

**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
KUMASI
COLLEGE OF SCIENCE**

**NUTRITIONAL INTERVENTION IN CHILDREN UNDERGOING
CHEMOTHERAPY FOR CANCER**

BY

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**NUTRITIONAL INTERVENTION IN CHILDREN UNDERGOING
CHEMOTHERAPY FOR CANCER**

By

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ABSTRACT

Burkitt's lymphoma (BL) and Wilm's tumour (WT) are the two most common childhood cancers. BL is a malignant tumour of the B cells of the lymphoid tissues that affects mainly children in Africa and WT is a malignant tumour that develops in the kidney from nephroblast. For most cancers, chemotherapy is the main form of treatment but each step of the cancer continuum from diagnosis to recovery poses nutritional challenges. Poor nutritional status predisposes an individual to disease and makes disease progression faster and recovery slower; nutritional decline among cancer sufferers also decreases the effectiveness of treatment and can slow recovery. The study aim was to investigate the impact of high protein-based supplement on nutritional status, and recovery among children undergoing treatment for Burkitt's lymphoma and Wilms tumour treatment at the Komfo Anokye Teaching Hospital (KATH), Ghana and also to determine the anti-proliferative effects of Isoflavanoids isolated from soyabean (SB) and soymilk powder (SMP) on Burkitt's lymphoma (DG 75) and other cell lines. The study was in 4 parts. In the first part, there was assessment of nutrient composition of the soymilk powder (SMP) used as a supplement. The second part, investigated the nutritional status of children suffering from cancer compared with an age and sex-matched non-cancer controls using a cross-sectional study design. The third part was a non-blinded randomized controlled trial of 32 children suffering from BL and WT who received soymilk powder (SMP) supplement and 32 non-supplemented cancer children. The intervention group was provided 2-weekly rations of the supplement measured to provide 80% RDA for protein for age and sex and were followed for 6 months, taking measurements at the 0, 3 and 6-months follow-up. During the follow-up nutritional status, some clinical parameters, deaths and recovery were monitored. Weight, height, mid-upper arm

circumference (MUAC) and triceps skinfolds (TSF) were measured and wasting, underweight and stunting levels determined using World Health Organization (WHO) standards. Biochemical/hematological parameters measured were zinc, reduced glutathione, prealbumin and Hb. In the last part of the study, Isoflavones were isolated from soybean (SB) and soymilk powder (SMP) and the anti-proliferative effect of these isoflavones on Burkitt's lymphoma (*DG-75*) and leukemia (*CEM*) cell lines and prostrate normal cell lines (*PNT 2*) were investigated. Proximate analysis on the SMP revealed a protein concentration of 49.62% and was also found to be safe for consumption after microbial assessments. A total of 96 children (64 with cancer and 32 non cancer group) below the age of 15 years were enrolled for the second part. Repeated measure anova was used to compare differences between groups and p-value less than 0.05 was significant. With the exception of height, the cancer children had significantly lower weight (18.7 versus 27.4 kg; $p < 0.0001$), MUAC (14.1 cm versus 17.8 cm; $p < 0.0001$), TSF (4.9 cm versus 6.1 cm; $p = 0.0332$), muscle arm circumference (MAC) (12.4 versus 15.8 cm; $p < 0.0001$) and body mass index (BMI) (14.6 kg/m^2 versus 18.4 kg/m^2 ; $p < 0.0001$). The cancer group had higher level of malnutrition by all indicators: Low BMI-for-age = 50%, stunting = 47%, wasting = 31% and underweight = 34% compared to non-cancer controls (low BMI for age = 4%, stunting = 25%, wasting = 8% and underweight = 7%). Among the cancer children 44% showed physical signs of wasting, 16% were oedematous and 44% had the sclera of the eye pale. The cancer group also had significantly lower levels of glutathione ($p < 0.0001$) and prealbumin ($p < 0.0001$) than their non-cancer counterparts. SMP intervention improved the various anthropometric, biochemical/hematological indices when the intervention group was more than the non-intervention group with height, weight and zinc being the exceptions. The SMP

intervention reduced low BMI- for-age by 39.4% and wasting by 25.6%. Anaemia was reduced by 84.2% in the intervention group and only 11.7% in the non-intervention group. Also, the percentage of children who were deficient in reduced glutathione improved by 21.9% in the intervention group compared to the 20.5% in the non-intervention group. In terms of recovery, the SMP improved recovery by 31%. In the final part of this work, the extract of isoflavones gave a higher concentration of genistein in SMP (5.5×10^{-3} mg/ml) compared to 5.86×10^{-4} mg/ml found in soyabean (SB). The concentration of daidzein in SMP was also higher (6.247×10^{-3} mg/ml) than that found in SB (3.92×10^{-4} mg/ml). The growth inhibitory effect of genistein on leukemia (*CEM*) cell line was stronger ($IC_{50} = 767.5$ mg/ml) than daidzein ($IC_{50} = 2542.3$ mg/ml). For the *DG 75*, genistein had an IC_{50} of 298.6 mg/ml and daidzein, $IC_{50} > 2542.3$ mg/ml. The IC_{50} for *SMP* on *DG-75* was 193.92 μ g/ml and that for *CEM*, $IC_{50} = 54.17$ μ g/ml. In terms of selectivity index (SI) the *SMP* was able to prevent the proliferation of *CEM* and *DG-75* than *SB*. In conclusion, this study revealed a high prevalence of undernutrition among cancer children compared to their non-cancer control. Nutrition supplementation using *SMP* improved the nutritional status of children with cancer. This is attributed to the higher protein, caloric and mineral composition of the *SMP* given to the intervention group. Furthermore, the anti-proliferative effect of isoflavanoids on lymphoma cell line *DG-75* also suggest some level of anticancer activity of *SMP*.

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LIST OF ABBREVIATIONS

BL	Burkitt's lymphoma
WT	Wilms Tumour
KATH	Komfo Anokye Teaching Hospital
KBTH	Korle Bu Teaching Hospital
NH	Non-Hodgkin's Lymphoma
CR	Cure rates
OS	Overall survival
NWTS	National Wilm's Tumour Study
COG	Children Oncology group
SIOP	International Society of Paediatric Oncology
UNICEF	United Nations Children fund
WHO	World Health Organization
NCI	National Cancer Institute
SMP	Soymilk powder
SB	Soybean

Publications

The manuscripts below are thesis that have been prepared out of this work.

Charles Apprey, Reginald A. Annan, Fareed K. N. Arthur, Samuel K. Boateng and Janet Animah. (2014). The assessment and prediction of malnutrition in children suffering from cancer in Ghana. *European Journal of Experimental Biology*. **4(4)**:31-37.

Apprey Charles, Christopher Larbie, Reginald A. Annan, Fareed K. N. Arthur, Samira Abdulai Saiku, Doris Berkoh and Alex Osei-Akoto (2015). Effects of chemotherapy and disease burden on Nutritional Status of Children undergoing chemotherapy for Burkitt's lymphoma. *Der Pharmacia letter* **7(2)**:95-99.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND.

Nutrition, infection and the immune function are synergistically interrelated (Katona and Katona-Apte, 2008). Malnutrition can predispose an individual to infection, diseases and make recovery from diseases slower and diseases progression more rapid. Likewise, good nutrition enhances immunity and ability to fight infection and diseases (Calder and Jackson, 2000). Infections and disease can lead to malnutrition and nutritional deficiencies by increasing nutrients requirements, utilization, nutrients losses and metabolism as the body tries to generate an immune response against the invading pathogen (Katona and Katona-Apte, 2008). Diseases therefore including cancers have the potential to cause malnutrition and nutrient deficiencies (Andreyev *et al.*, 1998).

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. It is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism) (Ferlay *et al.*, 2010). Worldwide, one in eight deaths is due to cancer; cancer causes more deaths than AIDS, tuberculosis, and malaria combined. It is also the leading cause of death in developed countries and the second leading cause of death in developing countries, following heart diseases (Ferlay *et al.*, 2010). The WHO (2007) indicated that, cancer was the leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008 and deaths from cancer worldwide were projected to continue rising, with an estimated 13.1 million deaths in 2030. The International

Agency for Research on Cancer (IARC), also estimated 12.7 million new cancer cases in 2008 worldwide, of which 5.6 million occurred in economically developed countries and 7.1 million in economically developing countries with 715,000 new cancer cases and 542,000 cancer deaths occurring in 2008 in Africa (Ferlay *et al.*, 2008). Cancer in childhood is quite rare compared with cancer in adults, but it still causes more deaths than any factor, other than injuries, among children from infancy to age 15 years (WHO, 2009).

Childhood cancers are becoming major reasons for hospital admissions in Ghana. A review of the cancer register in the Department of Child Health, Korle Bu Teaching Hospital (KBTH), over a 40-month period (January, 2008 to December, 2011) revealed that lymphomas (mainly Burkitt's lymphomas) was the commonest tumour (30.7%), followed by leukaemia (18.8%), retinoblastoma (15.5%) and Wilm's tumour (12.3%) out of 495 new cases (Segbefia *et al.*, 2013).

Burkitt's lymphoma can be described in simple terms as a malignant tumour of the B cells of the lymphoid tissues that affects mainly children in Africa (endemic BL) as well as adults and young adults of the temperate regions (sporadic BL) (Thorley-Lawson and Allday, 2008). It is a highly aggressive type of B-cell lymphoma that is endemic in children in equatorial Africa, where it accounts for over half of all childhood cancers and often starts and involves body parts other than lymph nodes (Cardy *et al.*, 2001). Wilms tumour also known as nephroblastoma (cancer of the kidney) was first classified by Max Wilms in 1899 at the Institute of Pathology in Bonn, Germany as an embryonal sarcoma which arise from remnant or immature kidney by (Poole, 2010). It is a malignant tumour that develops in the kidney from specialized cells called nephroblast and can affect only one kidney (unilateral) though

occasionally can affect both kidneys (bilateral). Treatments for both Burkitt's and Wilms are chemotherapy, radiotherapy, surgery, immunotherapy, bone marrow transplant and others depending on the type and stage of cancer (Chiu and Weisenburger, 2003).

