# PRO-INFLAMMATORY CYTOKINES AS MARKERS FOR THE DIAGNOSIS OF PROTEIN ENERGY MALNUTRITION



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by

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# DECLARATION

The experimental work described in this thesis was carried out by me at the Department of Molecular Medicine, KNUST. This work has not been submitted for any other degree.



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# ABSTRACT

*Protein-energy malnutrition (PEM) is a public health problem and is associated with high morbidity and mortality. There are altered biochemical and immunological parameters which may serve as indicators of PEM.The study was aimed at assessing the use of pro-inflammatory cytokines as diagnostic indicator for Protein energy malnutrition in children. A total of 115 children (35 healthy controls and 80 malnourished children) aged 8 – 36 months attending the Maternal and Child Health Hospital (MCHH), Kumasi were recruited for the study. The study was conducted between December 2009 and June 2010. Anthropometric measurements including weight, height and mid-upper arm circumference were taken for the study population and immunoassays on interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) and biochemical analysis of albumin and total protein) assayed. When categorized for PEM, 67.5% had marasmus, 18.8% had marasmic kwashiorkor and 13.8% had kwashiorkor. Mothers in the control group had attained higher education ($p=0.0021$) and were gainfully employed ($p=0.0038$) when compared to mothers of the subject group. Children in the subject group had a significantly low birth weight (<2.5kg, $p=0.0255$) as compared to controls and were more likely to have an exclusive breastfeeding period of less than three months ($p=0.058$). There were no statistically significant differences ($p>0.05$) in the mean total protein concentration of the controls (68.37± 1.43 g $L^{-1}$) when compared to that of the subjects before (66.27± 1.61 g $L^{-1}$) and after (69.55± 1.69 g $L^{-1}$) treatment. Serum albumin concentration in the control group (43.21 ± 0.90 g $L^{-1}$) was significantly higher than the concentration in the subject group before treatment (38.65 ± 0.90 g $L^{-1}$, $p=0.0027$). The mean concentration of IL-6 in the subjects at baseline (46.08 ± 7.48 pg $mL^{-1}$, $p=0.0008$) and after treatment (26.25 ± 5.19 pg $mL^{-1}$, $p=0.0148$) were significantly higher than those in the control group (7.01 ± 1.37pg $mL^{-1}$). A 43.8% decrease in the mean concentration of IL-6 was observed after treatment. TNF-α concentration before treatment (82.07 ± 6.02 pg $mL^{-1}$) was significantly higher when compared to the mean concentration in the control group (55.81 ± 2.20 pg $mL^{-1}$).The study observed increases in pro-inflammatory response in malnourished children with IL-6 concentration being a significant indicator of PEM in the subjects compared to TNF- α. The impact of dietary intervention on biochemical indices assessed in this study shows the ability of nutritional intervention to promote growth in malnourished children. Above all, the level of education and the socio-economicalstatus of the mothers of malnourished children in this study had a significant impact on malnourishment.*

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## LIST OF ABBREVIATION

AIDS – Acquired immune deficiency syndrome

APPs – Acute Phase Proteins

APR – Acute Phase Reaction

CRP – C - reactive protein



DNA – Deoxyribonucleic Acid

EDTA – Ethylene diaminetetraacetic acid

FAO – Food and Agriculture Organization

GDHS –Ghana Demographic Health Survey

Hb – Haemoglobin

HIV – Human Immunodeficiency Virus

IgA – Immunoglobulin A

IgG – Immunoglobulin G

IgM – Immunoglobulin M

IL – Interleukins

IL-6 – Interleukin 6

LBW – Low Birth Weight

MCHH – Maternal and Child Health Hospital

MUAC – Mid Upper Arm Circumference

PEM – Protein Energy Malnutrition

PEU – Protein Energy Under nutrition

RNA – Ribonucleic Acid

sIL6R – Soluble Interleukin 6 Receptor

SIRS – Systemic Inflammatory Response Syndrome

STD – Sexually Transmitted Disease

TNF – Tumour Necrosis Factor

TNF-*a* - Tumour Necrosis Factor – alpha

TWBC - Total White Blood Cell

UNICEF – United Nations Children's Fund

UNACC – United Nations Administrative Committee on Coordination

UNESCO - United Nations Educational, Scientific and Cultural Organization

WBC – White Blood Cells

WHO – World Health Organization

*Chapter 1*

# INTRODUCTION

## 1.1 GENERAL INTRODUCTION

The primary causes of morbidity and mortality among children aged less than 5 years are pneumonia, diarrhoea diseases, low birth weight, asphyxia and in some parts of the world, human immunodeficiency virus (HIV) infection and malaria. One out of every two such deaths has malnutrition as the underlying cause (Murray and Lopez, 1997). However malnutrition is rarely cited as being among the leading causes of death even though it is prevalent in developing countries (WHO, 2000b).



Malnutrition is currently the leading cause of global burden of disease (Ezzati *et al.*, 2002) and has been identified as the underlying factor in about 50% of deaths of children under 5 years of age in developing countries (Black *et al.*, 2003). The condition may result from lack of food or from infections that cause loss of appetite while increasing the body's nutrient requirements and losses. Children between 12 and 36 months old are especially at risk since they are the most vulnerable to infections such as gastroenteritis and measles (WHO, 2000b). It is estimated that, in developing countries, more than one-quarter of all children younger than 5 years of age are malnourished (UNACC, 2000).

A nationwide survey in Côte d'Ivoire indicated that chronic malnutrition affects an estimated 34% of children under five years, while an estimated 20.2% are underweight. In many countries in the Sahel region, notably Burkina Faso, Mali and Niger, the prevalence of acute malnutrition is between 10.6% and 18.6% (UNICEF, 2006a).

Protein energy malnutrition (PEM) is a potentially fatal body depletion disorder (Dulger *et al.*, 2002). The term protein energy malnutrition applies to a group of related disorders that include marasmus, kwashiorkor and intermediate states of

marasmic kwashiorkor. Marasmus involves inadequate intake of protein and calories and is termed "the sickness of the weaning" with no oedema (de Onis *et al.*, 1993). Kwashiorkor including marasmic kwashiorkor is characterized by massive oedema of the hands and feet, profound irritability, anorexia and desquamative rash, hair discolouration and a large fatty liver (Manary and Brewster, 1997). Hypoalbuminaemia and electrolyte imbalances have been put forward as possible causes of the oedema (Ahmed *et al.*, 2009).



The "radical theory" proposed by Golden and Ramdath postulates that the imbalance between the production of free radicals and their neutralization by scavengers play an important role in the development of the kwashiorkor syndrome. These radicals which are products of the inflammatory response generate peroxides, particularly in cell membranes (Golden and Ramdath, 1987). Prostaglandin E2 and cysteinyl leukotrienes, which are powerful agents in the inflammatory response increase in PEM upon *in vitro* stimulation (Mayatepek *et al.*, 1993).It has been suggested that acute phase response and pro-inflammatory cytokines directly affect the bone remodelling required for longitudinal growth (Stephensen, 1999).

Inflammation is a general name for reactions occurring after most kinds of tissue injury, infections or immunologic stimulation as a defense against foreign or altered endogenous substances. Inflammatory reactions involve a number of biochemical and cellular alterations, the extent of which correlates with the extent of the initial trauma. A number of cytokines, known collectively as pro-inflammatory cytokines because of the ability to accelerate inflammation, also regulate inflammatory reactions either directly or by their ability to induce the synthesis of cellular adhesion molecules or other cytokines in certain cell types. The major pro-inflammatory cytokines that are implicated in early responses are interleukin-1alpha (IL-1α), interleukin-1beta (IL-1β), interleukin 6 (IL-6) and

tumour necrosis factor-alpha (TNF-α) (Cytokine & Cells Online Pathfinder Encyclopedia, 2002).

Pro-inflammatory cytokines are produced predominantly by activated immune cells such as macrophages and are involved in the amplification of inflammatory reaction. These include IL-1, IL-6, TNF-α and TNF-β. Concentrations of (IL-6), C-reactive protein and the soluble receptors of TNF-α(sTNFR-p55 and sTNFR-p75) are greater in children with PEM, particularly in those with kwashiorkor, whereas soluble receptors of IL-6 (sIL6R-gp8O) and IL-l receptor antagonist concentrations are not significantly different from those of healthy children (Sauerwein *et al.*, 1997).



## 1.2 STATEMENT OF PROBLEM

Malnutrition is associated with abnormalities in the specific immune response and with susceptibility to infection. From early childhood itis associated with significant functionally increased impairment in adult life, reduced work capacity and decreasing economic productivity (Pelletier *et al.*, 1995). Children who are malnourished not only tend to have increased morbidity and mortality but are also more prone to suffer from delayed mental development, poor school performance and reduced intellectual achievement (Pelletier *et al.*, 1995).

PEM impairs the linear growth of children, leading to a further reduction in food intake, nutrient absorption, direct or catabolic nutrient losses and increased metabolic requirements. It has been suggested that acute phase response and pro-inflammatory cytokines directly affect bone remodelling required for longitudinal growth (Stephensen, 1999). Malnutrition is responsible, directly or indirectly for 54% of the 10.8 million deaths per year in children under five and contributes to every second death (53%) associated with infectious diseases among children under five years of age in developing countries (WHO, 2005).

Early diagnosis of protein energy malnutrition will prevent complications from occurring in children who fall victim to the condition. However, there is very little knowledge on early and precise diagnosis of PEM in Ghana, thus the outcome of this study would provide remedy for early detection and precise diagnosis.

## 1.3 JUSTIFICATION

Increased morbidity and mortality is found in children suffering from PEM and are also more prone to suffer from delayed mental development, poor school performance and reduced intellectual achievement (Pelletier *et al.*, 1995). The consequences of these conditions may not end in childhood but can continue to adolescent stage where stunting and thinness is common (Leenstra *et al.*, 2005). In West Africa, children under five and maternal mortality rates are amongst the highest in the world. One in three children under the age of five is undernourished and many are affected by acute and chronic malnutrition (UNICEF, 2006a). In Ghana PEM is common especially among those with poor living standards and most often in the rural areas. It has been suggested that acute phase response and pro-inflammatory cytokines directly affect the bone remodeling required for longitudinal growth (Stephensen, 1999). However, there is scarcity of data on pro-inflammatory cytokines and how they affect children with PEM in Ghana.

The presence of infection leads to the development of systemic inflammatory response syndrome which will cause metabolic deregulation and finally leads to the release of cytokines in critically ill child. During this process the immune system fails to respond to phagocytosis, chemotaxis and opsonization.

Pro-inflammatory cytokines which are produced would help the body defend itself against endogenous substances and further cause metabolic disorder in the protein, glucose and fat synthesis. When children are unable to adapt to these changes they become malnourished and is termed cytokine induced malnutrition.

Although anthropometric measurements, total protein and albumin concentrations are known indicators for PEM, the usage of the concentration of pro-inflammatory cytokines would be very essential in diagnosis of Protein Energy malnutrition.

## 1.4 STUDY HYPOTHESIS

With the burden of a low socio-economic background, could pro-inflammatory cytokines be used as diagnostic markers that would help in differentiation and management of protein energy malnutrition in children?



## 1.5 AIM

The main aim ofthe study was to assess the use of pro-inflammatory cytokines as diagnostic markers for protein energy malnutrition in children.

## 1.6 SPECIFIC OBJECTIVES

The specific objectives were to:

1. Determine the impact of the concentrations of serum total protein, albumin, IL-6 and TNF-α in malnourished children compared to controls.
2. Compare variations in the concentrations of the measured analytes in children with marasmus, marasmic kwashiorkor and kwashiorkor.
3. Determine the influence of parental socioeconomic and educational standards on the nutritional status of their children.
4. Assess the impact of nutritional intervention in resolving protein energy malnutrition.

*Chapter 2*

# LITERATURE REVIEW

## 2.1 MALNUTRITION

Malnutrition is globally the most important risk factor for illnesses and death, affecting especially hundreds of millions of pregnant women and young children. It is currently the leading cause of global burden of disease (Ezzati *et al.*, 2002). However evidence has shown that child death and malnutrition are not equally distributed throughout the world. They cluster in sub-Saharan Africa and south Asia, and in poor communities within these regions (de Onis and Blossner, 2003). Studies have also shown that malnutrition is the most common cause of immunodeficiency worldwide (Ezzati *et al.*, 2002).

The World Health Organization defines malnutrition as "the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions (Scrimshaw *et al.*, 1968). Severe malnutrition, typified by wasting, oedema or both, occurs almost exclusively in children (Brabin and Coulter, 2003).

There are two (2) elements of malnutrition: protein–energy malnutrition and micronutrient deficiencies. Apart from marasmus and kwashiorkor (the two forms of protein– energy malnutrition), deficiencies in iron, iodine, vitamin A and zinc are the main manifestations of malnutrition (Muller and Krawinkel, 2005). The degree and distribution of protein–energy malnutrition and micronutrient deficiencies in a given population depends on many factors: the political and economic situation, the level of education and sanitation, the season and climate conditions, food production (Brabin and Coulter, 2003), cultural and religious food customs, breast-feeding habits, prevalence of infectious diseases, the existence and effectiveness of nutrition programs and the availability and quality of health services (F.A.O., 2004).

