግብ በማደግ ላይ ላሉ ሀገሮች ዋናኛ ችግር ሲሆን ይህም ችግር በኢትዮጵያ ይታያል ። ዋነኛው በኢትዮጵያ የሚታየው ችግር የፕሮቲን ንጥረ ነገር አለመመጣጠን ሲሆን ነገር ግን ይህን ያልተመጣጠነ የምግብ ችግርን በአፋጣኝ እና በአስተማማኝ በኢትዮጵያ ሊላካ የሚች በላብራቶሪ የተደገፈ ዘዴ እስካሁን በተግባር ለመተግበር አልተቻለም።

ትራንስ ታይሬቲን በደም ውስጥ የሚገኝ ፕሮቴን ሲሆን በደም ውስጥ ለሁለት ቀን መቆየት የሚችል ሲሆን የዝህን ፕሮቲን በደም ውስጥ ያለውን መጠን በመለካት በቅርብ ቀን የተከሰተውን የምግብ አለመመጣጠንን መለካት ያስችላል ። እንዲሁም ይህ ፕሮቲን በተጨማሪ የፕሮቲን ንጥረ ነገር አለመመጣጠንን ለማወቅ የሚያስችል ሲሆን በአጠቃላይ በቅርብ ቀን ያለውን የሰውነታችን ሥነ-ምግብ ውህደት እንድናውቅ ይረዳናል ።

የዚህ ጥናት ዋና ዓላማ በደም ውስጥ ያለውን ትራንስታይሬቲን ፕሮቲንን በመለካት በቅርብ ቀን የተፈጠረው የምግብ አለመመጣጠን ለማወቅ ሲሆን ይህም ዘዴ ዋነኛው ላብራቶሪ ያማከለ ፈጣንና አስተማማኝ የሕፃናት የሥነ-ምግብ ሁኔታ ማወቅያ ዘዴ እና መለኪያ እንደሆን በማረጋገጥ ለወደፊት ይህ ዘዴ ተግባራዊነት እንዲያገኝ ለሚመለከተዉ አካል ለማቅረብ ነዉ።

<u>ተሳትፎ</u>

ለዚህ ጥናት ከግብ መድረስ የእናንተ በፍላጐት ፣በንቃት መሳተፍ ትልቅ ጉልህ ድርሻ አለው ስለዚህ የእናንተን ይሁንታ እና በፍላጐት መሳተፍን በማክበር እንጠይቃለን።

ከሕፃን ልጅዎ 3 ml መጠን የደም ናሙና በፍላጐት እንዲሰጡን እየጠየቅን ይህም ለጅዎትን የሥነ-ምግብ ሁኔታ ለማወቅ ያስችለናል። የደም ናሙናውንም ለመውሰድ የተቀቀለ እንዲሁም ከባክቴሪያ ነፃ የሆነና ወዲያውኑ የሚወገድ መርፌ እንጠቀማለን ስለዝህ ምንም ዓይነት የጤና መታወክ የማያስከትል መሆኑን እናረጋግጣለን ፡

ይህ 3ml የሚወሰደው ደም ምንም ዓይነት የጤና መታወክ በልጅዎ ላይ የማያስከትል ቢሆንም ደም በሚወሰድበት ጊዜ ወዲያው የሚጠፋ ህመም ሊኖር ይችላል። ነገር ግን ከዚህ በላይ የሚያስከትል ህመም ካለ በአፋጣኝ ልጅዎት ህክምና እንዲያገኝ እናደርጋልን።

<u>ለልጅዎ የሚያስገኝው ጥቅም</u>

ማንኛውም ስለልጅዎ ሁኔታ የሚያሳይ የላብራቶሪ ውጤት ልጅዎን ለሚከታተል ለጤና ባለሙያ እንዲሰጥ ይሆናል በተጨማሪም አስፈላጊው እርዳታ እንዲያገኝ ይረዳል።

ማረጋገጫ

የልጅዎ የላብራቶሪ ውጤት የተለየ መለያ ቁጥር ተሰጥቶት በሚስጥር ስለሚያዝ ማንኛውም አይነት የሞራል የሥነ-ምግባር ጥሰት በልጆዎ ላይ እንዳይካሄድ ይሆናል።