Nutrition encompasses all the processes that food goes through when eaten including digestion, absorption and metabolism to ensure health. It is a demand-led process influenced by an individual's physiological state such as age, gender, pregnancy, lactation and disease (Roth, 2010). Nutritional status is a balance between nutritional requirements and supply to meet the requirements and adequate nutrition is essential for growth and repair of tissues. Nutritional status can be assessed from dietary and nutrient history, clinical and physical examination, anthropometric measures and biochemical markers (Roth, 2010). Among the biochemical markers, plasma glutathione has been found to be markedly lower in malignancies and for this reason it is used as a nutritional marker in cancer patients. Pre-albumin is also a sensitive biochemical indicator of malnutrition and its concentration in plasma has been shown to respond quickly to nutrition support with a daily increase in value up to 1 mg/dl (Brose, 1990). Bauer *et al.* (2011), observed that up to 46% of children and young adults with cancer experience malnutrition resulting from tumour and other treatment regimen. This is because the growing tumour cells deplete the body nutrients reserve and intake. Thus, nutritional decline is often accepted as part of the cancer course and its treatment (Laviano *et al.*, 1996, Kirby and Teran, 1998) Pediatric patients especially those undergoing treatment for cancer, experience nutritional depletion and weight loss (Bauer *et al.*, 2011) and this makes malnutrition an index of research in cancer epidemiological studies (Dringen, 2000). Malnutrition goes to a large extent of affecting the tolerance to chemotherapy, immune status and survival (Jain *et al.*,

2003) .There is also evidence that malnutrition decreases the tolerance and hence effectiveness of chemotherapy (Mauer *et al.*, 1990). Cancer patients are therefore more likely to suffer from malnutrition, which in turn increases their chances of poorer response to treatment and ultimately recovery. It can therefore be concluded that if nutritional intake is improved in cancer patients, compliance to treatment and recovery will be enhanced.

Studies have shown that supplementation with diet rich in protein can alleviate the nutritional effect of chemotherapies, especially in children since protein supplementation is able to provide them with extra calories and also help them maintain and improve their nutritional status during treatments (Heys *et al.*, 1992). Although animal-based foods such as dairy milk are better sources of proteins, they are more costly and less accessible among poorer populations where most children with some childhood cancers may come from. A plant-based protein source such as soymilk powder (SMP) can therefore serve as a good alternative for a high protein nutritional supplement if processed in a way that removes its anti-nutritive constituents. Soybean is an important source of protein and other nutrients in many developing countries and also contains isoflavonoids which is associated with the reduction of risk of certain chronic diseases including cancer and cardiovascular diseases (Messina *et al.*, 1994, Anderson *et al.*, 1995). Three isoflavones are found in large amounts in soybeans; daidzein (4,7-dihydroxyisoflavone), genistein (4, 5, 7-trihydroxyisoflavone) and glycitein (6-methoxy-4, 7-dihydroxyisoflavone) (Barnes *et al.*, 2000). The primary objective of nutritional interventions in which nutritional assessment forms part in paediatric oncology is the maintenance of body store as close to the ideal or wasting management (Nieuwoudt, 2011), promotion of appropriate growth development by minimizing the impact of treatment-related side

effects (Isenring *et al.*, 2004), and to prevent nutrient deficiencies to provide good quality of life (Bauer *et al.*, 2011).

1.2 PROBLEM STATEMENT

To survive and grow, tumours must transform host stores into their energy fuel, not allowing host tissues to fully utilize the nutrients. Due to their increased proliferation rate, they affect host metabolism (and consequently nutritional status) in a variety of ways, even before it becomes clinically evident (Meguid *et al.*, 1992). Bauer *et al.* (2011), observed that up to 46% of children and young adults with cancer experience malnutrition resulting from tumour and other treatment regimen. Nutritional decline is therefore associated with cancer treatment (Laviano *et al.*, 1996, Kirby and Teran, 1998). Pediatric patients especially those undergoing treatment for cancer, experience nutritional depletion. This makes malnutrition an index of research in cancer epidemiological studies (Mosby *et al.*, 2009). Malnutrition goes to large extent of affecting the tolerance to chemotherapy, immune status and survival (Jain *et al.*, 2003). The cure rates for childhood cancer are currently only around 5% in Africa, compared with 75% to 80% in the United Kingdom and malnutrition has been implicated as one of the reasons for such low cure rate in developing countries (Afrox, 2011).

Soybean is an important source of protein and other nutrients in many developing countries and also contains isoflavonoids which is associated with the reduction of risk of certain chronic diseases including cancer and cardiovascular diseases (Messina *et al.*, 1994, Anderson *et al.*, 1995). Three isoflavones are found in large amounts in soybeans, daidzein (7,4-dihydroxyisoflavone), genistein (5, 7, 4-trihydroxyisoflavone) and glycitein (6-methoxy-7, 4-dihydroxyisoflavone) (Barnes *et al.*, 2000).

Barnes *et al.* (2000) in their study observed that genistein which can be obtained from the consumption of soya product was a naturally-occurring tyrosine kinase inhibitor and was a better anti-cancer agent as compared to the synthetic tyrosine kinase inhibitor which has higher level of toxicity. However not much has been done in the use of soyabean and its products for nutritional intervention study in children undergoing chemotherapeutic treatment due to cancer hence the need for this study. The study sought to investigate the impact of protein –based dietary supplement on the nutritional outcome, recovery and death rate of children undergoing chemotherapy due to cancer at the KATH and to further investigate the anti-proliferative effect of isoflavanoids isolated from in soybean.

1.3 GENERAL OBJECTIVE

The objective of this study was to investigate the impact of a high protein-based dietary supplement (soymilk powder) on nutritional status, recovery and death among children undergoing chemotherapy for Burkitt’s lymphoma (BL) and Wilms tumour (WT).

1.4 SPECIFIC OBJECTIVE

- To determine the mineral, proximate composition and microbial safety of the SMP.
- To determine the nutritional status of children undergoing chemotherapy treatment for Burkitt’s lymphoma (BL) and Wilms tumour (WT) and compare with their non-cancer control children.
- To investigate the effect of the Soyamilk powder (SMP) supplementation on anthropometric indicators, biochemical markers and nutrient intake among children with BL and WT compared with non-supplemented cancer children.

- To compare recovery and survival between the supplemented and non-supplemented children with cancer.
- To determine the concentration of isoflavonoids in SB and SMP and compare the anti-proliferative effect of these isoflavonoids on lymphoma (DG 75) and Leukemia (CEM) and prostrate normal (PNT2) cell lines.
- To compare these anti-proliferative effect of isoflavonoids isolated from SMP and SB with standard daidzein and genistein and explore any possible synergistic effect of the combined isoflavonoids using cell lines.

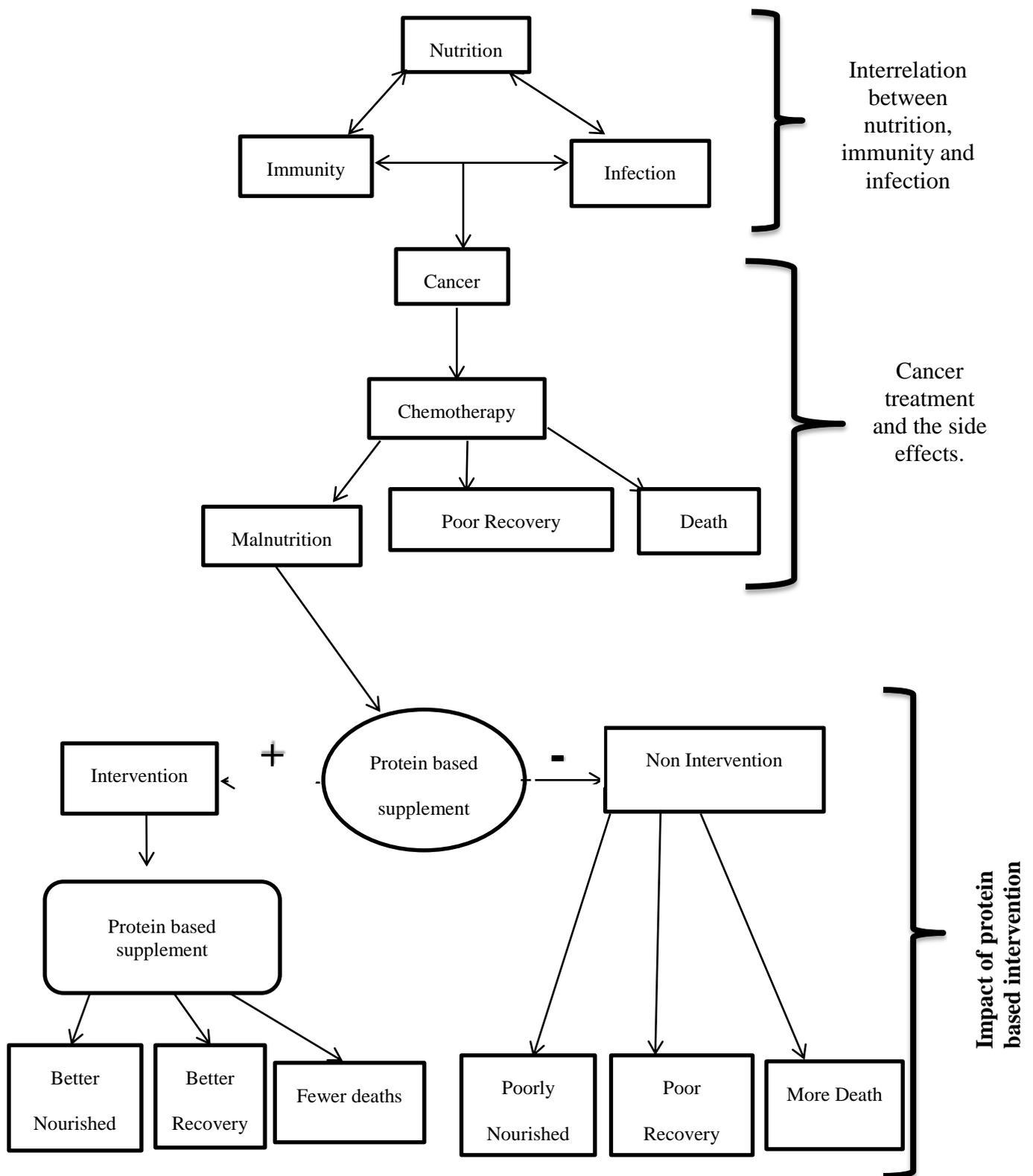


Figure. 1.1 Conceptual Framework of study

Fig. 1 is the conceptual framework of the study and it tries to link malnutrition with cancer and also shows why there is the need for the SMP intervention. In the figure, it could be seen that nutrition, infection and immunity are interrelated. Cancer (BL and WT) are forms of infection and the chemotherapy leads to malnutrition, poor recovery and death. To assess malnutrition, four main indices are employed, thus, anthropometric indices, biochemical indices, clinical signs and dietary intake. There is therefore the need to improve these nutritional indices since they are likely to fall due to the diseased condition as well as the chemotherapy. It is believed that intervention with a high protein based supplement would lead to better nourishment, better recovery as well as few deaths. In the case of non-intervention it is expected that there will be poorly nourished diseased children, poor recovery as well as more death. The effect of an intervention with high protein based supplement (SMP) for six months during the chemotherapy treatment is also investigated into since it is believed that the intervention group will be better nourished, have better recovery and also record fewer death as compared with their non-intervention counterparts.