## 2.2 PROTEIN ENERGY MALNUTRITION

Protein-energy malnutrition (PEM) may be present at any time during the life cycle, but it is more common in the extreme ages that is, during infancy/childhood and in the elderly (Castaneda *et al.*, 1995). The present review will be restricted mostly to the condition present during infancy and childhood. Protein energy malnutrition in children (PEM) is a pathologic depletion of the body's lean tissues caused by starvation, or a combination of starvation and catabolic stress (Castaneda *et al.*, 1995). It is the disease that develops when protein intake or energy intake, or both, chronically fail to meet the body's requirements for these nutrients (Hoffer *et al.*, 1999). The underlying mechanisms include decreased food intake because of anorexia, decreased nutrient absorption, increased metabolic requirements and direct nutrient losses(Gonzalez-Barranco and Rios-Torres, 2004).

Patients that lose 10–20 percent of their body weight may have moderate PEM. Losing 20 percent of body weight or more is generally classified as severe PEM (Gonzalez-Barranco and Rios-Torres, 2004; Hamer *et al.*, 2004). Primary PEM results from a diet that lacks sufficient sources of protein and/or energy. Secondary PEM usually occurs as a complication of chronic diseases such as AIDS, cancer, chronic kidney failure, inflammatory bowel disease, and other illnesses that impair the body's ability to absorb or use nutrients or to compensate for nutrient losses (Hamer *et al.*, 2004). Marasmus and Kwashiorkor are the two (2) forms of the protein– energy malnutrition.

Protein-energy malnutrition (PEM) is a problem in many developing countries, most commonly affecting children between the ages of 6 months and 5 years. The condition may result from lack of food or from infections that cause loss of appetite while increasing the body's nutrient requirements and losses. Children between 12 and 36 months old are especially at risk since they are the most vulnerable to infections such as gastroenteritis and measles (WHO, 2000b).

### *2.2.1 Kwashiorkor*

Kwashiorkor, also called wet protein-energy malnutrition, is a form of PEM characterized primarily by protein deficiency. This condition usually appears at the age of about 12 months when breastfeeding is discontinued, but it can develop at any time during a child's formative years (Manary *et al.*, 1998). Kwashiorkor usually manifests with fluid retention (oedema) usually starting in the legs and feet and spreading, in more advanced cases, to the hands and face. Oedema may be detected by the production of a definite pit as a result of moderate pressure for 3 seconds with the thumb over the lower end of the tibia and the dorsum of foot. Because of oedema, children with kwashiorkor may look "fat" so that their parents regard them as well fed (Manary *et al.*, 1998).

There is hair discoloration or loss of pigmentation; curly hair becomes straight easily pluckable. Coloured, dark skin may become dried and lighter in some places especially in the skin folds; outer layers of skin may peel off and ulceration may occur; the lesions may resemble burns (Cundiff and Harris, 2006). Children with Kwashiorkor are usually apathetic, miserable, and irritable. They show no signs of hunger, and it is difficult to persuade them to eat. There is hepatomegaly, lethargy, severe immune deficiency and early death occurs (UNACC, 2000). Hypoalbuminaemia and electrolyte imbalance have been put forward as possible causes of the oedema (Waterlow, 1992).

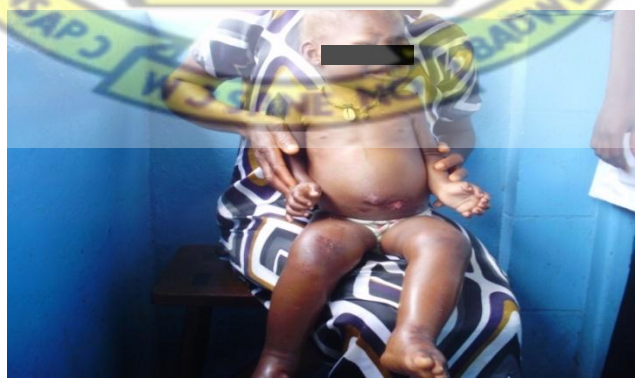

**Plate2.1Picture of a child with Kwashiorkor (Snapped at MCHH)**

### *2.2.2 Marasmic kwashiorkor*

This is a severe wasting in the presence of oedema. It is a mixed form of PEM, and manifests as oedema occurring in children who may or may not have other signs of Kwashiorkor (Manary and Brewster, 1997; Manary *et al.*, 1998).

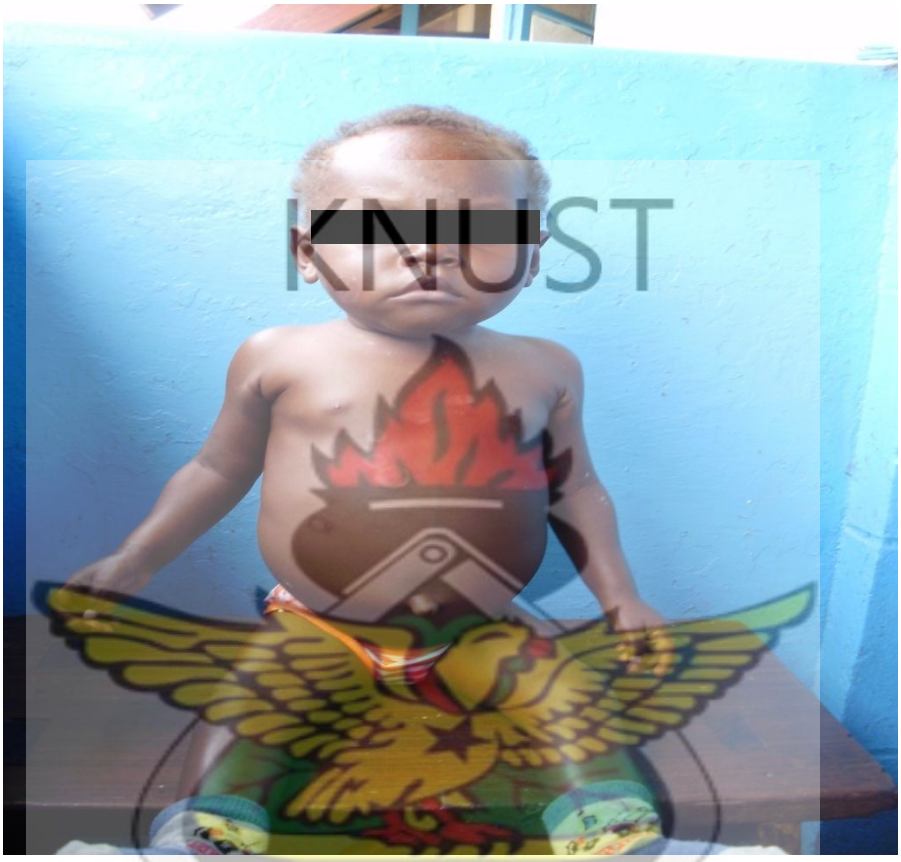

**Plate 2.2 Picture of a child with Marasmic Kwashiorkor (Snapped at MCHH)**

### *2.2.3 Marasmus*

Early marasmus occurs usually in the first year of life in children who have been weaned from breast milk or who suffer from weakening conditions like chronic diarrhoea. It is frequently associated with contaminated bottle-feeding in urban areas (Pinstrup-Andersen *et al.*, 1993). Primarily marasmus is caused by energy deficiency from prolonged starvation. It may also result from chronic or recurring infections with marginal food intake (de Onis *et al.*, 1993). Marasmus is characterized by stunted growth and wasting of muscle and tissue. Wasting

indicates recent weight loss, whereas stunting usually results from chronic weight loss. The major nutritional indicators studied are: stunting (low height-for-age); underweight (low weight-for-age); and wasting (low weight-for-height). Of the three (3), wasting is the most dangerous and signifies acute malnutrition (Muller and Krawinkel, 2005). The main sign is a severe wasting and the child appears very thin and has no fat. Most of the fat and muscle mass have been expended to provide energy. There is severe wasting of the shoulders, arms, buttocks and thighs, with no visible rib outlines. There is no oedema (swelling that pits on pressure) of the lower extremities (Manary and Brewster, 1997).



Clinical aspects typically include a triangular face, extended abdomen (from muscular hypotonia) and anal or rectal prolapse (from loss of perianal fat) (Manary *et al.*, 1998).


**Plate 2.3 Picture of a child with Marasmus (Snapped at MCHH)**

## 2.3 PATHOPHYSIOLOGY AND CLINICAL FEATURES OF PEM

Protein–energy malnutrition usually manifests early, in children between 6 months and 2 years of age and is associated with early weaning, delayed introduction of complementary foods, a low-protein diet and severe or frequent infections (Kwena *et al.*, 2003; Muller *et al.*, 2003). PEM is characterized by atrophy and weakness of the skeletal muscles (including the respiratory muscles), reduced heart muscle mass (Powell-Tuck, 1997), impaired wound healing, skin thinning with a predisposition to decubitus ulcers, fatigue, apathy and hypothermia. The extracellular fluid compartment characteristically expands in PEM, occasionally causing oedema (Hoffer, 2001). Synthesis of pigments in the hair and skin fails (e.g., hair colour may change and skin becomes hyperpigmented) because of a lack of substrate (e.g., tyrosin) and coenzymes (Muller and Krawinkel, 2005).

The other essential aspects of severe protein–energy malnutrition are the fatty degeneration of the liver and heart. This degeneration is not just a sign of severe malnutrition; it also causes subclinical or overt cardiac insufficiency, especially when malnutrition is accompanied by oedema. If the myocardial insufficiency is not corrected, iatrogenic fluid and sodium overload quickly escalate it into cardiac failure (Kwena *et al.*, 2003; Muller *et al.*, 2003). Another injurious aspect of PEM is the loss of subcutaneous fat, which markedly reduces the body's capacity for temperature regulation and water storage (Alam *et al.*, 2003). As a result, malnourished children become dehydrated, hypothermic and hypoglycemic more quickly and severely than others (Gracey, 1999).

Severe protein–energy malnutrition is associated with atrophy of the mucosa of the small bowel, leading to a loss of absorption as well as of digestion capacity (Alam *et al.*, 2003). Furthermore PEM is associated with chronic hypovolaemia, which leads to secondary hyperaldosteronism, and further complicates fluid and electrolyte balance (Kwena *et al.*, 2003; Muller *et al.*, 2003). PEM affected children do not show signs of hyperkalaemia. This is because the development of muscular

dystrophy mobilizes much of the body's potassium, which is then lost through urine (Manary and Brewster, 1997).

## 2.4 EPIDEMIOLOGY OF MALNUTRITION

Malnutrition is currently the leading cause of the global burden of disease (Ezzati *et al.*, 2002) and has been identified as the underlying factor in about 50% of deaths of children under 5 years of age in developing countries (Black *et al.*, 2003). In 1998 it was estimated that 9% of children below 5years of age globally suffer from wasting and this affects every fourth child world-wide: 150 million (26.7%) are underweight while 182 million (32.5%) are stunted (WHO, 1998).

Geographically, more than 70% of PEM children live in Asia. Most of these children were born by malnourished mothers (UNESCO/WHO, 2002). Nearly a third of these children who died with malnutrition as underlying factors were stunted and a quarter were underweight. This situation is expected to worsen in some parts of the world including sub-Saharan Africa (de Onis *et al.*, 2000; de Onis *et al.*, 2004) . These figures are indications of a serious public health crisis with long term effects on population, health, human capital accumulation and sustainability of developing countries. The commitment of the international community to reducing childhood malnutrition and mortality has been renewed recently through the Millennium Development Goals, but achieving this ambition requires further studies on how the determinants and the level of malnutrition respond to changing economic context, which has been the case in many developing countries experiencing high burden.

### *2.4.1 Malnutrition in Developed countries*

Malnutrition remains one of the most common causes of morbidity and mortality among children throughout the world (WHO, 1999). In developed countries, Protein Energy Undernutrition (PEU) is common among the institutionalized

elderly (although often not suspected) and among patients with disorders that decrease appetite or impair nutrient digestion, absorption, or metabolism. Although PEM is not prevalent among the general population of the developed world, it is often seen in elderly people who live in nursing homes and in children whose parents are poor. PEM occurs in one of every two surgical patients and in 48% of all other hospital patients (WHO, 1999).