ውጤቱን ስለማሳወቅ

የዚህ የጥናት ውጤት ለሚመለከተው አካል የሚቀርብ ሲሆን የዚህ ሪፓርት የልጆዎን ስም የማይጠቅስና በተለየ መለየ መለያ ቁጥር ስለሚቀርብ ምንም ዓይነት ስጋት የማያደርስ ሲሆን ውጤቱም እንዳስፈላጊነቱ ለህትመት ይበቃል፡፡

ጥያቄን ያለመቀበል መብት

በማንኛውም ሁኔታ ለጅዎት የደም ናሙና እንዳይሰጥ ከፈለጉ እንዲሁም ደም ለመስጠት ቃለ ገብተው ሀሳብዎን ከቀየሩ በፈለጉት ጊዜ አለመሰማማቶዎን በመገለጽ /ከምርምሩ እራስዎን በማግለል መተው የሚችሉ ሲሆን ይህም ምንም ዓይነት በሆስፒታሉ ለልጅዎ ህክምና ማግኘት ላይ ጉዳት እንደማያመጣ እና እንደማይገናኝ እናሳውቃለን፡፡

ጥናቱን የሚካሄደው አድራሻ

ማንኛውም ዓይነት ጥያቄ ካልዎት በሀይሉ ፀጋዬ በማንኛውም ሰዓት ማግኘት የሚችሉ ሲሆን አድራሻውም

በሀይሉ ፀጋዬ አዲስ አበባ ዩኒቨርሲቲ ህክምና ኮሌጅ ባዮ ኬሚስቲሪ ትምህርት ክፍል፡-

ስልክ 0937914134 , ኢሜል bhty99@yahoo.com

መስማማትን የሚገልጽ ቅጽ

አኔ ከዚህ በታች በፊርዬ የማረጋግጠው …….. የተባልኩ የኔን ልጅ ደም በመስጠት የጥናቱ ተሳታፊ እንድሆን ሰፈቅድ ሙሉ አላማሉን በግልጽ በመረዳት እንዲሁም የጥናቱ ሁኔታ በመረዳትና ሀሳቤን በቀየርኩበት ሰዓት ከጥናቱ እራሴ ማግለል እንደምችል ያለኝን መብት ተጠበቆልኝ ነው፡፡

እኔ ……የምባል ለዶ/ር /ሲስተር/ አቶ /ወሮ /ሪት …… መስማማቴን በፊረማዬ አያረጋገጥኩ እንዲሁም ለጥናቱ አስፈላጊውን ትብብር ለማድረግ መፈቀዴን አረጋግጣለሁ ፡፡ በተጨማሪም በፈለኩ ጊዜ ስምምነቱን ለመሰረዝ እንደምችል እንዲሁም ስምምነቱን አለመቀበል ምንም ዓይነት ጉዳት እንዲሁም ጥቅም እንደማያሳጣኝ በማወቅ /በመገዘብ/|ስምምነቱን ተቀበያለሁ፡፡

የታካሚው ሕፃን የተለየ ቁጥር/ኮድ ……የወላጅ ፊሪማ ……

ጥናቱን የሚያካሄደው የስምምነት ቅጽ

እኔ የህንን ጥናት የማካሄደው ሳይስና ሥነ-ምግባርን በጠበቀ ፣ የግለሰብን ሞራል እና ስብዕና ባገናዘበ መልኩ ሲሆን ለዚህም ተግባራዊነት ሙሉ ኃላፊነት በመውሰድ ነው፡፡ የጥናቱም ውጤት ለሚመለከተው አካል በየጊዜው እንደሚቀርብ በማሳወቅ ነው፡፡

ስም **በሀይሉ ፀጋዬ** ቀን ……..ፊርማ ……

የጥናቱ አማካሪዎች

ዶ/ር ሰለሞን ገነት ቀን …… ፊርማ ……

ዶ/ር አምሀ መካሻ ቀን……...ፊርማ……

Annex 3:- Sample collection sheet

Sample code	Sex		Age in year	Height Cm	weight Kg	MUAC Cm	Transthyretin Mg/l	Albumin Mg/dl	Health condition
	Male	Female							
C 0001									
C 0002									
C 0003									
C 0004									
C0005									
C 0006									
C 0007									
C0008									
C 0009									
C 0010									
C 0011									
C 0012									

Sample collection approval

Health professional Name ……

Signature……..

Principal Investigator Name **BehailuTsegaye** Signature ……

Advisor

Dr. Solomon Genet Signature …….

Dr. AmehaMekasha Signature …….

Dr.Muluwork Denberu Signature …….

Annex 4:- Transthyretin assay kit procedure