1.5 LIMITATIONS OF THE STUDY

The limitations encountered in this work were;

- Two childhood cancers (BL and WT) was a limitation since the two cancers have different treatment protocol and hence pose different nutritional challenges.
- For a prospective study of this nature, there were challenges of children defaulting as well as some expiring in the third and sixth month follow-up.
- Another limitation was the different time of recruiting. This brought about recruiting children in the intervention group first before that of the non-intervention group.

Hence randomization could not be done.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BURKITT'S LYMPHOMA

Burkitt's lymphoma (BL) was first described in Eastern Africa; initially it was thought to be a sarcoma of the jaw but later on it was confirmed as a distinct form of non-Hodgkin's lymphoma. It is named after Denis Parsons Burkitt, a surgeon who first described the disease in 1956 while working in equatorial Africa, specifically Uganda (Burkitt, 1962).

Burkitt's lymphoma can be described in simple terms as a malignant tumour of the B cells of the lymphoid tissues that affects mainly children in Africa as well as adults and young adults of the temperate regions (Thorley-Lawson and Allday, 2008). It is a highly aggressive type of B-cell lymphoma that is endemic in children in equatorial Africa, where it accounts for over half of all childhood cancers and often starts and involves body parts other than lymph nodes (Cardy *et al.*, 2001). As reported by (Rosenberg *et al.*, 1982), the tumour consists of high grade, diffuse, small non-cleaved B-cell lymphocytes.

2.1.1. Clinical variants of BL

Generally, there are three forms of BL according to its geographic distribution, incidence, magnitude and risk factors associated with them. However all the three forms are genetically characterized by a chromosomal translocation that results in deregulation of the *c-myc* oncogene (Orem *et al.*, 2007). The three forms are the endemic (eBL), sporadic (sBL) and the human immunodeficiency virus-related Burkitt's lymphoma.

Endemic BL (eBL) is the form of the disease originally described by Dennis Burkitt and it is largely found in Africa and very common in central Africa and Papua New Guinea with an estimated incidence of 5-15 cases per 100,000 (Hecht and Aster, 2000). The distribution of this form of the cancer has been linked to geographic and climate-associated features usually occurring in the regions of the 10° North and South of the equator with minimum annual rainfall and mean temperature of 50 cm and 15.6° C respectively. Patients with endemic Burkitt's lymphoma tend to be immunosuppressed, most commonly thought to be due to chronic malaria. Characteristically it affects the facial skeleton, extranodal sites such as the mandible or maxilla, ovaries or testes, the abdomen, and the ileocecal region of the bowel; however tumors of the jaw are a classic form of the presentation. Bone marrow and CNS may also be involved with estimated involvement of 10% and 20%-30% respectively (Hollender *et al.*, 2002). It occurs exclusively in children with a peak age range of between 5-8 years (Bosch, 2004). BL occur throughout tropical Africa except at high altitudes or in areas where the climate is relatively cool. It is also believed that occurrence of BL is greater in areas with greater rainfall and these geographic and climatic associations suggested an association with falciparum malaria (Ferry, 2006). There is also a strong relationship of BL with EBV and this is has been linked to the genetic instability of the EBV gene. This usually leads to greater risk of c-myc rearrangement and then to lymphoma (Knowles, 2003).

Sporadic Burkitt's lymphoma occurs in children and young adults in the temperate regions especially in America and Europe. It presents as a tumour of the lower abdominal masses, usually the ileum and sometimes a ring of lymphoid tissues formed by the tonsils called the "Waldeyer's ring" (Wright, 1999). This form of the disease is rare with 2-3 cases per million. Less than 20% of all cases is believed to be

associated with the EBV and the average age of children affected is 12.2 (Banthia *et al.*, 2003) but it may also affect all ages . Rochford *et al.* (2005) reported the average age to be 12.2 years but people of all ages can contract the disease.

Immunodeficiency – related BL, also referred to as AIDS – BL, has no specific geographical distribution and is seen to occur in all ages of people with HIV infections (Orem *et al.*, 2007) but also occurs in allograft recipients (Gong *et al.*, 2003) and individuals with congenital immunodeficiency (Xicoy *et al.*, 2003) and this risk is usually related to deficient T-cell functions (Sandlund *et al.*, 1996). The diagnosis of Burkitt's lymphoma in an HIV-positive individual often represents the first AIDS-defining criterion and is believed that HIV-associated Burkitt's lymphoma shares some patho-genetic features with endemic Burkitt's lymphoma (Knowles, 2003). Martínez-Maza and Breen (2002) observed that HIV is not directly involved in lymphoma genesis but is indirectly involved via cytokine deregulation, chronic antigenic stimulation, and decreased immune surveillance.

2.1.2 Epidemiology and Burden of BL in Ghana and Africa

BL is of greatest importance in sub-Saharan Africa, where it is the most common childhood cancer, accounting for up to 36% of childhood cancers and 70% of childhood lymphomas (Parkin *et al.*, 1998, Aderele and Anita, 1983, Daniel, 1990). Parkin *et al.* (1998) have also implicated BL as the commonest childhood cancer in tropical Africa with the area of highest risk for BL appearing to be between 10° north and 10° south of the equator and in Papua New Guinea, where BL is said to be endemic and very common in relation to other types of childhood cancer. The annual incidence of BL in western countries is about 20-40 times lower than in endemic regions.

According to Ferlay *et al.* (2010), Uganda is the country with the highest BL incidence in Africa, recording approximately 20 cases per year though this cannot be said of other countries because of the lack of cancer registry in most African countries. In Ghana, Nkrumah and Olweny (1985), reported an annual BL incidence of approximately 40 cases over an 11-year study conducted in Accra, whereas Owusu *et al.* (2009b) reported an annual incidence of 75 cases at the Komfo Anokye Teaching Hospital, Kumasi. Data from the cancer register in the Department of Child Health-Korle Bu Teaching Hospital, Accra suggest lymphoma was the commonest tumour constituting 67% of 254 cases recorded over a period of 40 months with Burkitt's lymphoma being the commonest subtype (Welbeck and Hesse, 1997).

Shapira and Peylan-Ramu (1998) observed in their review that the most outstanding differences between eBL and sBL are: the median ages of patients at the time of diagnosis, the higher morbidity among males as compared to females, and the differences in the primary sites of the tumour (abdominal or head-neck).

2.1.3 Aetiology/ risk factors of Burkitt's lymphoma

The exact cause of BL is still unknown; however it is believed to affect the body's lymphatic system and results in tumours composed of lymphocytes (Meremikwu *et al.*, 2005). In 1961, Burkitt's made the acquaintance of Epstein, an experimental pathologist, and shared samples of the lymphoma, In his analysis a virus that has come to be known as Epstein-Barr virus (EBV) was identified (Burkitt, 1958). Despite intensive research for more than 30 years, the precise role of EBV in the pathogenesis of BL is still unclear. However, the ubiquitous distribution of early EBV infection (Moormann *et al.*, 2005) and the non-random occurrence of eBL within

holoendemic malaria regions suggest that other cofactors contribute to the etiology of the cancer (Mwanda *et al.*, 2004, Rainey *et al.*, 2007).

EBV is a lymphotropic gamma human herpes virus widely spread among humans and its transmission takes place early in life and is associated with human contact through saliva (Lazzi *et al.*, 1998). A report by International Agency for Research in Cancer (IARC, 1997) on cancer concluded that there was sufficient evidence for the carcinogenicity of EBV in the causation of BL. This stems from the fact that EBV is able to stimulate B-cell proliferation resulting in malignant phenotype which strongly supports the etiology of EBV in BL (Zur Hausen, 1991). At the molecular level BL is commonly characterized cytogenetically by chromosomal translocations that involve the *c-myc* oncogene located on chromosome 8. The *c-myc* oncogene is known to regulate transcription of a number of genes controlling progression through the cell cycle and in apoptosis. Translocation between chromosome 8 and chromosomes 2, 14, or 22 serve to juxtapose *c-myc* with immunoglobulin regulatory elements, which result in dysregulation of *c-myc* expression (Hecht and Aster, 2000).

Plasmodium falciparum is not an oncogenic agent, however, it has been suggested that the geographic distribution of eBL and holoendemic malaria is shared thus both diseases show highest incidence rates in the lymphoma belt or malaria belt (Araujo *et al.*, 1996).

As observed by Rochford *et al.*, (2005), malaria could disrupt immune response against EBV, leading to BL though a population model to understand the specific role of *P. falciparum* in the causation of BL is yet to be identified. Arbovirus, a RNA virus transmitted by insect vectors has also been implicated as a cofactor in the etiology of eBL by inducing cell growth and B-cell hypermutation (Van den Bosch, 2004).

The common neoplasm that has been found in most HIV infected patients is BL, though this cannot be said for other forms of immuno-depression. In most cases, HIV-associated BL display an activation of *c-myc* by chromosome translocations that show structural similarities to those found in patients with sporadic BL (Franceschi et al., 1999). Immuno suppression is the main determinant of the increased risk of non-Hodgkin's lymphoma in AIDS patients. The sap of the milk bush (*Euphorbia tirucalli*) and other *Euphorbiaceae* species have also been implicated as possible environmental risk factors for BL due to their ability to activate the viral replication cycle in the latent phase of EBV-infected cells and this is supported by the finding from Malawi (Van den Bosch *et al.*, 1993).