### *2.4.2 Malnutrition in Developing countries*



Malnutrition is a major public health problem throughout the developing world, particularly in southern Asia and sub-Saharan Africa (Schofield and Ashworth, 1996); (WHO and UNICEF, 2004). Diets in populations in these countries are frequently deficient in macronutrients (protein, carbohydrates and fat, leading to protein–energy malnutrition), micronutrients (electrolytes, minerals and vitamins, leading to specific micronutrient deficiencies) or both (Millward and Jackson, 2004). Apart from deficiencies in single nutrients, such as vitamins, essential fatty acids, amino acids, iron, and trace elements (Woodward, 1998), the high prevalence of bacterial and parasitic diseases in these developing countries contributes greatly to malnutrition there (de Onis *et al.*, 1993; Stoltzfus *et al.*, 2004). The World Health Organization report (WHO, 2000b) indicated that malnutrition was responsible, directly or indirectly, for 54 per cent of the 10.8 million deaths per year in under-five children and contributes to every second death (53%) associated with infectious diseases in developing countries. In the year 2000 it wasestimated that, in developing countries, more than one-quarterof all children younger than 5 years of age were malnourished (UNACC, 2000). Brabin and Coulter (2003) reported that of all the children under the age of 5 years in developing countries, about 31% were underweight, 38% had stunted growth and 9% showed wasting. According to United Nations Children's Fund (UNICEF, 2006b) report, 27% of children < 5 years of age in developing countries suffer from wasting. This is

against the background evidence that malnutrition contributes to 54% of all deaths among children < 5 years of age.

2.4.2.1Malnutrition in Ghana

Although malnutrition continues to be a major public health problem throughout the developing world, in Ghana there has been substantial scope of progress in reducing the average malnutrition level. The prevalence of underweight fell progressively from 30% in 1988 to 21% in 2003. The prevalence of stunting fell from 29% to 26% between 1988 and 1993, and then increased again to 29% during the period 1993-2003. The level of wasting remained unstable during the period 1993-2003 (GDHS, 1998-2003). However the 2003 Ghana demographic and health survey report indicated that, malnutrition accounted for 40% of under 5 mortality in Ghana (GDHS, 2003). UNICEF (2006a) reported that 22.4% of the under-five children were stunted and 17% were underweight. Nti and Lartey, (2006) also stated that the prevalence of stunting among children between 12 and 36 months in the Manya Krobo District of Ghana was as high as 20%. The prevalence of wasting/malnutrition identified in a study in Kumasi in the Ashanti Region was 21.2%(Antwi, 2008). These results were consistent with the 22.1% reported by the World Food Programme – Ghana in 2005 (WHO, 2005). The report further stated that one (1) out of every 5th child in Ghana was malnourished.

## 2.5 PROTEIN ENERGY MALNUTRITION AND INFECTION

Malnutrition and infection interact in a vicious cycle and the presence of one easily leads to the development of the other (Chandra, 1991). Apart from deficiencies in single nutrients, such as vitamins, essential fatty acids, amino acids, iron, and trace elements, under nutrition greatly increases susceptibility to infectious diseases, especially in children belonging to the lower socio-economic strata (Woodward, 1998). Infection causes energy loss on the part of the individual, which reduces

productivity on the community level and perpetuates the alarming spiral of malnutrition, infection, disease, and poverty (Pelletier *et al.*, 1995).

Patients with severe tissue injury develop a hypermetabolic response termed as systemic inflammatory response syndrome (SIRS), which is defined by the presence of thefollowing: fever (or profound hypothermia), tachycardia, tachypnoea and leukocytosis (or increased numbers of band forms) (Davies and Hagen, 1997), changes in acute-phase serum protein concentrations (Gabay and Kushner, 1999) increased energy expenditure, increased whole-body protein turnover, anorexia and protein wasting (Davies and Hagen, 1997). In response to infection, the immune system first executes innate and then subsequently acquired host defense functions of high diversity. Both processes involve activation and propagation of immune cells and synthesis of an array of molecules requiring DNA replication, RNA expression and protein synthesis and secretion, and therefore consume additional anabolic energy (Elizabeth, 2009). Mediators of inflammation further increase the catabolic response. The resulting negative nitrogen balance is usually proportional to the intensity of the injury (Coss-Bu *et al.*, 2001).

Nutritional status of the host critically determines the outcome of infection. Severe malnutrition during childhood affects thymic development, which compromises immunity in children by a long-term reduction of peripheral lymphocyte counts (Bhan *et al.*, 2003).Most children with severe protein–energy malnutrition have asymptomatic infections because their immune system fails to respond with chemotaxis, opsonization and phagocytosis of bacteria, viruses or fungi. The system is rundown and therefore the body cannot produce even the fever that is typical of inflammation (Bhan *et al.*, 2003).

## 2.6 MALNUTRITION AND IMMUNITY

PEM impairs cell-mediated immunity, phagocytic function, and the complement systems leading to immunologic deficiency in the humoral and cellular subsystem and lack of immune mediators (e.g., tumour necrosis factor). It also diminishes immunoglobulin (IgA, IgM, and IgG) concentrations and cytokine production (Lesourd, 1995). Pro-inflammatory cytokines, produced in response to local inflammation, travel through the blood and stimulate liver cells to synthesize andsecrete acute phase proteins (APPs) such as C-reactive proteins (Grimble, 1996). Systemic inflammation elicits changes in body composition, alters the use of various macronutrients (that is, fats, carbohydrates and protein) and increases cellular consumption of important vitamins and minerals (that is, micronutrients). Systemic inflammation also promotes the breakdown of protein and fat and loss of muscle mass, and it stimulates the liver to produce more APPs. These changes increase the body's demand for nutrients from food, particularly in malnourished people (Enwonwu and Ritchie, 2007).

The acute phase reaction (APR) also promotes production of specific acute phase proteins (APPs), with increased release of many inflammatory mediators, proliferation of immune cells and several metabolic changes (Gabay and Kushner, 1999).In the process, micronutrients such as vitamin A, iron, copper, selenium and zinc are compartmentalized to the tissues, lost from the body or blocked from cellular use. Pro-inflammatory cytokines stimulate APR and promote major changes in protein and amino acid metabolism (Phillips *et al.*, 2005). Amino acids released from muscle and other tissues may be inadequate for synthesis of the APPs and essential proteins and thus, must be supplemented from dietary sources. In particular, requirements for specific amino acids such as arginine (a substrate for nitric oxide synthesis), sulphur amino acids, cysteine and methionine may be increased. Tissue repair after an inflammatory process also may increasethe

requirement for the non essential amino acid glycine, which is an important component of collagen.

Cytokines are substances that play an important role in coordinating the inflammatory response of the body to various external and internal stimuli (Savino, 2002). There are two classes of cytokines: pro-inflammatory and anti-inflammatory. The pro-inflammatory cytokines are essential for initiation of defense against various pathogens. The anti-inflammatory cytokines down-regulate the inflammatory process by suppressing production of the pro-inflammatory cytokines when there is over production. Present evidence suggests that cytokines, as intercellular mediators, play a key role in the nutrition-infection complex. Protein-calorie malnutrition, deficiency of fatty acids, vitamins and trace elements impair cytokine production. On the other hand, infections increase pro-inflammatory cytokine production interfering with nutritional status by impairing metabolic activity and by inducing anorexia. Elevated cytokine levels in human milk represent a physiological phenomenon and are not necessarily associated with infectious processes (Muñoz *et al.*, 1995).

As a nonspecific antigen proliferative factor for all T lymphocyte subpopulations, IL-2 is an immune regulator playing a major role in inflammatory reactions as well as in tumor control. During inflammation, IL-2 stimulates secretion of pro-inflammatory cytokines such as IL-1, TNF-*a*, and TNF-*β* (Rhodus *et al.*, 2005). IL-6 is a pleotropic cytokine that influences the antigenspecific immune responses and inflammatory reactions. Together with IL-1 and TNF-*a* (which also stimulate IL-6 secretion), it belongs to the group of main pro-inflammatory cytokines (Yamano *et al.*, 1999). Malnourished patients preserve the capacity to release inflammatory markers such as CRP and IL-6, which can be considered favorable for combating infections. Conversely, this release might also have a significant impact on nutritional status during hospitalization (Delgado *et al.*, 2008).

## 2.7 EFFECT OF MALNUTRITION ON CHILDREN

Chronic PEM has many short-term and long-term physical and mental effects, including growth retardation, lowered resistance to infection, and increased mortality rates in young children (Pelletier *et al.*, 1995). It was recognized in the 1950s that the severe forms of protein-energy malnutrition, kwashiorkor and marasmus, were associated with marked cognitive effects (Scrimshaw *et al.*, 1968) although the lasting effects on survivors were unknown. Effects of malnutrition in early childhood can be devastating and permanent. Whether or not children are well-nourished during the prenatal period and the first years of life can have a profound effect on their health status, as well as their ability to learn, communicate, socialize, reasoning and adapt to their environment (Pelletier *et al.*, 1995).



## 2.8 DIAGNOSIS OF PEM

The diagnosis of malnutrition is generally based on objective measurements of nutritional status, including assessments of oral intake, weight loss, anthropometric data, and determination of cell-mediated immunity, biochemical parameters, physical examination and body composition analysis (Hulst *et al.*, 2004).

### *2.8.1 Anthropometricmeasurements*

In children, protein–energy malnutrition is defined by measurements that fall below 2 standard deviations under the normal weight for age (underweight), height for age (stunting) and weight for height (wasting) (Pinstrup-Andersen *et al.*, 1993).

Reduced height-for-age reflects the slowing of skeletal growth, and is considered to be a reliable indicator of long-standing malnutrition in childhood. Low weight-for-height, on the other hand, indicates a deficit in tissue and fat mass. This

measure is more sensitive to temporary food shortages and episodes of illness. A low weight-for-age is also used in the literature to indicate malnutrition, however this does not discriminate well between temporary and more permanent malnutrition (Zere and McIntyre, 2003).

2.8.1.1Weight

It is a measure of overall nutritional status with age, sex and height required for optimal interpretation. Weight is determined using digital or beam balance scale. It is recorded to the nearest 0.01Kg in infants and 0.1Kg in older children (Duggan *et al.*, 2004).



2.8.1.2 Mid-upper arm circumference (MUAC)

This is a quick and simple way to determine whether or not a child is malnourished using a simple colored plastic strip. MUAC and triceps skin fold (TSF) are also used as part of the assessment to determine body fat and protein stores in children with chronic disease (Duggan *et al.*, 2004). MUAC is suitable to use on children from the age of 12 months up to the age of 59 months.

**Figure 2.1 Mid-Upper Arm Circumference (MUAC)Tape**

2.8.1.3Height or Length

Measurement of length in particular, but also of height, requires great care to be of value. Both remain the reserve for assessment of linear skeletal growth. Height or

length generally correlates better with socioeconomic status than soft tissue measurement such as weight. Although relatively insensitive to short-term nutritional deficits, height or length reflects long-standing nutritional experience. Length is usually indicated for children up to 24 months of age, and height is used thereafter. Readings are recorded to the nearest 0.1 cm (Neumann *et al.*, 1982).

## 2.9 BIOCHEMICAL MARKERS OF PEM

Biochemical parameters provide valuable information for the over-all management and act as very sensitive indicators. Different biochemical parameters are altered during protein energy malnutrition (Mishra *et al.*, 2009). In case of severely malnourished wasted children, serum total protein and albumin are normal or reduced and fractions of the glycoproteins responsible for binding drugs are decreased (Muller and Krawinkel, 2005). The serum albumin concentration remains normal in successfully adapted PEM and it falls when adaptation fails. A normal serum albumin concentrationin a PEM patient is a favourable prognostic finding. It is an indication of a successful adaptation and, the absence of metabolic stress (Hoffer, 2001).

Because albumin and pre-albumin are negative acute-phase proteins, their serum levels fall in response to metabolic stresseven in the absence of PEM. The reductions of total serum protein and albumin are more marked in kwashiorkor with oedema than in marasmus. Lowering of these serum total protein and albumin values in PEM could be explained on the basis of generalized protein deficiency leading to impaired synthesis (Mishra *et al.*, 2009). It could also be due to the redistribution of albumin into an expanded extracellular fluid compartment that occurs in acute severe inflammation (Hoffer, 2001). In kwashiorkor the oedema may clear during nutritional rehabilitation without any change in serum albumin concentration (Kazeem *et al.*, 2009). Studies by Rahman *et al.*,(2007) observed that the mean serum total protein and albumin level in normal children

12-59 months of age was significantly higher than that of malnourished children. However, mean of serum globulin level was higher in malnourished children than that of normal children. Raised globulin level is anticipated in malnourished children since malnutrition is commonly associated with infections (Rahman *et al.*, 2007).

## 2.10 INTERVENTIONS

Interventions to prevent protein–energy malnutrition range from promoting breast-feeding to food supplementation schemes. Micronutrient deficiencies are best addressed through food-based strategies such as dietary diversification and fortification of salt with iodine has been a global success story. To be effective, all such interventions require accompanying nutrition-education campaigns and health interventions (Muller and Krawinkel, 2005). Malnutrition in the young child may be 'prevented' by identifying the individuals at risk and for the period when the risk is greatest, modifying their environment, or even removing them from it in order to ensure that, as individuals, they are spared the sequelae of undernutrition.