Socio-economic factors should be regarded as a surrogate marker for exposure to those factors believed to play an etiological role; low socioeconomic factors may also be associated with a poor defense response toward environmental exposures due to poor nutrition and/or poor hygienic conditions (Magrath *et al.*, 1992).

2.1.4 Staging and treatments of BL

Identifying the exact stage of the disease at diagnosis is very necessary before the onset of treatment. All staging systems basically reflect the extent of the disease and the most frequently used is the St. Jude Children's Research Hospital staging system though the Ann Arbor system could also be used (Kasamon and Swinnen, 2004). Like all other non – Hodgkin's lymphoma, BL progresses through four main stages of the disease on the bases of limited versus extensive disease.

Table 2.1: Stages of BL and the symptoms that characterize each stage (Shapira and Peylan – Ramu, 1998).

STAGE	SYMPTOMS
STAGE 1	Non-Hodgkin’s Lymphoma is limited to one lymph node group (e.g., neck, underarm, groin, etc.) or tumor outside of the abdomen or mediastinum (middle chest)
STAGE 2	NHL is limited to one tumor with local lymph node involvement, or NHL is limited to two or more tumors or lymph node groups on the same side of the diaphragm, or NHL is limited to a primary tumor of the gastrointestinal tract with/without involvement of local lymph nodes
STAGE 3	NHL includes tumors or lymph node groups on both sides of the diaphragm, or any primary NHL tumor within the thorax (trunk) or extensive NHL within the abdomen, or any NHL around the spine or the outermost membrane of the brain and spinal cord.
STAGE 4	NHL is in the bone marrow or central nervous system (CNS), with/without other sites of involvement. Bone marrow NHL is defined as 5% malignant cells in an otherwise normal bone marrow with normal blood counts and smears. By contrast, lymphoblastic lymphoma that produces more than 25% malignant cells in the bone marrow is defined as leukemia.

It is estimated that 30% of patients present with limited-stage disease (I or II), while 70% present with widespread disease (stage III or IV) using the St. Jude system (Jaffe *et al.*, 2011). Aggressive chemotherapeutic regimens administered to patients with BL has led to good outcomes with cure rate (CR) rates of 65%–100% and overall survival (OS) rates of 50%–70% (Kasamon and Swinnen, 2004). As observed by Levine (2002), 50%-80% of adult patients with BL can be cured with intensive chemotherapy regimens with pediatric patients having a higher cure rates. Cyclophosphamide plays a central role in the management of Burkitt’s lymphoma and several treatment regimens have been devised around this drug with different classes of drugs employed to manage tumour lysis syndrome (TLS) associated with cytotoxic chemotherapy. There is also 20%-30% risk of developing central nervous system involvement hence

the inclusion of CNS prophylaxis in the treatment regimen (Magrath *et al.*, 1996). In the CODOX-M/IVAC regimen (Magrath protocol)—two cycles of the chemotherapy in 14 days of CODOX-M (cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate, and intrathecal therapy) alternating with IVAC (ifosfamide, etoposide, high-dose cytarabine, and intrathecal therapy)—for high-risk disease, and for those with low risk disease (e.g., one extranodal site or completely resected abdominal disease with normal LDH), three cycles of CODOX-M, represented a major step forward in the treatment of Burkitt's lymphoma (Magrath *et al.*, 1996). The United Kingdom Lymphoma Group, for example, used this protocol with slight modifications, such as decreases in vincristine (Mead *et al.*, 2002).

The Dana-Farber Cancer Institute has treated patients with a modified Magrath protocol, aimed at decreasing toxicity while maintaining good outcome. In this modification, the schedule of fractionated cyclophosphamide was altered and the vincristine dose was capped, but the dose of doxorubicin was escalated (Lacasse *et al.*, 2004).

2.2 WILMS TUMOUR

Wilms tumour also known as nephroblastoma was first classified as an embryonal sarcoma which arise from remnant or immature kidney by Max Wilms in 1899 at the Institute of Pathology in Bonn, Germany (Poole, 2010). It is believed that two forms of the diseases exist thus the sporadic and the familial form, though the latter form is much less common and only 1% of Wilms' tumour patients have positive family history (Tay, 1995). It is a malignant tumour that develops in the kidney from specialized cells called nephroblast and can affect only one kidney (unilateral) though

occasionally can affect both kidneys (bilateral). The tumour can sometimes spread (metastasize) to other parts of the body, such as the lungs or liver.

2.2.1 Epidemiology and Burden of Wilms Tumour in Ghana and Africa

Breslow et al. (2006), indicated that more than 80% of cases are diagnosed before 5 years of age, with a median age of 3.5 years and constitute about 95% of all diagnosed renal cancers as well as 6% of all childhood cancers. Male to female ratio in unilateral disease is 0.92 and 0.6 in bilateral diseases (Petruzzi and Green, 1997). In terms of racial distribution, incidence rate is about three times higher for blacks in the United States and Africa than for East Asians, with rates for white populations in Europe and North America intermediate between these extremes (Stiller and Parkin, 1990).

In Africa, it accounts for about 4–26% of all malignant solid tumours in childhood. Aguehoude *et al.* (1993) and hospital-based reports suggest that 4 to 10 new cases of Wilms' tumour are seen every year in most tertiary referral centres (*Abuidris et al., 2008, Amel et al., 2003*). In Ghana, data from the cancer register of the Department of Child Health, Korle Bu Teaching Hospital (Accra) suggests that Wilms tumour constitute 7.8% of 254 malignancies diagnosed over a 40 months period (Welbeck and Hesse, 1997). The incidence in Africa and Ghana is probably underestimated, however, because some of the affected children are not brought to the attention of trained medical practitioners.

2.2.2 Aetiology/ Risk factors of Wilms Tumour (WT)

WT is believed to be brought about by mutation of at least four different genes but the best defined gene is the *WT1* tumor suppressor gene on chromosome *11p13*; both alleles are deleted or inactivated in Wilms' tumors initiated by mutation at this gene (Miller et al., 1964). Also, it occurs sporadically though 1% of cases is familial. Loss

of heterozygosis of chromosome *16q* has been observed in 15–20% of patients with WT and found to be associated with a 3.3 times higher relapse risk and a 12 times higher risk of death (Grundy *et al.*, 2005). A number of recognized syndromes have been associated with an increased predisposition towards developing WT and these are classified into two main groups, namely overgrowth and non-overgrowth syndrome. The common overgrowth syndrome includes Beckwith-Wiedemann Syndrome (BWS) (visceromegaly, hypoglycaemia, macroglossia, midline defects); and isolated hemihypertrophy. The most recognized non-overgrowth syndromes include WT, aniridia, genito-urinary anomalies, mental retardation (WAGR) syndrome and Denyse Drash syndrome (DDS) (protein-losing nephropathy, disorders of sexual development, WT) (Grønskov *et al.*, 2001, Muto *et al.*, 2002, Breslow *et al.*, 2003).

The role of parental environmental exposure is still not conclusive since no consistent positive findings have emerged from a series of case control studies. However, Sanders *et al.* (1981), observed an increased proportional mortality rate among kidney tumor patients whose fathers were employed in hydrocarbon-related industries at the time of the patient's death.

2.2.3 Pathology and Clinical presentation

The gross appearance of the kidney, distorted by the presence of the tumor, seldom gives external evidence pointing to the underlying histopathology, however, histology has been the most important prognostic indicator for Wilms tumour. In histology of classic WT, three main cell types, blastemal, stromal, and epithelial are observed in varying proportions depending on the extent of the diseases though they are all not present in every case (Schmidt and Beckwith, 1995). Presenting symptoms usually

include abdominal pain leading to the risk of preoperative rupture and bleeding. Gross hematuria may be a sign of tumor extension into the collecting system or ureter (Ritchey *et al.*, 2007). In physical examination of WT the location and size of the abdominal mass and its movement with respiration is necessary since it helps differentiate Wilms tumour from splenomegaly. Laboratory evaluation usually follows a physical examination and this includes a complete blood count, a differential white blood cell count, a platelet count, liver function test, renal function tests, serum calcium which is elevated in patients with mesoblastic nephroma (Green, 1985). A radiographic evaluation is also done to assess the presence of an intrarenal mass and a normal functioning contra lateral kidney. Radiography also helps demonstrate the presence or absence of pulmonary metastases (Green, 1985).

2.2.4 Staging and Treatment

The staging system used to describe children with Wilms tumor considers several physical features of the tumor that increase the risk of local or distant recurrence and therefore dictate treatment modifications. The outcome of children with WT are usually based on staging and histopathology. Staging criteria for Wilms' tumor are based exclusively on the anatomic extent of the tumor, without consideration of genetic, biologic, or molecular markers (Kalapurakal *et al.*, 2004). There are currently two staging systems available reflecting treatment differences; a pre-chemotherapy/up-front, surgery-based system developed by the National Wilms' Tumor Study Group (NWTSG) and post-chemotherapy based system developed by the International Society of Pediatric Oncology (SIOP). The difference in surgical timing confounds stage-for-stage comparisons between the two systems though both staging systems have proven valuable in predicting outcomes.

Table 2.2: National Wilms' Tumour Study Group staging system

STAGE	DEFINITION
I	Tumour confined to the kidney and completely resected; no penetration of the renal capsule or involvement of renal sinus vessels
II	Tumour extends beyond the kidney but is completely resected (negative margins and lymph nodes; at least one of the following has occurred: Penetration of the renal capsule Invasion of the renal sinus vessels Biopsy of tumour performed before removal Spillage of tumour locally during removal
III	Gross or microscopic residual tumour remains postoperatively, inducing inoperable tumour, positive surgical margins, tumour spillage involving peritoneal surfaces, regional lymph node metastases, or resected tumour thrombus
IV	Haematogenous or lymph node metastases outside the abdomen (e.g. lung, liver, bone, and brain)
V	Bilateral renal Wilms' tumours

The treatment of Wilms' tumour is a prime illustration of success achieved with multimodality therapy in paediatric oncology. This multimodality therapy includes radiation, surgery and chemotherapy. The two major treatment strategies are that of the National Wilms' Tumour study (NWTs) now the Children Oncology Group (COG) (USA), where initial surgery is performed with treatment according to the post-surgical stage. The second treatment strategy is that of the International Society of Paediatric Oncology (SIOP, Europe) that gives the child neo-adjuvant chemotherapy for 4-8 weeks to shrink the tumour. Surgery is then performed with postoperative treatment according to the post-surgical stage (Poole, 2010).