## 2.11 MANAGEMENT AND CONTROL

In spite of the various dietary approaches to manage severe malnutrition (Khanum *et al.*, 1994) patients with kwashiorkor (including marasmic kwashiorkor) continue to die much more frequently than those with marasmus alone in developing countries (Ahmed *et al.*, 1999). An additional concern is that many of these children with severe malnutrition are also infected with HIV (Ambrus and Ambrus, 2004). Therefore there is the need for a systematic approach to the severely malnourished patient that goes beyond an appropriate diet. Essential management steps include intake of a reduced volume of protein and sodium during the first phase while emergency measures are taken to reduce the risk of hypoglycemia, hypothermia and dehydration (WHO, 1999). Oral, enteral and parenteral volume loads must be

checked carefully to avoid imminent heart failure. Thus, continuous monitoring of central venous blood pressure is very desirable. In the early phase of rehabilitation, a protein intake exceeding 1 g/kg body weight in combination with impaired liver function (with breakdown of the urea cycle) and little urine excretion (a result of dehydration) easily exceeds the malnourished child's metabolic capacity to rid himself or herself of excess ammonia (WHO, 2000a).



*Chapter 3*

# MATERIALS AND METHODS

## 3.1 STUDY DESIGN

This hospital-based case control study was conducted at the Maternal and Child Health Hospital (MCHH) in the Subin Sub-Metro in the Kumasi Metropolitan area. All children between the ages of 8 months to 36 months attending the child welfare clinic and the rehabilitation center of MCHH during the period of December 2009-June 2010 were recruited after fulfilling the inclusion criteria.



## 3.2 STUDY AREA

Subin Sub-metro is one of the four sub-metros in the Kumasi Metropolitan area. It is strategically located at the centre of the metropolis and it forms 10.9% of the population with an estimated population of one hundred and seventy two thousand, three hundred and forty four (172,344) with 25,335 children under 5 years. The Subin sub-metro covers a landmark of 28sqm with a growth rate of 3.4%.It shares boundaries with Bantama to the East, Asokwa to the West and Tafo to the North. The major economic activities of the populace are trading and government sector work.

The sub-metro has the highest immigrants from the Northern and the two Upper Regions doing mainly minor work such as washing of dishes at chopbars, head potters (Kayayee) and chopbar attendants. Because they do not have a proper place of abode, they resort to the streets. As a result they fall prey to rapists, which sometimes lead to HIV/AIDS and other STDs, teenage pregnancy, which affects their health.The catchment area has a floating population of 5,000 which is very high during the day (obtained from the Kumasi Metro Health Directorate).

## 3.3 STUDY SITE

The Maternal and Child Health Hospital (MCHH) is specialized in the management of under nutrition and its associated conditions. It serves as a referral centre for children with Protein energy malnutrition. It is situated in the Subin Sub-district in Kumasi near the Komfo Anokye Teaching Hospital with staff strength of about 230 including paediatricians, general practitioners, nurses, biomedical scientists and health aids. The Paediatric ward and the Rehabilitation centre at MCHH admit between 480 and 550 cases annually with a monthly average of 35 patients. The Paediatric ward has about 50% of all children admitted being malnourished. MCHH is the only hospital with a Rehabilitation centre in the Kumasi metropolis for protein energy malnutrition. In 2007 the hospital recorded a total of 588 admissions with 39% rehabilitation rate. In 2008 and 2009, 560 and 722 admissions with 35.8% and 56.2% rehabilitation rates were respectively recorded(obtained from the records department of the Kumasi Metro Health directorate).

## 3.4 STUDY POPULATION

Children between the ages of 8 – 36 months who attended the Maternal and Child Health Clinic during the study period were included in this study after fulfilling the inclusion criteria. Signed informed consent was obtained if parent or guardian demonstrated understanding of the study and was willing to enroll the child. The interview was conducted in Twi which is the local language in the region. The study was approved by the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi.

### *3.4.1 Inclusion and exclusion criteria*

3.4.1.1Malnourished children

A total of 80 children who attended the hospital with anthropometric measurements of weight for age <70% (Z-scores)and weight for height < 80% (Z-Scores)were included in this study.Children who were on either micronutrient supplementation or on other medications were excluded from the study.

3.4.1.2Healthy children (Control)

A total of 35 children who attended the child welfare clinic for routine checkups with weight for age >90% (Z-scores)and weight for height >90% (Z-Scores) were used as controls.



## 3.5 SAMPLING METHODS

Blood samples were collected from all the children who fulfilled the inclusion criteria. Simple random sampling was used to select both the malnourished and healthy children for the study.

### *3.5.1 Anthropometric measurements*

The anthropometric measurements used for nutritional assessment were weight, height and mid-upper arm circumference. Weight was measured to the nearest 0.1kg in light clothing. Height was measured to the nearest centimeter (cm) against a wall-mounted ruler. Mid-upper arm circumference was obtained with a WHO standardized tape. Nutritional assessment was based on weight for age; height for age and mid-upper arm circumference of the controls and subjects.

## 3.6 LABORATORY INVESTIGATIONS

Three millilitres (3ml) of blood samples was collected from both the malnourished and healthy subjects who fulfilled the inclusion criteria. Two millilitres (2.0ml) of the blood sample was put into vacutainer plain tubes and allowed to clot. The

clotted samples were then centrifuged for 10 minutes at 1250 x g and serum stored at -80$^{o}$C. The serawere used for biochemical and immunological assays. The remaining 1ml of the blood sample was put into monovet® ethylene diaminetetraacetic acid (EDTA) tubes and used for haematological analysis and preparation of blood films for malaria parasites.

### *3.6.1 Haematological analysis*

The haematological analysis performed on the EDTA blood samples were: haemoglobin concentration (Hb) and total white blood cell count (WBC) using the Sysmex 2000i xt (Sysmex Corporation, Kobe, Japan).



#### 3.6.1.1Total White Blood Cell Count (WBC)

The principle underlying the estimation of WBC is based on flowcytometry. The flow cytometer measures multiple characteristics of individual cells flowing in single file in a stream of fluid. Light scattering at different angles distinguish differences in size and internal complexity of cells, whereas light emitted from fluorescent labeled antibodies identifies a wide array of cell surface and cytoplasmic antigens. The emitted lights are collected via optics that direct the light to a series of filters and dichroic mirrors that isolate particular wavelength bands. The light signals are detected by photomultiplier tubes and digitized for computer analysis. The resulting information usually is displayed in histogram or two dimensional dot-plot formats(Sysmex, 2007).

#### 3.6.1.2Haemoglobin

This method for estimating haemoglobin is based on that described by Oshiro*et al.,* (1982) utilizing sodium laurel sulphate (SLS),a non-cyanide reagent. SLS converts hemoglobin into methaemoglobin in the order of oxyhaemoglobin, haemochrome and methaemoglobin and its oxidative activity. The end product is a colored compound that is measured spectrophotometrically at a wavelength of 540nm. Haemoglobin determinations are performed from a dilution and in its own

separate chamber so there is no interference from high WBC counts, lipaemia or abnormal proteins.

### *3.6.2 Biochemical analysis*

Serum total protein and albumin were analyzed using the Vitalab Flexor E (Vital Scientific N V Netherland) chemistry analyser.

#### 3.6.2.1Total protein

The present method is based on the modifications of Gornall*et al.,*(1949). Protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of proteins present when compared to a solution with known protein concentration.

$$Protein + Cu^{2+} \xrightarrow{alkali} Coloured\ complex$$

#### 3.6.2.2Albumin

The method used for this assay is based on that of Doumas*et al.,*(1971) where at a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of the colour at 630nm is directly proportional to the albumin content.

$$BCG + Albumin \xrightarrow{controlled} Green\ BCG - Albumin\ Complex$$

### *3.6.3 Immunological Assay*

IL-6 and Tumor Necrosis Factor-alpha (TNF-α) were analyzed with the Quantikine enzyme immunosorbent assay kit (ELISA) (R&D Systems Abingdon UK).

#### 3.6.3.1Principle of assay for IL-6 and TNF-α

The assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-6 or TNF-α has been pre-coated onto a micro plate. Standards and samples (100 µL for IL-6 and 200 µL for TNF-α respectively)

were pipetted into the wells and any IL-6 or TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 or TNF-α was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour develops in the proportion to the amount of IL-6 or TNF-α bound in the initial step. The colour development is stopped and the intensity of the colour was measured at 450nm.



## 3.7 TREATMENT

The children were put on a diet regimen for a period of six (6) weeks. They were put on starter (F75) and catch up (F100) formulae. These products contain 75 and100 Kcal per 100ml respectively and are high in energy, fat and protein thereby providing a large amount of nutrients.

## 3.8 STATISTICAL ANALYSIS

Continuous data are expressed as mean ± SD whilst categorical data are expressed as proportions. Statistical comparisons were analyzed using one-way ANOVA and corrected with Bonferroni's Multiple Comparison test (*post-hoc)*. Student's *t*-test (paired) was used to compare means in subjects before and after treatment. The chi square test statistics was used to compare the statistical significance of proportions. A *Pvalue* of less than 0.05 was considered significant. All statistical analysis was performed using GraphPad prism version 5.0 for windows.

*Chapter 4*

# RESULTS

## 4.1 SOCIO-ECONOMIC FAMILY PROFILE AND BIRTHWEIGHT

Table 4.1 presents a general socio-economic family profile and birth profile of the study population. A proportional comparison of the marital status of parents of the control group to parents of the subject group showed no statistical significance (p=0.1240) but females in the control group happened to have married parents when compared to females in the subject group (p=0.0386). With an average of about two children, no significant differences were observed in the number of children per family when the control group was compared to the subject group (p>0.05). A further assessment of the educational background of the parents of the study population showed mothers (p=0.0021) and fathers (p=0.0005) in the control group attaining higher education compared to mothers and fathers of the subject group. Likewise, mothers of females in the control group have attained higher education compared to mothers of females in the subject group (p=0.0053) and fathers of females in the control group also have higher education compared to fathers of females in the subject group (p<0.0001). In the control group, fathers of females have attained higher education when compared to fathers of males (p=0.0200).

Employment details show mothers of the control group being gainfully employed and earning salaried incomes when compared to mothers of the subject group (p=0.0038) who had a significantly higher unemployment rate (p=0.0212). Mothers of females in the control group were also gainfully employed (p=0.0006) compared to mothers of females in the subject group who were significantly unemployed (p=0.0326). Fathers of the control group were gainfully employed when compared to fathers of the subject group (p=0.0156). Furthermore, fathers of females in the

**Table 4.1General characteristics of the study population stratified by gender**

| | CONTROL | | | SUBJECTS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variables** | **Total** | **Male** | **Female** | **Total** | **Male** | **Female** | **P value** | **P value*** | **P value**** | **P value***** | **P value******** |
| ***N*** | ***35*** | ***23*** | ***12*** | ***80*** | ***35*** | ***45*** | | | | | |
| **Parent demographic** | | | | | | | | | | | |
| Married (%) | 30(85.7) | 19(82.6) | 11(91.7) | 58(72.5) | 26(74.3) | 32(71.1) | 0.1240 | 0.4673 | 0.8469 | 0.4571 | 0.0386 |
| No. of children in Family | 2.71 ± 0.24 | 2.87 ± 0.32 | 2.41 ± 0.36 | 2.66 ± 0.21 | 2.57 ± 0.34 | 2.73 ± 0.27 | 0.8853 | 0.3873 | 0.7063 | 0.5502 | 0.5697 |
| Child birth weight (kg) | | | | | | | | | | | |
| <2.5 | 0(0.0) | 0(0.0) | 0(0.0) | 11(13.8) | 6(17.1) | 5(11.1) | 0.0255 | | 0.4371 | 0.0360 | 0.2267 |
| 2.5 to 4 | 31(88.6) | 19(82.6) | 12(100.0) | 55(68.8) | 22(62.9) | 33(73.3) | 0.0243 | 0.1248 | 0.3159 | 0.1060 | 0.0441 |
| >4 | 4(11.4) | 4(17.4) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0.0021 | 0.1248 | | 0.0106 | |
| Exclusive breastfeed (months) | | | | | | | | | | | |
| <3 | 4(11.4) | 4(17.4) | 0(0.0) | 22(27.5) | 7(20.0) | 15(33.3) | 0.058 | 0.1248 | 0.1852 | 1.0000 | 0.0198 |
| 3 to 6 | 26(74.3) | 17(73.9) | 9(75.0) | 56(70.0) | 27(77.1) | 29(64.4) | 0.6401 | 0.9443 | 0.2189 | 0.7786 | 0.4907 |
| >6 | 3(8.6) | 1(4.3) | 2(16.7) | 0(0.0) | 0(0.0) | 0(0.0) | 0.0063 | 0.2166 | | 0.2134 | 0.0053 |
| **Mother** | | | | | | | | | | | |
| High education | 4(11.4) | 2(8.7) | 2(16.7) | 0(0.0) | 0(0.0) | 0(0.0) | 0.0021 | 0.4817 | | 0.0758 | 0.0053 |
| Occupation | | | | | | | | | | | |
| Civil Servants | 5(14.3) | 2(8.7) | 3(25.0) | 1(1.3) | 1(2.9) | 0(0.0) | 0.0038 | 0.1907 | 0.2539 | 0.3260 | 0.0006 |
| Self employed | 21(60.0) | 15(65.2) | 7(30.4) | 40(50.0) | 18(51.4) | 22(48.9) | 0.3228 | 0.6891 | 0.8217 | 0.2996 | 0.5609 |
| Unemployed | 9(25.7) | 7(30.4) | 2(16.7) | 39(48.8) | 16(45.7) | 23(51.1) | 0.0212 | 0.3764 | 0.7022 | 0.2446 | 0.0326 |
| **Father** | | | | | | | | | | | |
| High education | 5(14.3) | 1(4.3) | 4(33.3) | 0(0.0) | 0(0.0) | 0(0.0) | 0.0005 | 0.0200 | | 0.2134 | <0.0001 |
| Occupation | | | | | | | | | | | |
| Civil Servants | 9(25.7) | 4(17.4) | 5(41.7) | 7(8.8) | 4(11.4) | 3(6.7) | 0.0156 | 0.1608 | 0.4546 | 0.5194 | 0.0019 |
| Self employed | 23(65.7) | 18(78.3) | 5(41.7) | 56(70.0) | 23(65.7) | 33(73.3) | 0.6484 | 0.0304 | 0.4607 | 0.3045 | 0.0387 |
| Unemployed | 3(8.6) | 1(4.3) | 2(16.7) | 17(21.3) | 8(22.9) | 9(20.0) | 0.0988 | 0.2166 | 0.7566 | 0.0568 | 0.7949 |