2.3 NUTRITION AND CANCER

2.3.1 Nutrition, Nutritional Status and Nutritional Markers

Nutrition encompasses all the processes that food goes through when eaten including digestion, absorption and metabolism to ensure health. It is a demand-led process influenced by an individual's physiological state such as age, gender, pregnancy, lactation and disease. Infections and disease increase the demand for nutrients because of increased utilization and loss as the body mounts its response against the invading pathogens. Nutritional status is a balance between nutritional requirements and supply to meet these requirements and adequate nutrition is essential for growth and can be assessed from dietary and nutrients history, clinical and physical examination, anthropometric measures and biochemical marker (Roth, 2010). Nutritional markers generally used to in determining the nutritional status of individual. The ideal nutritional marker should readily respond to changes in nutrient intake, be uninfluenced by other disease processes and be measurable (Banh, 2006).

2.4. NUTRITIONAL ASSESSMENT

Nutritional health is maintained by a state of equilibrium in which nutrient intake and requirements balance. Malnutrition occurs when net nutrient intakes (nutrient intake corrected for abnormally large fecal or urinary losses) is less than requirements. The evaluation of the nutritional status is a broad area and mostly there is the need to use an ideal method which is clinically important. For nutritional assessment to be clinically useful, the method used should be able to specifically assess the risk of morbidity and mortality resulting from malnutrition as well as to be able to identify and separate the causes and consequences of malnutrition and disease in the individual patient.

Albina *et al.* (2002) observed that nutritional status, which is a global term, encompasses a number of specific components some of which includes nutritional screening, nutritional assessment, nutritional care plan and specialized nutritional support. Nutritional assessment is therefore a comprehensive approach to defining the nutritional status of an individual that uses medical, nutritional and medication histories, physical examination, anthropometric measurements and biochemical analysis.

Nutritional assessment is an integral part of patients care especially in the pediatric setting since children undergo complex process of growth and development which are influenced by the genetic makeup of the individual and coexisting medical illness in addition to nutritional status (Maka and Murphy, 2000). According to Gibson (2005), the purpose of nutritional assessment is to identify individuals or population groups at risks of becoming malnourished, identify individuals or population groups who are malnourished, develop health care programs that meet the community needs which are defined by the assessment and measure the effectiveness of the nutritional programs and intervention once initiated. There are various methods available to assess the nutritional status of an individual. It is often helpful to combine multiple methods to obtain a more comprehensive and accurate assessment.

The two main forms of assessing nutrition are the direct and the indirect methods. The direct methods deal with the individual and measure objective criteria, while indirect methods use community health indices that reflect nutritional influences. The direct ones are usually summarized as ABCD thus anthropometric methods, biochemical/laboratory methods, clinical methods and dietary evaluation methods.

2.4.1 Anthropometric Methods

Anthropometry (the use of body measurements to assess nutritional status) is a practical and immediate applicable technique for assessing children's development patterns during the first years of life. It is an important tool in the study and understanding of human biological variability, including, of course, morphological variation as universally applicable, non-invasive and inexpensive methods (WHO, 2004). It can be simply defined as the measurement of the body height, weight and proportions. Evaluation of the growth of children also provides useful insights into the nutrition and health situation of entire population groups. Anthropometric indicators are less accurate than clinical and biochemical techniques when it comes to assessing individual nutritional status. In many field situations where resources are severely limited, however, anthropometry can be used as a screening device to identify individuals at risk of under nutrition, followed by a more elaborate investigation using other techniques. The four main building block of anthropometry are age, sex, length (height) and weight. Each of the four variables provides a piece of information about an individual, however, when used together can provide important information about a person's nutritional status. When two of the variables are used together they are called index. The three main indices that are commonly used in assessing nutritional status are weight for age, length for age (Height for age), weight for length (weight for height).

2.4.1.1 BMI

Body Mass Index (BMI) is a simple, safe, non-invasive and cheap way of estimating body fat percentage and assessing a person's health and nutritional status (Jéquier, 1987). It is index of weight-for-height that is commonly used to classify underweight,

overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2) and an internationally accepted index for defining obesity (Eknayan, 2008).

Table 2.1: The International Classification of adult underweight, overweight and obesity according to BMI (WHO, 2004).

Classification	BMI (kg/m^2) Cut off Points
Underweight	< 18.50
Normal range	18.50- 24.99
Overweight	\geq 25.00
Obese	\geq 30.00

It is the most commonly used measure of adiposity, however, it also plays a lot of role in determining the nutritional status of an individual.

Though BMI calculation for children and adults do not differ, the interpretations of the results differ. In adults, the BMI classifications do not depend on age or sex however for children and adolescent aged 2 to 20 years old, the BMI is interpreted in relations to the age and sex of the child and this is mainly so as the amount of body fat changes with age and sex (Mei *et al.*, 2002).

2.4.1.2 Weight-for-age

Weight-for-age index is used in identifying the condition of being underweight for a specific age and it is able to reflect both past (chronic) and/ or present (acute) under nutrition though it is unable to distinguish between the two. Underweight determination is based on weight- for- age and is a composite measure of stunting and wasting and it is a recommended indicator to assess changes in the magnitude of malnutrition over time (Cogill, 2003).

2.4.1.3 Height-for-age

The height-for-age index is an indicator of past undernutrition or chronic malnutrition. It cannot measure short term changes in malnutrition and for children below 2 years of age. Deficits in length-for-age or height-for-age are signs of stunting. Stunting is mostly due to low length-for-age, stemming from the slowing in the growth of the foetus and the child. This brings about children who are unable to achieve the expected length as compared to their healthy and well-nourished child of the same age. It is usually associated with factors such as chronic insufficient proteins, energy intake, frequent infections, sustained inappropriate feeding practices and poverty. The effect is usually irreversible in children over 2 years of age hence any intervention for stunting is more responsive in children under two years than children above 2 years (Cogill, 2003). However, Adair (1999) indicated catch-up growth to reverse stunting as a possibility though this outcomes is not predominant in children who remain in the same environment.

2.4.1.4 Weight- for- height

Weight- for- height is the third index. It is used to identify children suffering current or acute undernutrition or wasting. Weight-for-length (in children under 2 years of age) or weight- for- height (in children over 2 years of age) is appropriate for examining short-term effects such as seasonal changes in food supply or short-term nutritional stress brought about by illness (Cogill, 2003). Wasting is said to occur when the weight of a child falls significantly below the weight expected of a child of the same length or height. It is a good indicator of current or acute malnutrition resulting from failure to gain weight or actual weight loss. Inadequate food intakes, incorrect feeding practices, disease, and infection or, more frequently, a combination

of these factors have been implicated as the cause of wasting. Collins *et al.* (2000) observed that wasting in individual children and population groups can change rapidly and shows marked seasonal patterns associated with changes in food availability or disease prevalence to which it is very sensitive. The three indices are therefore used to identify three nutritional conditions thus, underweight, stunting and wasting.

Oedema which is a clinical sign of severe malnutrition is also recorded when using weight- for height for surveillance and screening purposes. It is the presence of excessive amounts of fluid in the intracellular tissue and can be diagnosed by applying moderate thumb pressure to the back of the foot or ankle. The impression of the thumb remains sometime when edema is present and is diagnosed if both feet show the impression for some time. A child with edema is automatically included with children counted as severely malnourished independently of its wasting, stunting, or underweight status. This is due to the strong association between edema and mortality (Cogill, 2003).

2.4.1.5 Mid upper arm circumference

Mid upper arm circumference (MUAC) is relatively easy to measure and a good predictor of immediate risk of death, it is more sensitive, less prone to mistakes and also increases the link with the beneficiary community (Collins *et al.*, 2000). It is used for rapid screening of acute malnutrition from the 6-59 month age range. MUAC can also be used for screening in emergency situations and is recommended for assessing acute undernutrition and for estimating prevalence of undernutrition at the population level (Velzeboer *et al.*, 1983).

2.4.1.6 Tricep skin fold

Skinfold thicknesses are important and valid anthropometric indicators of nutritional status, body composition and relative subcutaneous fat distribution (regional and total body fatness), especially in research settings (Lohman *et al.*, 1988, Sinha *et al.*, 2008). The triceps skinfold provides information about fat reserves of the body and is the most reliable one, because oedema is not often seen in the upperarm.

2.4.1.7 Mid arm circumference

Mid arm circumference is an indicators for the quantity of fat and muscle mass in the body. It is a measure for mid upper arm circumference of the body measured together with the triceps skinfold (Frisancho, 1981). In this method the assumption is that the measured muscle circumference is representative for the rest of the body. It is given by the formula below;

$$MAC = MUAC - 0.1 (TSF \times 3.14) \quad (\text{Symreng, 1982})$$

The disadvantage of this method is that the results are not reliable patients with oedema in the upper extremities or people who are immobile by muscle disease or something else (Ravasco *et al.*, 2002).

2.4.2 Biochemical Assessments

Checking a patient's blood for biochemical markers (whole blood or serum levels of nutrients) can be useful in aiding the nutritional assessment process. It is often appropriate for serum levels of nutrients such as vitamins, proteins (pre-albumin, albumin, retinol-binding protein, fibronectin, c-reactive protein), minerals (zinc and iron), glutathione (an antioxidant) alongside haemoglobin to be taken (Ripley, 2006).

Such readings can identify nutrients that the patient may be lacking in, and then a plan to address this can be implemented.

2.4.2.1 Albumin

Albumin is a negatively charged protein that is synthesized by the liver and its main function is to maintain osmotic pressure as well as transport other circulating molecules (Rosenoer *et al.*, 2014). Serum albumin levels have long been used as a major measure of malnutrition in both developed and developing countries and low values are always indicative of malnutrition (Covinsky *et al.*, 2002). The level of albumin are sometimes highly predictive of patient mortality in the hospital (Guijarro *et al.*, 1996) and mortality in the general population (Klonoff-Cohen *et al.*, 1992). Goldwasser and Feldman (1997) in their research found out that for every 2.5 g/L decrease in serum albumin concentration, there is about 24% - 56% increase in the likelihood of dying. Albumin has a half-life of approximately 18 days (Wells *et al.*, 1985). It functions by maintaining plasma osmotic pressure and transport substances in plasma and the serum level reflects the net results of hepatic synthesis, plasma distribution and net loss (Omran and Morley, 2000). Chronic alteration in patients with kidney failures and diseases affecting hepatic production of albumin (liver diseases and congestive heart failure) and rate of albumin loss (nephrotic syndrome and protein-losing enteropathy) (Huang *et al.*, 1996, Kaysen *et al.*, 2004).