***Continuous data were compared were compared using unpaired t-test whilst categorical data were compared using Chi-square analysis. P value = control vrs patients, P value* = control male vrs control female, P value** = male subjects vrs female subjects, P value *** = control male vrs male subjects, P value**** = control female vrs female subjects.***

control group were gainfully employed when compared to fathers of females in the subject group (0.0019). Fathers of males in the control group (0.0304) andfathers of females in the subject group (0.0387) happened to be self-employed in comparison to fathers of females in the control group whilst fathers of males in the subject group happened to be unemployed when compared to fathers of males in the control group (0.0568).

An analysis of birth weight showed children in the subject group having a significantly low birth weight (<2.5kg) when compared to children of the control group (p=0.0255) who have significantly high birth weight (>4.0kg) (p=0.0021). Additionally, low birth weight was observed in a significant proportion of the male children in the subject group (p=0.0360) compared to male children in the control group with birth weight above 4kg at birth (p=0.0160). Furthermore, children and females in the subject group had an exclusive breastfeeding period of less than three months compared to children in the control group (p=0.058) and females in the control group (p=0.0198) respectively who had an exclusive breastfeeding period spanning more than six months (p=0.0063) and (p=0.0053) respectively.

## 4.2 DEMOGRAPHIC AND ANTHROPOMETRIC MEASUREMENTS

Demographic and anthropometric measurements of the study population stratified by protein energy malnutrition (PEM) into marasmus (67.5%), marasmic kwashiorkor (18.8%) and kwashiorkor (13.8%) based on weight for age, weight for height and height for age estimations are presented in Table 4.2. An analysis of age showed children with kwashiorkor being significantly older (23.73 ± 2.01 months) than marasmic children (15.85 ± 0.85 months) and children in the control group (16.74 ± 1.19 months) (F=4.899; p=0.0031). A greater percentage (31.5%) of the children with marasmus were below 12 months of age while 60.0% of the children with marasmic kwashiorkor were between the ages of 12 to 23 months and 54.5% of the children with kwashiorkor were between the ages of 24 to 36. A comparison of birth weight in the three PEM categories to the control group showed a significant value (p=0.0255) of children in the three categories having birth weight below 2.5kg. Furthermore, a

**Table 4.2One-way ANOVA of demographic and anthropometric measurements in the study population stratified by PEM**

| | | SUBJECTS | | | | |
|---|---|---|---|---|---|---|
| **VARIABLES** | **CONTROL** | **MARASMUS** | **MARASMIC KWASHIORKOR** | **KWASHIORKOR** | **P value** | **F Test** |
| **N** | **35** | **54** | **15** | **11** | | |
| Age (months) | 16.74 ± 1.19 | 15.85 ± 0.85 | 19.47 ± 1.84 | 23.73 ± 2.01*†† | 0.0031 | 4.899 |
| <12 | 10(28.6) | 17(31.5) | 2(13.3) | 0(0.s0) | | |
| 12 – 23 | 18(51.4) | 29(53.7) | 9(60.0) | 5(45.5) | | |
| 24 – 36 | 6(17.1) | 8(14.8) | 4(26.7) | 6(54.5) | | |
| >36 | 1(2.9) | 0(0.0) | 0(0.0) | 0(0.0) | | |
| No. of Children | 2.71 ± 0.24 | 2.43 ± 0.22 | 3.20 ± 0.60 | 3.09 ± 0.76 | 0.3883 | 1.016 |
| **Child Birth weight** | | | | | | |
| <2.5 | 0(0.0) | 6(11.1)‡ | 3(20.0)‡‡ | 2(18.2)‡‡ | | |
| 2.5 to 4.0 | 31(88.6) | 41(75.9) | 8(53.3)‡‡ | 6(54.5)‡ | | |
| >4.0 | 4(11.4) | 0(0.0) | 0(0.0) | 0(0.0) | | |
| **Exclusive Breastfeeding** | | | | | | |
| <3 | 4(11.4) | 12(22.2) | 5(33.3) | 5(45.5)‡ | | |
| 3 to 6 | 26(74.3) | 41(75.9) | 9(60.0) | 6(54.5) | | |
| >6 | 3(8.6) | 0(0.0) | 0(0.0) | 0(0.0) | | |
| MUAC (cm) | 14.45 ± 0.19 | 10.83 ± 0.18*** | 11.02 ± 0.32*** | 11.65 ± 0.50*** | <0.0001 | 59.65 |
| Height (cm) | 81.21 ± 1.16 | 72.27 ± 0.65*** | 75.69 ± 1.52* | 76.18 ± 1.09 | <0.0001 | 18.34 |
| Weight (kg) | 10.28 ± 0.27 | 6.68 ± 0.16*** | 7.31 ± 0.25*** | 8.20 ± 0.50***†† | <0.0001 | 50.10 |

***MUAC = mid-upper arm circumference; *p<0.01; ***p<0.0001; ††p<0.001; ‡p<0.05, ‡‡p<0.001***

**** indicates the level of significance when control was compared to subjects (post hoc test with Bonferroni's Multiple Comparison test); † indicates the level of significance when marasmic subjects were compared to kwashiorkor subjects (post hoc test with Bonferroni's Multiple Comparison test); ‡ indicates the level of significance when control was compared with subjects (Chi-square test)***

significant percentage of the children with marasmic kwashiorkor (53.3%) and kwashiorkor (54.5%) had birth weights between 2.5 to 4.0kg when compared to the control group. On exclusive breastfeeding, a significant proportion ($p<0.001$) of the children withkwashiorkor happened to have been breastfed for less than 3 month when compared to the control group. A comparison of the mean mid-upper arm circumference (MUAC) within the three categories of PEM and control group showed significantly reduced values in the subject group ($F=59.65$; $p<0.0001$) likewise the mean weight ($F=50.10$; $p<0.0001$). The mean height in children with marasmus and marasmic kwashiorkor was significantly lower when compared to that of children in the control group ($F=18.34$; $p<0.0001$).



## 4.3 CHANGE IN CONCENTRATION OF ANALYTES

Percentage changes in the concentration of measured analytes in the control group compared to those of the subjects at baseline (before treatment) and after treatment are presented in Table 4.3. The mean haemoglobin concentration in the control group ($12.02 \pm 0.18$ g $dL^{-1}$) was significantly higher than that in the subjects before ($8.14 \pm 0.15$ g $dL^{-1}$; $p<0.0001$) and after treatment ($8.46 \pm 0.16$ g $dL^{-1}$; $p<0.0001$). A haemoglobin concentration increase of 3.2% and a -6.2% decrease in the proportion of children with haemoglobin concentration <11.0 g $dL^{-1}$ was observed in the subjects after treatment. Conversely, the mean total white blood cell counts (TWBC) of $12.39 \pm 0.65$ k $\mu L^{-1}$ and $11.18 \pm 0.62$ k $\mu L^{-1}$ in the subjects before and after treatment respectively were significantly higher than the mean TWBC of $8.81 \pm 0.36$ k $\mu L^{-1}$ in the control group ($p=0.0006$ and $p=0.0153$). A decrease in TWBC of -9.9% and a -13.7% decrease in the proportion of children with TWBC >12.0 k $\mu L^{-1}$ was observed in the subjects after treatment. The proportion of children in the control group who tested positive for malaria parasites was significantly higher when compared to the subject group before ($p=0.0080$) and after ($p=0.0486$) treatment.A biochemical analysis of the mean concentration of total protein in the

control group (68.37 ± 1.43 g $L^{-1}$) compared to that in the subjects before (66.27 ± 1.61 g $L^{-1}$) and after treatment (69.55 ± 1.69 g $L^{-1}$) showed no statistically significant differences ($p>0.05$). However, a percentage increase of 5.8 was seen in the mean concentration of total protein in the subjects after treatment compared to the baseline concentration. Serum albumin concentration in the control group (43.21 ± 0.90 g $L^{-1}$) was significantly higher than the concentration in the subject group before treatment (38.65 ± 0.90 g $L^{-1}$) ($p=0.0027$). A 6.8% increase in the mean concentration of serum albumin concentration was observed in the subjects after treatment.



**Table 4.3Percentage change in the concentration measured analytes in the study population**

| | | SUBJECTS | | | | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **CONTROL** | **BEFORE** | **AFTER** | **%Δ** | **p** | **p*** | **p**** |
| N | 35 | 80 | 80 | | | | |
| **Haematology** | | | | | | | |
| Haemoglobin | 12.02 ± 0.18 | 8.14 ± 0.15 | 8.46 ± 0.16 | 3.2 | <0.0001 | <0.0001 | 0.1573 |
| <11.0g $dL^{-1}$ | 5(14.3) | 80(100.0) | 75(93.8) | -6.2 | <0.0001 | <0.0001 | 0.0231 |
| TWBC | 8.81 ± 0.36 | 12.39 ± 0.65 | 11.18 ± 0.62 | -9.9 | 0.0006 | 0.0153 | 0.1831 |
| <4.0k $\mu L^{-1}$ | 0(0.0) | 1(1.3) | 2(2.5) | 1.2 | 0.5065 | 0.3453 | 0.5600 |
| >12.0k $\mu L^{-1}$ | 3(8.57) | 36(45.0) | 25(31.3) | -13.7 | 0.0001 | 0.0091 | 0.0734 |
| Malaria parasites | 3(8.57) | 0(0.0) | 1(1.25) | 1.3 | 0.0080 | 0.0486 | 0.3158 |
| **Biochemistry** | | | | | | | |
| Total Protein | 68.37 ± 1.43 | 66.27 ± 1.61 | 69.55 ± 1.69 | 5.8 | 0.4226 | 0.6615 | 0.1612 |
| <60g $L^{-1}$ | 3(8.6) | 27(33.8) | 14(17.5) | -16.3 | 0.0047 | 0.2145 | 0.0186 |
| Albumin | 43.21 ± 0.90 | 38.65 ± 0.90 | 41.14 ± 0.86 | 6.8 | 0.0027 | 0.1476 | 0.0479 |
| <35g $L^{-1}$ | 4(11.4) | 26(32.5) | 13(16.3) | -16.2 | 0.0179 | 0.5027 | 0.0167 |
| **Cytokines** | | | | | | | |
| IL-6(pg $mL^{-1}$) | 7.01 ± 1.37 | 46.08 ± 7.48 | 26.25 ± 5.19 | -43.8 | 0.0008 | 0.0148 | 0.0320 |
| >14pg $mL^{-1}$ | 5(14.3) | 42(52.5) | 37(46.3) | -6.2 | 0.0001 | 0.0011 | 0.4292 |
| TNF-$\alpha$ (pg $mL^{-1}$) | 55.81 ± 2.20 | 82.07 ± 6.02 | 72.53 ± 6.93 | -11.4 | 0.0053 | 0.1110 | 0.2992 |
| >8.1pg $mL^{-1}$ | 35(100.0) | 80(100.0) | 80(100.0) | 0.0 | | | |

***TWBC = total white blood cells, MUAC = mid-upper arm circumference, IL-6 = interleukin 6, TNF-α = Tumour necrotic-alpha, %Δ = percentage change, p = defines the level of significance when control was compared to subjects (before); p* = defines the level of significance when control was compared to subjects (after); p** = defines the level of significance when subjects (before) was compared to subjects (after)***

Furthermore, the proportion of children in the subject group with a total protein concentration <60 g $L^{-1}$ decreased by -16.3% after treatment whilst the percentage proportional decrease in children with albumin concentration <35 g $L^{-1}$ was -16.2%. Significant elevations in the concentration of pro-inflammatory cytokines were observed. The mean concentration of interleukin-6 (IL-6) in the subjects at baseline (46.08 ± 7.48 pg $mL^{-1}$) and after treatment (26.25 ± 5.19 pg $mL^{-1}$) were significantly higher than that in the control (7.01 ± 1.37pg$mL^{-1}$) group (p=0.0008 and p=0.0148 respectively) with a -43.8% decrease in the mean concentration of IL-6 being observed after treatment. The proportion of children with IL-6 concentration >14pg $mL^{-1}$ also decreased by 6.2% in the subject group after treatment. Tumour necrosis factor-alpha (TNF-α) concentration in the subject group before treatment (82.07 ± 6.02 pg $mL^{-1}$) was significantly higher when compared to the mean concentration (55.81 ± 2.20 pg $mL^{-1}$) in the control group but not statistically significant difference was observed in the TNF-α concentration in the subject group before and after (72.53 ± 6.93 pg $mL^{-1}$) treatment. A percentage decrease of 11.4% was observed in the mean TNF-α concentration of the subjects after treatment.

## 4.4 CONCENTRATION OF ANALYTES IN CONTROLS AND PEM CATEGORIES

Figure 4.1 presents a general overview of comparison of the concentration of measured analytes in the control group and the subject group stratified into the three categories of PEM before treatment. The mean haemoglobin concentration in the control group was significantly higher when compared to children with marasmus (p<0.0001), marasmic kwashiorkor (p<0.0001) and kwashiorkor (p<0.0001) Fig. 4.1A; the mean TWBCs' in the marasmic (p=0.0006), marasmic kwashiorkor (p=0.0157) and kwashiorkor (p=0.0007) children were significantly higher compared to the count in children in the control group Fig. 4.1B; no significant differences in the mean total protein concentration was observed when

controls were compared to children with marasmus (p=0.4303), marasmic kwashiorkor (p=0.6125) and kwashiorkor (p=0.3814) Fig. 4.1C; the mean serum albumin concentration in the control group was significantly higher than that in children with marasmus (p=0.0371), marasmic kwashiorkor (p=0.0005) and kwashiorkor (p=0.0006) Fig. 4.1D; IL-6 was significantly lower in the control group when compared to that in children with marasmus (p=0.0013), marasmic kwashiorkor (p=0.0002) and kwashiorkor (p=0.0001) Fig. 4.1E and finally the mean TNF-$\alpha$ concentration in the control group was significantly lower when compared to that in children with marasmus (p=0.0145), marasmic kwashiorkor (p=0.0112) and kwashiorkor (p<0.0001). TNF-$\alpha$ concentration in patients with kwashiorkor was significantly higher when compared to children with marasmus (p=0.0510) Fig. 4.1F.

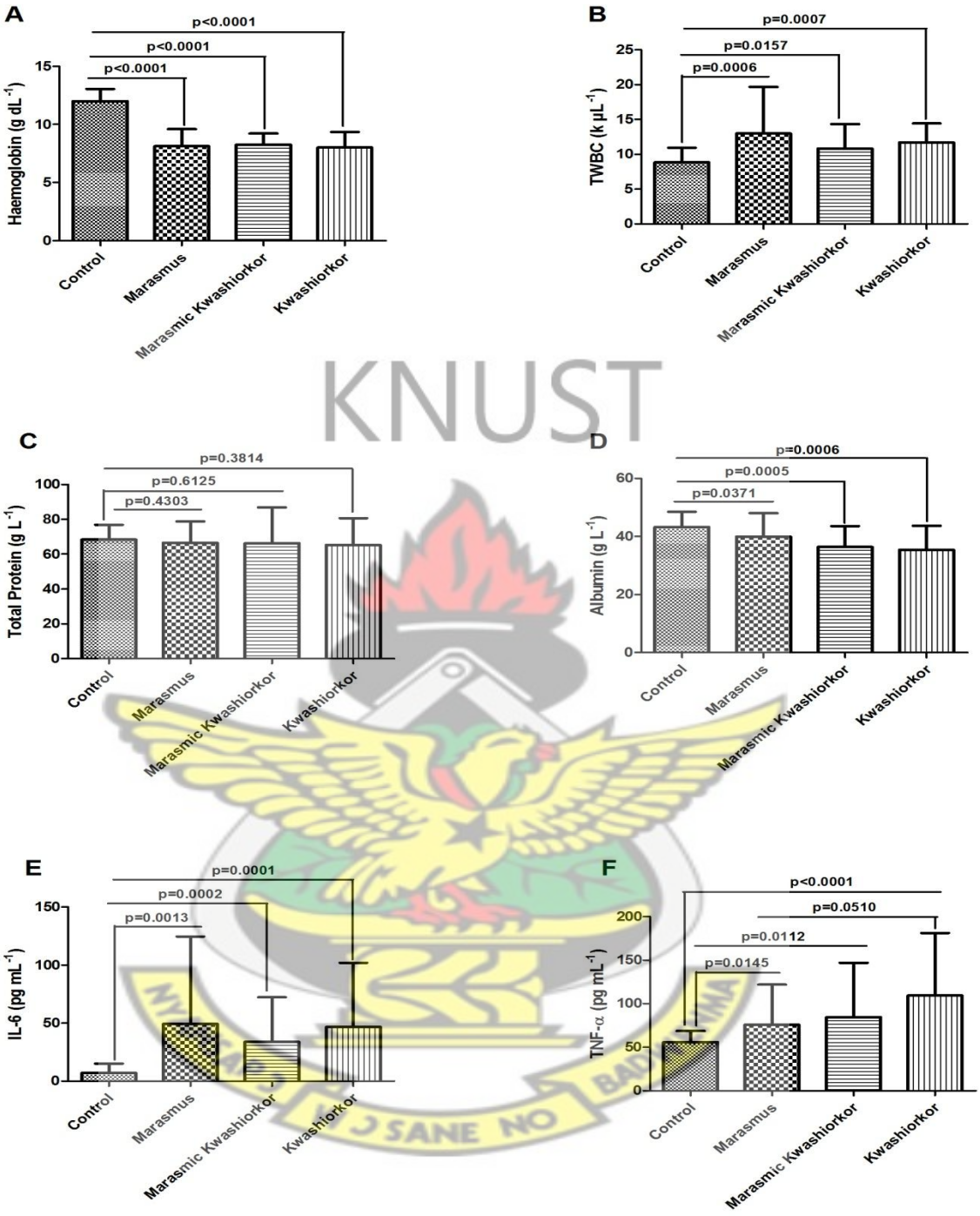


**Figure 4.1Comparison of concentration of analytes between controls and the three categories of PEM (Before Treatment)**

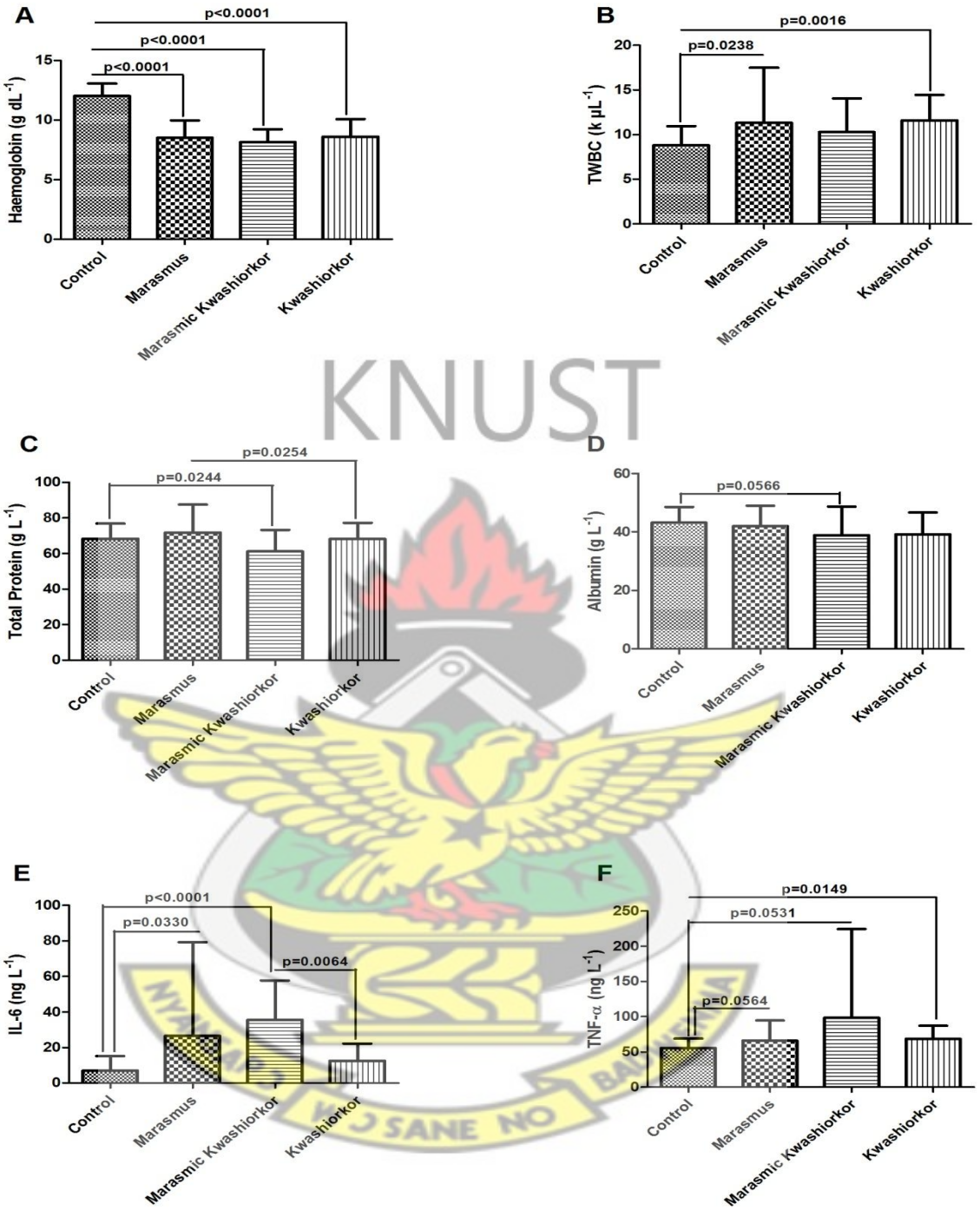


**Figure 4.2 Comparison of concentration of analytes between controls and the three categories of PEM (After Treatment)**

Figure 4.2 presents a general overview of comparison of the concentration of the measured analytes in the control group and the subject group stratified by the type of PEM after treatment. The mean haemoglobin concentration in the control group was significantly higher when compared to those in children with marasmus ($p<0.0001$), marasmic kwashiorkor ($p<0.0001$) and kwashiorkor ($p<0.0001$) Fig. 4.2A; the TWBC in the control group was significantly lower when compared to those in children with marasmus ($p=0.0238$) and kwashiorkor ($p=0.0016$). No statistically significant difference in TWBC was observed when children with marasmic kwashiorkor were compared to children in the control group Fig. 4.2B; the mean total protein concentration in the control group was significantly higher when compared to those in children with marasmic kwashiorkor ($p=0.0244$) and kwashiorkor ($p=0.0254$) Fig. 4.2C; Serum albumin concentration in children with marasmic kwashiorkor showed a low marginally significant value ($p=0.0566$) when compared to the control group Fig. 4.2D; the concentration of IL-6 was significantly higher in children with marasmus ($p=0.0330$) and marasmic kwashiorkor ($p<0.0001$) compared to the control group. A significantly reduced IL-6 concentration was further observed in children with kwashiorkor when compared to children with marasmic kwashiorkor ($p=0.0064$) Fig. 4.2E; TNF-$\alpha$ on the other hand was significantly lower in the control group when compared to children with kwashiorkor ($p=0.0149$) whilst the concentration in children with marasmus ($p=0.0564$) and marasmic kwashiorkor ($p=0.0531$) showed marginally high significant TNF-$\alpha$ concentration when compared to the control group Fig. 4.2F.