2.4.2.2 Prealbumin

Prealbumin or Transthyretin (TTR) is an important marker for assessing protein deficiency. It is produced in the liver and it is a serum and cerebrospinal fluid carrier of the thyroid hormone thyroxine (T4) and retinol binding protein bound to retinol. The name transthyretin comes from the fact that prealbumin mainly transports

thyroxine and retinol. The liver secretes transthyretin into the blood, and the choroid plexus secretes TTR into the cerebrospinal fluid (Razavi *et al.*, 2003). Prealbumin was one of the earliest laboratory indicator of nutritional status and has emerged as the preferred marker for malnutrition because it correlates with patient outcomes in a wide variety of clinical conditions (Ingenbleek *et al.*, 1975). Beck and Rosenthal (2002) observed that determining the level of prealbumin, a hepatic protein, is a sensitive and cost-effective method of assessing the severity of illness resulting from malnutrition in patients who are critically ill or have a chronic disease and the levels have been shown to correlate with patient outcomes and are an accurate predictor of patient recovery. Historically, albumin levels have been used as a determinant of nutritional status, but they are relatively insensitive to changes in nutrition. Albumin has a relatively large body pool and a half-life of 20 days. Serum albumin concentrations are affected by the patient's state of hydration and renal function. The level typically takes 14 days to return to normal when the pool has been depleted. This makes prealbumin the preferred marker for protein malnutrition. It has a shorter half-life of 2-4 days and is less affected by liver disease than other serum proteins. Prealbumin has one of the highest ratios of essential to nonessential amino acids of any protein in the body, making it a distinct marker for protein synthesis (Spiekeman, 1995). Studies have indicated that determination of the prealbumin level may allow for earlier recognition of and intervention for malnutrition (Mears, 1996). In one study conducted by (Bleiberg-Daniel, 1992), prealbumin production decreases after 14 days of consuming a diet that provides only 60 percent of required proteins. Ingenbleek *et al.* (1975) and Morlese *et al.* (1996) also showed that synthesis of prealbumin increases above baseline levels within 48 hours of protein supplementation in children with severe protein calorie malnutrition and returns to normal levels within eight

days. These observations and others led to the recommendation that prealbumin levels should rise 2 g per dL (20 g per L) per day with adequate nutritional support (Spiekeman, 1995). In the Mears (1996), study, patients at severe risk [i.e., prealbumin levels below 10 mg per dL (100 mg per L)] averaged hospital stays of 22 days compared with an average of six days in patients at moderate risk [prealbumin levels between 10 and 17 mg per dL (100 and 170 mg per L)]. Serum prealbumin is considered the relatively more sensitive parameters of the efficacy on nutrition interventions this is mainly because of its shorter half-life (2-3 days) and in cancer patients who are provided nutrition therapy including protein sparing diets supplemented with proteins, an increase in serum hepatic proteins could signify an anabolic response (Guerra *et al.*, 2009). Neyra *et al.* (2000) observed that prealbumin changes to short-term interventions and are also unaffected by hydration status and this makes it a good indicator of early monitoring.

2.4.2.3 Transferrin

Transferrin is a serum beta globulin protein synthesized primarily in the liver, but unlike albumin it is located intra-vascularly as a transporter of iron with a half-life of (8–10 days), and responds more rapidly to changes in protein status. The level of transferrin are usually affected by iron status, it is one of the strongest predictor of cancer patient mortality and morbidity (Beguin, 2003, Guerra *et al.*, 2009). Its main function is to bind and transport ferric iron to cell membranes, where transferrin molecules are captured, internalized, acidified, stripped of iron, and extruded iron free and intact (Thorstensen and Romslo, 1990). Transferrin also helps minimize bacterial infections by decreasing the biologically active free ionized iron concentration necessary for bacterial growth (Bullen, 1981, Symeonidis and Marangos, 2012). It is a cardinal point in diagnosing iron deficiency anemia and has a smaller body pool

compared to that of albumin (Ingenbleek et al., 1975, Short and Domagalski, 2013). Transferrin levels of greater than 200 mg/dL indicate an adequate nutritional state, and concentrations of less than 100 mg/dL indicate severe visceral protein depletion (Spiekeman, 1995). Though transferrin is an early indicator of protein of Protein malnutrition, it is less specific and the concentration of >1.5 g/L is usually an indicative of protein malnutrition. However, this can significantly be influenced by the amount of iron, acute inflammation diseases and chronic hepatopathy (Hrnciarikova *et al.*, 2006).

2.4.2.4 Transferrin receptors

Transferrin receptor is a disulphide dimer which is made up of two identical subunits of 95 kDa. It mostly found on immature erythroid and malignant cells. It functions mainly by internalizing absorbed iron into target cells (Nadeem *et al.*, 2011). The concentration of transferrin is dependent on the body stored of iron. Thus, when there is low body stored of iron, there is increased expression of receptors and vice-versa. Clinically, the serum level of transferrin receptor is used to evaluate the progress of iron deficiency anaemia as well as the rate of erythropoiesis (Worwood, 2002) .

2.4.2.5 Retinol-binding Protein

Retinol-Binding protein (RBP) is a single chain protein that circulates in the plasma as the retinol–RBP complex and its main function is to protect the retinol molecule from oxidative damage while transporting it from the liver (Graham *et al.*, 2006). RBP is closely related to prealbumin and usually forms a complex in a 1:1 ratio. This complex is necessary to stabilize the RBP-retinol binding and prevents the loss of RBP through the renal glomerular barrier. Since RBP is required for retinol

transportation, its serum-level decrease significantly in cases of retinol (vitamin A) deficiency due to a block in its hepatic secretion (Vaisbuch *et al.*, 2010). In this case RBP is synthesized and stored in the liver until retinol is repleted. RBP has a small pool size and a short half-life of 12 h, which makes it a legitimate marker for protein deprivation (Johansson *et al.*, 2001). Retinol binding protein is also used to determine protein malnutrition but it could be confounded by inflammation, infection (Gamble *et al.*, 2001).

The normal range is between 30 and 65 mg/L however, the level increases in conditions such as renal failure, acute hepatic diseases and decrease during vitamin A deficiency (Shetty *et al.*, 1979).

2.4.2.6 Serum cholesterol

Cholesterol is a member of a large group of substances called steroid, which include vitamin D. It is an essential component of cell membrane, brain and nerve cells, and bile, where it plays a vital role in fat absorption (Tripathy *et al.*, 1970). Though the body can produce all the cholesterol in needs in making vitamin D, estrogen, testosterone and cortisol they are also obtained in food (Onitiri and Boyo, 1975). Serum cholesterol levels lower than 160 mg/dL have been considered a reflection of low lipoprotein and thus of low visceral protein (Coward and Lunn, 1981). Hypocholesterolemia have been found to affect mortality via pressure sores, cancer, rheumatoid arthritis, and use of enteral feeding (Huang *et al.*, 1996). However, hypocholesterolemia usually happens at the late stage of malnutrition and this limits the value of cholesterol as a screening tool. Its usefulness as a prognostic tool still remains of value (Rudman, 1984). Hypercholesterolemia has also been reported to be

associated with higher mortality risk in the elderly mostly due to cardiovascular diseases (Verdery and Goldberg, 1991).

2.4.2.7 Reduced glutathione

Glutathione (GSH) is a tripeptide with a gamma peptide linkage between the amine group of cysteine (which is attached by normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al.*, 2003). Intracellular and blood glutathione concentrations are in the millimolar range, whereas plasma concentrations are in the micromolar range. The tripeptide can be free (GSht), reduced (GSH), oxidized (GSSG), or bound to proteins (Shaw *et al.*, 1983). In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules, such as reactive oxygen species. In donating an electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress. Han and Park (2009) indicated that higher level of GSH reduces the level of reactive oxygen species which may reduce cancer development. However, once a cancer has already developed, by conferring resistance to a number of chemotherapeutic drugs, elevated levels of reduced glutathione in tumour cells are able to protect cancerous cells in bone marrow, breast, colon, larynx, and lung cancers (Balendiran *et al.*, 2004).

2.4.2.7 Zinc

Zinc, as an essential trace element for health, plays various biological roles in human body functions and recent clinical and experimental findings have reinforced the link among zinc deficiency, malnutrition and diarrhoea diseases (Wapnir, 2000) and because there is a strong association between protein and zinc content in virtually all types of foods, insufficient protein intake may often be the cause of zinc deficiency. This is the reason why protein and zinc deficiencies are strong negative determinants for normal cellular immunity. It is also an intrinsic constituent of superoxide dismutase, a major scavenger of free radicals, present in the cytoplasm of many types of cells and in the extracellular space. Superoxide dismutase converts the super oxygen free radical to hydrogen peroxide, which is further decomposed by catalase into water and oxygen (Leung, 1998).

2.4.3 Clinical Assessments

The clinical assessment of nutritional status attempts to identify the initial nutritional state and the interplay of the factors influencing the progression or regression of nutritional abnormalities. Therefore, a clinical nutritional assessment is a dynamic process that is not limited to a single “snapshot” at the moment of measurement but provides a picture of current nutritional status and insight into the patient’s future status (Kondrup *et al.*, 2003). The clinical assessment of nutritional status involves a focused history and physical examination in conjunction with selected laboratory tests aimed at detecting specific nutrient deficiencies to identify patients who are at high risk for future nutritional abnormalities (Shirodkar and Mohandas, 2005).

2.4.3.1 Nutritional history

In clinical assessment of nutritional status, the evaluation of nutritional history is also done to identify whether there has been a recent unintentional change in body weight, if dietary intake is adequate and if there is a change, what has caused the change in dietary intake. This includes change in appetite, disturbance in taste, smell and the ability to chew or swallow food. Questions relating to gastrointestinal challenges such as early satiety, postprandial pain, nausea or vomiting, any medication that affects food intake and evidence of malabsorption are all noted. Symptoms of specific nutrients deficiencies including macronutrients, micronutrients and water are all observed as part of clinical examination of nutritional history (Stratton *et al.*, 2004).

2.4.3.2 Physical Examination

This is done to collaborate and add to the findings obtained by the history method of clinical assessment. Anthropometry is the main form of physical assessment of nutritional status. Fluid status appraisal which includes evaluation of dehydration, (hypotension, mucosal xerosis, dry skin, swollen tongue and excess body fluid), oedema presence should be made. Evaluation for specific nutrient deficiencies and in this assessment, rapidly proliferating tissues such as that of the oral mucosa, hair, and skin are assessed since they are often more sensitive to nutrient deficiencies than tissues that turnover more slowly (Kondrup *et al.*, 2003, Stratton *et al.*, 2004).

2.4.4. Dietary Assessment

This is a method of assessing dietary intake of an individual and it includes diet record, 24- hour dietary recall and food frequency questionnaire. In dietary assessment, the collection of the data as well as proper analysis of the dietary report is very critical since the results of the assessment should be accurate and complete. Once

the assessment has been obtained, dietary analysis is performed to translate food intake into nutritional recommendations for individuals and populations. Some of the options available for analyzing food intake in dietary assessments include software programmes and food database. The cost and output of the software as well as the quality of the nutritional database are factors that are taken into account when choosing a food analysis method. Other considerations such as new and ethnic foods, along with fluid and supplement analysis (snacking) are made and included in the dietary analysis process. Reliable and accurate ways to assess food intake includes use of food diaries, 24- hour dietary recalls and the use of food frequency questionnaires (Driskell and Wolinsky, 2010).

2.4.4.1 Diet Records

A diet record consists of all the food and beverage a person consumes in a certain amount of time. Three- day diet records are most often used (preferable two weekdays and one weekend) to determine an individual's daily food and beverage consumption. The seven-day food dairies are more time consuming and are also not accurate and concise diet records though it gives a complete diet of the individual's diet (Miller, 2007).

2.4.4.1.1 Twenty-four hour dietary recall

A 24-hour dietary recall is a retrospective method of dietary assessment where an individual is interviewed about their food and beverage consumption during a defined period of time, typically the previous day or the preceding 24 hours since a recall of intake over a long period is problematic due to the limitation of memory (Brustad *et al.*, 2002). A single 24-hour recall is not considered to be representative of habitual diet at an individual level but is adequate for surveying intake in a large group and

estimating group mean intakes. To assess a typical diet of an individual, 7-day daily recall is always recommended though very time consuming and also not accurate and concise. In an Australia study conducted by Jackson *et al.* (2008), to observe the variation in micronutrient intake, Sunday was the day for greatest variation. The researchers therefore recommend that dietary recall is done in two working days and a weekend to compensate for the variation in micronutrients intake. Both quantity and food preparation play a major role when performing a 24-hour dietary recall. An advantage of this method is that it can be done in person or over the telephone in a brief amount of time. A main concern with a 24-hour dietary recall is its misrepresentation of the usual diet. It is important to ask the individual if the diet consumed within the last 24 hours is a normal diet or if it was a variation from the norm.

2.4.4.2 Food Frequency questionnaire

Food frequency questionnaire can assist in determining, on average, the amount of a specific macro- or micronutrient an individual consumes with a given time. It is highly dependent on the individual's memory and ability to estimate the quantity of a particular food or food group. This is usually done by providing a list of foods to the individual and he or she is asked to determine how often each food is consumed during a specific period, usually ranging from one day to several months. A limitation of food frequency questionnaire is its specificity to certain populations (Magkos and Yannakoulia, 2003). Over reporting can be a major factor in this type of diet analysis (Buzzard *et al.*, 2001).

2.3.3 Nutrition, Infection and the Immune System

The immune system consists of all cells and tissues that protect the host from invading pathogens and from noxious environmental agents. It is divided into an innate or native and adaptive parts. The innate immunity consists of barriers like the skin and mucosa, and also antimicrobial compounds in the mucosa, immune cells such as phagocytic cells (macrophages and neutrophils) and natural killer cells, proteins called cytokines produced by these cells, and blood borne proteins called complement factors. The adaptive immunity is very specific and can differentiate between substances even if their structure is similar. The cells in the adaptive immune system are called lymphocytes and the major types are the B and T cells. These cells are involved in antibody production, memory, destruction of infected cells, and control of inflammatory response (Abbas *et al.*, 1994).

Infection and malnutrition have always been intricately linked and malnutrition has been linked as the primary cause of immunodeficiency worldwide with infants, children, adolescent and the elderly being the most affected group. A strong relationship has always been drawn between malnutrition and infection and infant mortality because poor nutrition leaves children underweight, weak and vulnerable to infections, primarily because the integrity of the epithelial cell is lost as well as inflammation (Keusch, 2003). According to UNICEF, (2006) there are five major infectious diseases; pneumonia, diarrhoea, malaria, measles and AIDS which account for more the one-half of all deaths in children under 5years of age and these infections are mostly linked with poor nutritional status of the children under 5years of age. Keusch (2003), also showed an extensive, synergistic, antagonistic and clinical interactions between malnutrition and infection.

It has been observed by Müller *et al.* (2003) that malnutrition makes individuals more susceptible to infections and infections also contribute to malnutrition thus causing a vicious cycle. An inadequate dietary intake usually brings about weight loss, lowered immunity, mucosal damage, invasion by pathogen and impaired growth and development in children. When an individual is sick, the nutrition condition is further aggravated by conditions such as diarrhoea, malabsorption, loss of appetite, diversion of nutrients for the immune response and urinary loss of nitrogen. All the above mentioned conditions lead to nutrient loss and further damage to the defense mechanism which in turn causes reduce dietary intake. Malnutrition in the form of macronutrient or micronutrients all brings about deficiency diseases such as Kwashiorkor, marasmus, vitamin A deficiency, zinc deficiency etc. and in most of these deficiency diseases, the immune function is impaired and other infectious diseases takes over and this brings to bear the inseparable nature of the infection, malnutrition and the immune system as depicted in the figure 2.2.

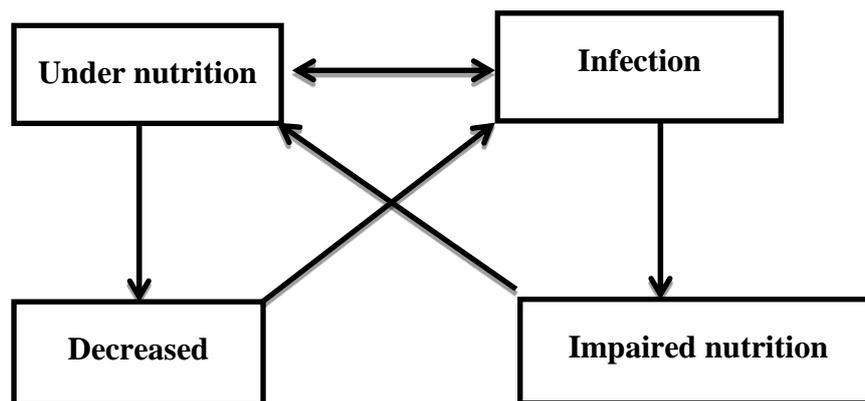


Figure 2.2: Interaction between Malnutrition and Infection

(Katona and Katona-Apte, 2008).

2.3.4 Nutritional Concerns of Cancer Treatment

At the time of presentation, up to as many as 40% of cancer patients are already malnourished, before the onset of any medical or surgical treatment (Shike, 1996). Nutritional decline is often accepted as part of the cancer course and its treatment (Laviano *et al.*, 1996, Kirby and Teran, 1998). Pediatric patients especially those undergoing treatment for cancer, experience nutritional depletion and weight loss. This makes malnutrition an index of research in cancer epidemiological studies (Platek *et al.*, 2011). Bauer *et al.* (2011), also observed that up to 46% of children and young adult with cancer experience malnutrition resulting from tumour and other treatment regimen. Despite the 80% increase in the treatment of childhood cancers over the decade using combined therapy, it is believed that the therapeutic modalities themselves, including chemotherapy, radiation therapy, and surgery, can cause gut malabsorption and derangements of metabolism, which can further worsen a patient's nutrition status (Fearon, 2001).

As observed by Corli *et al.* (1992), the etiology of malnutrition in patients with cancer is multifactorial and these may include the systemic effects of the tumor, the local effects of the tumor, or the side effects of anticancer treatment (Rivadeneira *et al.*, 1998). Systemic effect of the tumor such as anorexia and altered metabolism seen with cachexia, are multiple and differ in type and severity depending on the form of cancer. The local effect of the tumor also brings about malabsorption, obstruction, diarrhoea, and vomiting (Bloch, 1990). Capra *et al.* (2001) also observed that fatigue, depression, anxiety, or pain (resulting from the treatment or the cancer itself can interfere with dietary intake.

2.3.5 Anorexia and Cachexia

Cancer-anorexia– cachexia syndrome remains the most common documented cause of cancer death and is a result of the altered metabolism of fat, carbohydrate, and protein that occurs in patients with cancer (Lefton *et al.*, 2009). Anorexia, or the involuntary decline of food intake, occurs in at least half of newly diagnosed cancer patients (Capra *et al.*, 2001). Cancer cachexia is a complex metabolic syndrome in which patients experience anorexia, early satiety, weakness, anaemia, sarcopenia, and severe weight loss (Herrington *et al.*, 1997). Cancer cachexia also results from inadequate energy intake, increased energy expenditure, altered metabolism, and proinflammatory cytokine-induced abnormalities (Bartlett *et al.*, 1995, Tisdale, 1997, Falconer *et al.*, 1994). Cachexia is detrimental since it results in an accelerated loss of skeletal muscle in relation to adipose tissue due of altered metabolism. However, in simple starvation fat instead of glucose is the preferred fuel to spare lean body mass (Chlebowski *et al.*, 1996, Tisdale, 1997).

Cancer cachexia has been the main cause of poor treatment outcomes in cancer patients especially in children. It affects morbidity, mortality and also leads to lower quality of life and to some extent change in self-image (Grant *et al.*, 1994, Brown and Radke, 1998). Further, a patient with cancer cachexia has a poor response and diminished tolerance to antineoplastic treatments such as chemotherapy, radiotherapy, and surgery (Corli *et al.*, 1992, Laviano *et al.*, 1996, Butters *et al.*, 1996, Rivadeneira *et al.* (1998)) . This has led to an overwhelming evidence that weight loss and malnutrition are adverse prognostic factor in treating patients with cancer especially children.