## 4.5 CHANGES IN ANALYTE CONCENTRATION BY PEM CATEGORY

Tables 4.4, 4.5 and 4.6 present the percentage changes in the concentrations of measured analytes in the study group stratified into the three categories of PEM before and after treatment. A 4.5% and 4.7% increases in mean

haemoglobinconcentration was observed in children with marasmus and kwashiorkor respectively after treatment. Contrarily, a percentage decrease of 3.1 in mean haemoglobin concentration was seenin children with marasmic kwashiorkor after treatment. Proportional decreases of 1.9%, 13.3% and 18.2% in the number of children with haemoglobin<11.0 g dL-1 was observed in the marasmic, marasmic kwashiorkor and kwashiorkor groups respectively after treatment. The mean TWBC in children with marasmus, marasmic kwashiorkor and kwashiorkor decreased by 12.7%, 5.1% amd 1.6% respectively. Proportions of children with marasmus and marasmic kwashiorkor having TWBC >12.0 k $\mu L^{-1}$ decreased by 16.6% and 13.3% respectively whilst no percentage decrease was observed in children having kwashiorkor with TWBC >12.0 k $\mu L^{-1}$ after treatment.

The mean serum total protein concentration in children with marasmus and kwashiorkor increased by 8.0% and 8.8% respectively after treatment whilst the percentage change in total protein in children with marasmic kwashiorkor decreased by 5.7% after treatment. However, proportional decreases of 18.5%, 13.4% and 9.1% in children with total protein concentration <60g $L^{-1}$ was observed in the marasmic, marasmic kwashiorkor and kwashiorkor groups respectively after treatment. Likewise, the mean serum albumin concentration increased by 5.3%, 9.4% and 13.3% in children with marasmus, marasmic kwashiorkor and kwashiorkor respectively after treatment. The proportion of children with serum albumin concentration <35g $L^{-1}$ in the marasmus, marasmic kwashiorkor and kwashiorkor groups decreased by 14.8%, 13.4% and 27.3% after treatment respectively.

The mean concentration of IL-6 decreased considerably by 46.3% and 70.9% in children with marasmus and kwashiorkor respectively after treatment whilst a percentage IL-6 decrease of only 2.0 was observed in children with marasmic kwashiorkor after treatment. The proportion of children with IL-6 concentration >14pg $mL^{-1}$ decreased by 9.3%, 20.0% and 27.2% in the marasmic, marasmic

kwashiorkor and kwashiorkor groups after treatment. The mean TNF-α concentration in children with marasmus and kwashiorkor decreased by 12.2% and 37.5% respectively after treatment whilst the TNF-α concentration in children with marasmic kwashiorkor showed a percentage increase of 15.9% after treatment.

**Table 4.4Percentage change in the concentration of analytes in subjects with Marasmus**

| | MARASMUS | | | |
|---|---|---|---|---|
| VARIABLE | BEFORE TREATMENT | AFTER TREATMENT | %Δ | p |
| **N** | **54** | **54** | | |
| **Haematology** | | | | |
| Haemoglobin (g $dL^{-1}$) | 8.14 ± 0.20 | 8.50 ± 0.20 | 4.5 | 0.2002 |
| <11.0g $dL^{-1}$ | 54(100.0) | 53(98.1) | -1.9 | 0.3151 |
| TWBC (k $\mu L^{-1}$) | 12.96 ± 0.91 | 11.32 ± 0.84 | -12.7 | 0.1873 |
| <4.0k $\mu L^{-1}$ | 1(1.9) | 1(1.9) | 0.0 | 1.0000 |
| >12.0k $\mu L^{-1}$ | 26(48.1) | 17(31.5) | -16.6 | 0.0769 |
| Malaria Parasites | 0(0.0) | 0(0.0) | 0.0 | |
| **Biochemistry** | | | | |
| Total Protein (g $L^{-1}$) | 66.49 ± 1.67 | 71.82 ± 2.14 | 8.0 | 0.0519 |
| <60g $L^{-1}$ | 17(31.5) | 7(13.0) | -18.5 | 0.0206 |
| Albumin (g $L^{-1}$) | 39.94 ± 1.10 | 42.04 ± 0.95 | 5.3 | 0.1521 |
| <35g $L^{-1}$ | 14(25.9) | 6(11.1) | -14.8 | 0.4937 |
| **Cytokines** | | | | |
| IL-6 (pg $mL^{-1}$) | 49.37 ± 10.23 | 26.53 ± 7.19 | -46.3 | 0.0704 |
| >14pg $mL^{-1}$ | 29(53.7) | 24(44.4) | -9.3 | 0.3358 |
| TNF (pg $mL^{-1}$) | 75.79 ± 6.28 | 65.88 ± 4.04 | -12.2 | 0.1926 |
| >8.1pg $mL^{-1}$ | 54(100.0) | 54(100.0) | 0.0 | |

***%Δ = percentage change in the concentration of analytes (Before &After Treatment); p = indicates the level of significance when analytes (before) were compared to analytes (after); IL-6 = Interleukin-6, TNF-α = Tumour Necrosis Factor-alpha; TWBC = total white blood cell.***

**Table 4.5Percentage change in the concentration of analytes in subjects with Marasmic Kwashiorkor**

| | MARASMIC KWASHIORKOR | | | |
|---|---|---|---|---|
| VARIABLE | BEFORE TREATMENT | AFTER TREATMENT | %Δ | p |
| N | 15 | 15 | | |
| **Haematology** | | | | |
| Haemoglobin (g $dL^{-1}$) | 8.25 ± 0.25 | 8.15 ± 0.30 | -3.1 | 0.7834 |
| <11.0g $dL^{-1}$ | 15(100.0) | 13(86.7) | -13.3 | 0.1432 |
| TWBC (k $\mu L^{-1}$) | 10.83 ± 0.90 | 10.29 ± 1.04 | -5.1 | 0.6996 |
| <4.0k $\mu L^{-1}$ | 0(0.0) | 1(6.7) | 6.7 | 0.3091 |
| >12.0k $\mu L^{-1}$ | 6(40.0) | 4(26.7) | -13.3 | 0.4386 |
| Malaria Parasites | 0(0.0) | 1(6.7) | 6.7 | 0.3091 |
| **Biochemistry** | | | | |
| Total Protein (g $L^{-1}$) | 66.30 ± 5.29 | 61.15 ± 3.36 | -5.7 | 0.4349 |
| <60g $L^{-1}$ | 7(46.7) | 5(33.3) | -13.4 | 0.4561 |
| Albumin (g $L^{-1}$) | 36.39 ± 1.86 | 38.92 ± 2.70 | 9.4 | 0.4373 |
| <35g $L^{-1}$ | 7(46.7) | 5(33.3) | -13.4 | 0.4561 |
| **Cytokines** | | | | |
| IL-6 (pg $mL^{-1}$) | 33.81 ± 9.92 | 35.54 ± 6.18 | 2.0 | 0.8877 |
| >14pg $mL^{-1}$ | 7(46.7) | 10(66.7) | 20.0 | 0.2690 |
| TNF (pg $mL^{-1}$) | 84.60 ± 16.09 | 98.13 ± 32.64 | 15.9 | 0.7128 |
| >8.1pg $mL^{-1}$ | 15(100.0) | 15(100.0) | 0.0 | |

***%Δ = percentage change in the concentration of analytes (Before &After Treatment); p = indicates the level of significance when analytes (before) were compared to analytes (after); IL-6 = Interleukin-6, TNF-α = Tumour Necrosis Factor-alpha; TWBC = total white blood cell.***

**Table 4.6Percentage change in the concentration of analytes in subjects with Kwashiorkor**

| | KWASHIORKOR | | | |
|---|---|---|---|---|
| VARIABLE | BEFORE TREATMENT | AFTER TREATMENT | %Δ | p |
| **N** | **11** | **11** | | |
| **Haematology** | | | | |
| Haematology (g $dL^{-1}$) | 8.02 ± 0.40 | 8.61 ± 0.47 | 4.7 | 0.3470 |
| <11.0g $dL^{-1}$ | 11(100.0) | 9(81.8) | -18.2 | 0.1380 |
| TWBC (k $\mu L^{-1}$) | 11.69 ± 0.83 | 11.59 ± 0.90 | -1.6 | 0.9351 |
| <4.0k $\mu L^{-1}$ | 0(0.0) | 0(0.0) | 0.0 | |
| >12.0k $\mu L^{-1}$ | 4(36.4) | 4(36.4) | 0.0 | 1.0000 |
| Malaria Parasites | 0(0.0) | 0(0.0) | 0.0 | |
| **Biochemistry** | | | | |
| Total Protein (g $L^{-1}$) | 65.17 ± 4.65 | 68.23 ± 2.85 | 8.8 | 0.5910 |
| <60g $L^{-1}$ | 3(27.3) | 2(18.2) | -9.1 | 0.6109 |
| Albumin (g $L^{-1}$) | 35.36 ± 2.51 | 39.20 ± 2.36 | 13.3 | 0.2819 |
| <35g $L^{-1}$ | 5(45.5) | 2(18.2) | -27.3 | 0.1697 |
| **Cytokines** | | | | |
| IL-6 (pg $mL^{-1}$) | 46.69 ± 16.71 | 12.65 ± 3.04 | -70.9 | 0.0710 |
| >14pg $mL^{-1}$ | 6(54.5) | 3(27.3) | -27.2 | 0.1933 |
| TNF-$\alpha$ (pg $mL^{-1}$) | 109.4 ± 21.68 | 68.44 ± 5.56 | -37.5 | 0.0820 |
| >8.1pg $mL^{-1}$ | 11(100.0) | 11(100.0) | 0.0 | |

***%Δ = percentage change in the concentration of analytes (Before &After Treatment); p = indicates the level of significance when analytes (before) were compared to analytes (after); IL-6 = Interleukin-6, TNF-α = Tumour Necrosis Factor-alpha; TWBC = total white blood cell.***

*Chapter 5*

# DICUSSION

The diagnosis of PEM is generally based on measurements of nutritional status, including assessments of oral intake, weight loss, anthropometric data and determination of cell-mediated immunity, biochemical parameters, physical examination and body composition analysis.



## 5.1 EFFECT OF SOCIO-ECONOMIC FAMILY PROFILE AND WEANING

The socio-economic and family profile of the study children showed that lack of education, unemployment and parental poverty singly or in combination affected the nutritional status of the subjects compared to the controls. These findings corroborate with results of previous studies, (Devi Yasoda and Geervani, 1994; Delpeucha *et al.*, 1999) which suggested a strong influence of socio-economic status of parents on the nutritional status of children in developing countries. The study demonstrated higher level of illiteracy and unemployment rate among mothers of female subjects' compared to the control group. A high female to male ratio (1.3:1) was observed in subjects in this study which compares well with the findings of Meremikwu *et al.*, (1992) and Odebode and Odebode, (2005) who reported gender ratios of 1.5:1 and 1.7:1 respectively showing a high impact of PEM in females compared to males.The importance of a minimum level of education for mothers to adequately care for their children and thus ensure satisfactory growth has been put forward by Ali *et al.*,(2005). The observed lack of education and unemployment in mothers of female subjects could be a major factor impacting on the nutritional deficiency observed in the female subjects. Contrarily, Owor*et al.*,(2000) and Oyedejo*et al.*,(1996) in their studies in Uganda and Nigeria respectively, could not find any correlation between formal education of the

mothers and nutritional status of their children in that despite high education levels of mothers, children still had severe forms of malnutrition.

It has generally been found that urban women breast-feed for shorter duration than rural women (WHO, 1991; Niger *et al.*, 2010). The sampled parental population distribution inthis study was mainly urban and peri-urban with about 48.8% of the mothers' of the subjects being unemployed and 50% engaged in petty trading and apprenticeship. The burden of unemployment (51.1%) and self employment (48.9%) was more in mothers' of female subjects and this coupled with the occupational stress and load of work associated with urban life would end up in reduced quality of care and time for breastfeeding. The study showed that about 27.5% of the malnourished children were exclusively breast fed for less than three months without any of them being breast fed for more than six months compared to the control group.



## 5.2 EFFECT OF LOW BIRTHWEIGHT

Stunting is a cumulative process that starts in-utero and studies have shown that intrauterine growth is a strong predictor of postnatal growth (Berkowitz and Papiernik, 1993). Low birth weight (LBW) greatly increases the risk of neonatal death. In addition to its impact on infant mortality, LBW has been associated with higher probabilities of infection, malnutrition and handicapping conditions during childhood, including cerebral palsy, mental deficiencies and problems related to behaviour and learning during childhood (Dunin-Wasowicz *et al.*, 2000). Significant proportions of the subjects with LBW (<2.5kg) in this study were distributed across the three categories of PEM and agree well with the findings of Dunin-Wasowicz.

Conley and Bennett (2001) and Duncan (1996) in their studies reported that low socioeconomic level is the most important risk factor for LBW, independent of

other factors such as reproductive and nutritional characteristics, smoking, morbidity during pregnancy, and accessibility to health services and prenatal care. The burden of low socio-economic levels in this study was more in mothers and as such could be a major factor for the observed LBW in the malnourished and these results are similar to those in literature describing a positive relationship between socioeconomic condition and effects on health.

## 5.3 HAEMATOLOGICAL AND BIOCHEMICAL CHANGES

Hematological analysis of the subjects and controls in this study showed significantly reduced haemoglobin concentration in the subjects compared to controls at baseline with 100% of the subjects being anemic. However, a 6.2% decrease in the proportion with anemia was observed after treatment (nutritional intervention with starter formula) showing the ability of diet intervention to improve upon haemoglobin concentration and this finding compares well with that of Mishra *et al.,* (2009). Gabay and Kushner (1999) also reported on the effect of infections on erythropoiesis and the general lack of response to haematinics in the presence of active infection in children with PEM. A significant proportion of the subjects (45.0%) had elevated levels of total white blood cells (TWBC) when compared to the controls (8.6%) and this proportion decreased by about 13.7% after nutritional intervention. Bhan*et al.,*(2003) attributed elevated TWBCs in children with severe PEM to asymptomatic infections and severe nutritional deficiency is imminent in the failure of the immune system to respond to chemotaxis, opsonization and phagocytosis of bacteria, viruses or fungi. Children with PEM in this study might therefore have asymptomatic infections as evidenced by the elevated TWBCs which could have had a negative impact on erythropoiesis hence the resultant decreases in haemoglobin concentration observed in the subjects at baseline.

Differential biochemical parameters are altered during protein energy malnutrition and as such biochemical parameters provide valuable information and act as sensitive indicators for overall management of PEM (Mishra *et al.*, 2009). In their study on biochemical indicators in children with PEM, Mishra *et al.,*(2009) reported that a significant proportion of children with protein energy malnutrition had altered biochemical parameters which were related to food intake and biochemical metabolism mandatory during growth and development of children less than five years of age. There was significantly higher proportion of hypoproteinaemia, hypoalbuminaemia and anaemia in children with PEM when compared to normally nourished children in their study.



Mishra *et al.,*(2009) further showed a strong association of hypoproteinaemia in their PEM group compared to the control group with the risk of protein energy malnutrition being 3.7; meaning that developing hypoproteinaemia was approximately associated with a fourfold increase in risk of developing PEM when compared with control group. Likewise, significantly higher decline in serum albumin level in the PEM group compared to the control group gave a relative risk of 5.2; meaning that developing hypoalbuminaemia was associated with a fivefold increase in risk of developing PEM when compared with the control group showing the strong association of hypoalbuminaemia and PEM. A significant proportion of the subjects (33.8%) with PEM in this study developed hypoproteinaemia in comparison to the controls (8.6%) at baseline and this proportion decreased by about 16.3% after nutritional intervention. Also, 32.5% developed hypoalbuminaemia compared to 11.4% of the controls at baseline and this significant proportion decreased by 16.2% after nutritional intervention. These findings prove the contribution of hypoproteinaemia and hypoalbuminaemia in PEM and agree well with that of Mishra *et al.,*(2009). Sullivan (2001) in his study on serum proteins related hypoalbuminaemia to increased vascular permeability to albumin probably mediated by cytokines (IL-6 and TNF-α). This study observed increased concentrations of IL-6 in the subjects at baseline which decreased by

6.2% after nutritional intervention and as such could have contributed to the significant decrease in serum albumin at baseline.

## 5.4 PRO-INLFAMMATORY CYTOKINES

Different studies have produced varying reports on pro-inflammatory cytokines in the malnourished. Whilst Vaisman*et al.,*(1989), Stenvinkel*et al.,*(2002) andAzevedo*et al.,*(2005)have reported increases, reports from Muñoz *et al.,*(1994) indicate that pro-inflammatory cytokine levels in the malnourished are reduced. Abo-Shousha*et al.,*(2005) showedthat the levels of pro-inflammatory cytokines (GMCSF,IL-8, and IL-6) isolated from 46 malnourishedchildren were lower compared to healthy controls. They found that inflammatory response was severely impairedin oedematous compared with non-oedematousmalnourishedchildren. Cederholm*et al.,* (1997)demonstrated that production of IL-6 is increased in malnourished subjects. Morlese*et al.*, (1996)suggested that increase in the pro-inflammatory cytokines could be due to stimulations either by the presence of endotoxin, bacterial exotoxin, fungi or viruses. This corroborate with a study conducted byMalave*et al.,*(1998), who showed that CRP and IL-6 increased to approximately similar levels in sera from undernourished and control children with overt infections. These cytokines, during acute generalized infections initiate acute-phase reactions which include fever, malaise, myalgia, headaches, cellular hypermetabolism and multiple endocrine and enzyme responses (Beisel, 1995). The acute-phase reaction and its cytokine-driven hypermetabolism have high nutritional costs (Beisel *et al.*, 1977; Roubenoff *et al.*, 1994; Constans *et al.*, 1995). Cytokine-induced malnutrition is therefore initiated by hypermetabolism(Beisel *et al.*, 1977; Roubenoff *et al.*, 1994) with its high basal metabolic rates. Body nitrogen and other elements are lost quickly, while body water and sodium are being retained (Beisel *et al.*, 1977). Glucose and urea synthesis are both increased during cytokine-induced malnutrition, but ketone production is slowed (Beisel *et al.*, 1977). Oxidation of

branched-chain amino acids is increased and acute-phase plasma glycoproteins are created (Beisel *et al.*, 1977) thereby activating the immune system. Opposite responses to such metabolic instances are typical of uncomplicated starvation (Beisel, 1995). Significantly increased concentration of IL-6 was observed in subjects (52.5%) in this study when compared to controls (14.6%) at baseline and because starvation is rarely uncomplicated in children, the resultant malnutrition observed in subjects in this study could be generally influenced by cytokine-induced (IL-6) components.



Tumour necrosis factor (TNF) plays essential role in the development of the metabolic and pathological consequences of the stress response(Fong *et al.*, 1990). It has been detected in the serum of patients experiencing various diseases, such as parasitic or bacterial infections, tumour-bearing disease, burns and acute hepatic failure(Marano *et al.*, 1990). Giovambattista*et al.*, (2000) observed that basal TNF serum concentrations were significantly higher in malnourished children than in controls. The concentration of TNF-α in the subjects before (82.07 ±6.02) and after treatment (72.53 ± 6.93) were significantly higher (p=0.0053) than that in the controls. However there was no significant difference between values before and after nutritional intervention. This finding is in agreement with that of Dulger*et al.*,(2002), who reported no significant difference in the concentration of TNF-α in children with PEM compared to controls in their study on pro-inflammatory cytokines in Turkish children with PEM.

*Chapter 6*

# CONCLUSIONS

This study observed increases in inflammatory response in children with PEM with IL-6 concentration being a significant indicator of PEM in the subjects compared to TNF-α concentration. The impact of dietary intervention on haematological and biochemical indices assessed in this study shows the ability of nutritional intervention to achieve immunomodulation, promote growth, and improved immunity, general well-being and development of malnourished children less than five years of age. Above all, the impact of the level of education and the socio-economical status of the mothers of malnourished children in this study was clearly evident.



## 6.1 LIMITATIONS

Limitations of the present study include:

- The hitherto short period of the study (6 months) did not necessitate recording of anthropometric measurements which could show the direct impact of growth.
- The effect of subtle subclinical conditions was not factored into the variables for the heterogeneous group of malnourished children recruited for this study.

## 6.2 RECOMMENDATIONS

- In future, similar studies should be conducted in a larger cohort of malnourished children with homogeneous conditions to explore the relationship between cytokines, malnutritionandspecific diseases.

# APPENDIX

## QUESTIONNAIRE

**PRO-INFLAMMATORY CYTOKINE IN GHANAIAN CHILDREN WITH PROTEIN ENERGY MALNUTRITION BEFORE AND AFTER TREATMENT**

MOTHER/CAREGIVER-FORM

**SECTION A: IDENTIFICATION**

Hosp. Number……
Study Number……..
Mother ID……..
Name of Interviewer……

**SECTION B: GENERAL INFORMATION**

1. Name of Mother/Caregiver……

2. Age of Mother……..

3. Marital Status
1. Single 2. Married
3. Separated 4. Widowed
5. Divorced

4. What is your highest education?
1. Primary 2. JSS/Mid
3. SSS/Sec 4. Tertiary
5. None

5. What is your main Occupation?
1. Unemployed 2. Farming
3. Artisan 4.Trading
5. Gov't worker 6. Apprenticeship

6. What is the highest education level of your ward's Father?
1. Primary 2. JSS/Mid
3. SSS/Sec 4. Tertiary
5. None

7. What is your ward's Father's Occupation?
1. Unemployed 2. Farming
3. Artisan 4.Trading
5. Gov't worker 6. Apprenticeship

8. Where do you stay? ……

9. (a) How many people are in the house in which you live?
1. 0 - 10 2. 11- 20
3. 21- 30 4. 31- 40
5. >40

(b) What type of housing?
1. Mud 2. Bricks
3. Blocks 4. Bamboo

10. Number of people that sleep in the room?
……..
11. What is your religion?
1. Christian 2. Moslem
3. Traditionalist 4. Eckankar
5. Others (Specify)…….

12. When do you wash your hands?
1. After visiting the toilet
2. After removing your baby's diaper
3. When cooking
4. Taking your bath
5. Before eating

13. Do you have infections hazard near the home?
1. Rubbish dump
2. Public toilet
3. Stagnant water
4. Don't know

14. Mother's ethnicity…….

15. Father's ethnicity…….

**FAMILY HISTORY**

16. How many children do you have? ……..

17. How many are alive? .....

18. What was the cause of death in the others?
If Known……

19. a) Is there any disease that runs through your family?
□ Yes □ No
If yes which one of these is present?
I. Diabetes
II. Hypertension
III. Heart disease
IV. Sickle cell disease
Other specify……

**PERINATAL HISTORY**

20. Progress of pregnancy
1. Normal
2. Complications
3. Don't know

21. Where did you deliver your ward?
1. Church
2. Fetish
3. Home
4. Hospital
5. Farm
Others (Specify)…….

22. The type of delivery you underwent?
1. Cesarean section
2. Self delivery
3. Pre-term delivery
4. Vacuum extraction
Others (Specify)……

23. Birth weight of your ward?
1. Below 2.5kg
2. 2.5-4.0kg
3. Above 4.0kg

24. Developmental history
1. Child growing well
2. Not gaining weight
3. Losing weight

25. Have you been visiting the Child welfare Clinic with this particular child?
YES □ NO □

**IMMUNOLOGICAL HISTORY**

26. How many times have you immunized the child in the first one year on the following below?

| No. | Immunization | No. of times done |
|---|---|---|
| 1. | Tuberculosis (BCG) | |
| 2. | Poliomyelitis | |
| 3. | Diphtheria/Pertussis/ Tetanus/Hepatitis B/ H. Influenza B | |
| 4. | Measles | |
| 5. | Yellow fever | |

27. Has he/she fallen ill before?
Yes □□ No

28. How many times?
1. twice
2. thrice
3. Quadrate
4. > four times

29. What was the cause of illness?
……..

**NUTRITIONAL HISTORY**

30. How long did you exclusively breastfeed your ward?
1. 0 – 3 months
2. 4 – 6 months
3. > 6 months

30. How long did you breastfeed your child?
1. 0 - 3 months
2. 4 – 6 months
3. 7 – 9 months
4. > 1year

5. Still Breastfeeding

31. Mention some of the complementary foods you give your child (at least three)……..

**KNOWLEDGE**

33. When did you start complementary feeding?
1. 0 - 3months
2. 4 - 6months
3. 7 - 9months
4. >1years
5. Others Specify……

34. Why have you brought your child here?
……

35. What do you think caused the condition?
……

36. What is Kwashiorkor?
……

37. What causes the above condition?
1. Witchcraft
2. Improper breastfeeding
3. Imbalance meals
4. Early introduction of complementary foods
5. Others (specify)……

**ATTITUDE**

38. Do you think that Kwashiorkor could be a problem?
Yes □□ No

39. Why didn't you bring your ward early to the hospital?
……

How long has the child suffered from the condition?
1. 1week
2. 2weeks
3. 3weeks
4. ≥4weeks

41. Did you seek treatment from elsewhere before coming here?
□ Yes □ No

If yes where
1. Herbalist
2. Prayer camp
3. Malam
4. Others (specify)……

42. How long were you there? .....

**SECTION C: SUBJECT IDENTIFICATION**

Name of the child……
ID Number …….

**ANTHROPOMETRIC MEASUREMENT OF SUBJECT**

Age: ……
Mid Upper Arm Circumference:……
Height: ……..
Weight: ……..

**OTHER INFORMATION ABOUT SUBJECT**

43. Type of Protein energy malnutrition (PEM)
1. Kwashiorkor 2.Marasmic kwashiorkor
3. Marasmus

44. Blood sample taken before treatment.
□Yes □No

45. Has blood sample for Haemoglobinand WBC testing been taken?
□Yes □No
Result……..

46. Have you been referred? ……..

47. From which hospital/ health centre have you been referred?

48. Second blood sample taken after treatment (b/n 8th and 16th day)
□Yes □No

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