2.3.6 Cancer Therapy and Nutrition

Chemotherapy is any artificially manufactured drug and could also apply to antimicrobials used as anti-neoplastic agents. It works by a variety of mechanisms to either induce cell death through apoptosis (cytotoxic) or to prevent cell division (cytostatic) and usually given in combinations (McKnight, 2003). The main target of any cytotoxic chemotherapy treatment is rapidly dividing cells, which include normal cells as well as cancer cells and this usually brings about acute and long-term side effects. Normal cells that are mostly affected are cells with a high turnover rate, such as cells in the bone marrow, hair follicles, oral, esophageal, and gastrointestinal mucosa, and reproductive system (Bloch, 1990, Sanford, 2005). The drug type, dosage, treatment duration and frequency as well as individual characteristics determine the side effect observed during chemotherapy. For instance, Shike (1996) and Rivadeneira et al. (1998) observed that antineoplastic agents such as cisplatin, doxorubicin, and fluorouracil can indirectly affect food intake, absorption, or can induce severe gastrointestinal symptoms such as nausea, vomiting, anorexia, abdominal pain, diarrhoea, fever, stomatitis, mucositis, and food aversions. Antineoplastic agents such as dactinomycin, daunorubicin, cisplatin, nitrogen mustard, streptozotocin, mechlorethamine, nitrosoureas, and dacarbazine produce severe nausea and vomiting (Bloch, 1990, Mitchell and Schein, 1982). Also oral antibiotics, which are used frequently by immunosuppressed patients undergoing high-dose chemotherapy, can cause nausea and vomiting (Bloch, 1990). This leads to electrolyte imbalance, dehydration, weight loss, and weakness if nausea and vomiting are not controlled. Conditions such as stomatitis, cheilosis, glossitis, pharyngitis, and esophagitis can occur with chemotherapy and severely affect dietary intake (Mitchell and Schein, 1982). Mucositis is commonly associated with methotrexate, antibiotic

antitumor agents such as actinomycin-D, 5-fluorouracil, and the vinca alkaloids (Donaldson and Lenon, 1979).

When the structure and function of the cell lining the small and large intestines are altered by chemotherapeutic drugs such as cytosine arabinoside and 5-fluorouracil conditions such as diarrhoea, abdominal pain, and protein-losing enteropathy arises (Mitchell and Schein, 1982). Bloch (1990) therefore observed that nutrition supplements may help to maintain gastrointestinal mucosal integrity during chemotherapy and assist with tissue-repair after chemotherapy.

2.3.7 Radiotherapy and its Nutritional Concerns

Radiotherapy has also been one of the major forms of treatments of cancer and it involves the use of radiation. The severity and extent to which patients undergoing radiation treatment experience side effects depend on the tumor and treatment site, duration of treatment, and radiation dose (Capra *et al.*, 2001). Radiotherapy is toxic to tumor cells and normal host cells within the area of treatment. As with chemotherapy, rapidly dividing tissue cells, such as blood cells, hair follicles, and cells lining the gastrointestinal tract, are the most susceptible to radiation damage (Bloch, 1990).

Though the effect of radiotherapy is usually site specific, children undergoing radiotherapy treatments usually suffer from generalized fatigue, anorexia, and emotional stress, which can contribute to decreased food intake (Herrington *et al.*, 1997). Weight loss is found in approximately 90% of patients receiving radiation to the head, neck, or abdomen and this usually leads to poor nutritional status of patients with head-and-neck cancer (Grant *et al.*, 1994).

2.5 SOYBEAN

Soybean (*Glycine max*) is an annual leguminous crop grown to provide food for humans, feed for animals and raw materials for industries. There are many varieties with different growing periods. The matured soybean plant stands up to 60 to 100 cm with the colour of the pods ranging from light yellow to grey, brown or black. Each pod contains two or three seeds which are round or oval in shape. Seed colour may be yellow, green, brown or speckled depending on the variety (Kure *et al.*, 1998).

Soybean is an important source of protein and other nutrients in many developing countries due to its proteins' positive nutritional profile, being nearly equal to casein in biological value, low cost, availability and in Africa, soybeans are used extensively to fortify cereal-based weaning foods (Parman, 1974, Njoki and Faller, 2001). Soybean is the world most valuable and cheapest source of protein compared to other protein-rich foods such as meat, fish and egg and also an important source of human dietary protein with an average of 40% content, 30% carbohydrate and essential fatty acids and oil content of 20% and high content of essential mineral and vitamins (Kure *et al.*, 1998, Adu-Dapaah, 2004, CSIR, 2005). The seeds are on high demand and is estimated to be the world most valuable source of protein. However, soybean contain some level of anti-nutrition factors such as trypsin inhibitors, lipoxidase enzymes, goitrogens and phytate which are all heat labile hence are destroyed through adequate heat treatment and other processing techniques (Liener, 1994). Some heat stable factors such as flatulence factors, saponins and isoflavones are found in soyabean and these have health-promoting properties (Knight and Eden, 1996, Plahar, 2006).

2.5.1 Isoflavanoids in Soybean

Isoflavones are phytochemicals found in a variety of plants including soybean and usually contribute to the many health benefits attributed to the consumption of these foods or their products (Crouse *et al.*, 1999). They are naturally occurring polyphenolic compounds found in the *Fabaceae* family and exist in twelve different chemical forms (Lee *et al.*, 2004, Romani *et al.*, 2003, Barnes, 2010). They usually exist in four forms; the aglycones form (daidzein, glycitein and genistein), the β -glycoside form (daidzin, glycitin and genistin), the 6-O-malonyl glucosides (malonyl daidzin, malonyl glycitin and malonyl genistin) and the 6-O-acetyl glucosides (acetyl daidzin, acetyl glycitin and acetyl genistin) (Lee *et al.*, 2004). The glycosides form are the most predominant in soybean though these are hydrolyzed to aglycones component which is essential for absorption and this makes the aglycones form the biologically active form of isoflavone (Day and Williamson, 2001). Genistein (4',5,7 trihydroxyisoflavone), the most abundant isoflavone found in soybeans, is believed to be a potent anticancer agent (Mills *et al.*, 1989, Knight and Eden, 1996). The content of isoflavones in soybeans ranks 1.2 to 2.4 mg of total isoflavones per gram of sample distributed in different concentrations in the tissues of the seed, being higher in the embryo than in the endosperm (Rostagno *et al.*, 2004, Kim *et al.*, 2004).

2.5.2 Isoflavanoids and Cancers

Epidemiological studies have shown that consumption of soyabean and soy products may reduce the risk of cardiovascular disease, improve bone health, and inhibit the growth of human cancers (Messina *et al.*, 1994). Among Asian populations with a high intake of soy and soy products, extensive epidemiological, *in-vitro*, and animal

data suggests lower incidence of cardiovascular disease (Barač *et al.*, 2005), hormone-dependent cancers of the breast and prostate (Giovannucci, 1995, Zhang *et al.*, 2013).

Genistein which is the predominant isoflavanoid has been found to inhibit cell growth of tumour cell lines from the various malignancies including breast, melanoma, prostate, head and neck squamous cell carcinoma, leukaemia and lymphoma (Sarkar and Li, 2004, Pagliacci *et al.*, 1994, Kyle *et al.*, 1997, Spinozzi *et al.*, 1994, Alhasan *et al.*, 1999). It is believed that these isoflavones exert their anticancer activities by various mechanism such as inhibition of protein tyrosine kinase (Akiyama *et al.*, 1987), inhibition of DNA topoisomerase II (Markovits *et al.*, 1989), inhibition of angiogenesis (Fotsis *et al.*, 1995), antiproliferation and cell arrest (Matsukawa *et al.*, 1993) and induction of apoptosis (Constantinou *et al.*, 1998). The similarities between these oestrogen and isoflavanoids suggests that isoflavanoids exert their anticancer property through inhibition of the oestrogen receptor.

These are nuclear receptors and transcription factors which, when activated by agonists, undergo conformational changes and bind to chromatin (DNA plus protein) in various promoter regions on DNA, activating the transcription of the respective genes. ER α stimulates transcription and cellular proliferation, while ER β inhibits the activation of ER α (Paech *et al.*, 1997). Barkhem *et al.* (1998) found out that Genistein has a 30-fold greater affinity for ER β than for ER α and is a partial agonist.

About 50% of known oncogenes (mutated forms of proto-oncogenes which can overstimulate cell division) code for proteins which catalyse or undergo phosphorylation by tyrosine kinases, and many cancers show increased tyrosine kinase activity. Numerous study have reported tyrosine kinase inhibition by genistein and one way in which genistein inhibits tyrosine kinase activity is by competing with

ATP for the enzyme's catalytic domain (Akiyama *et al.*, 1987). Another way in which genistein can decrease tyrosine kinase activity is by inhibiting the enzymes' transcription (Chen *et al.*, 2003).

CHAPTER THREE

3.0 SUBJECTS, MATERIALS AND METHODS

3.1 PREPARATION, PROXIMATE, MINERAL AND MICROBIAL SAFETY ASSESSMENT OF THE SOYMILK POWDER

3.1.1 PREPARATION OF SOYMILK POWDER

The raw soybean '*Anidaso*' variety was purchased from grains board, Kumasi. This was to ensure the authenticity of the source of soybean as well as the uniformity of the variety. The soymilk powder (SMP) was prepared by an expert who has gained experience in the preparation of soymilk powder for commercial use. The description of the SMP has not been captured in this text since the producer deemed it a trade secret.

3.1.2 PROXIMATE ANALYSIS OF THE SOYMILK POWDER

Proximate compositions of the SMP were determined by the Association of Analytical Communities (AOAC), (2000) standard method. The proximate parameters included moisture content, crude fat content, total ash content, crude protein content, crude fiber content and total carbohydrate. Details of the methodology are captured in the Appendix A.

3.1.3 MINERAL ANALYSIS OF THE SOYMILK POWDER

The soymilk powder was also analyzed for the concentration of Zn and Fe in duplicates and this was done using Atomic Absorption Spectrophotometry (AAS).

3.1.3.1 Principle

Atoms are able to absorb light and the wavelength of absorption is specific to each element. The amount of light absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases, and is proportional to the concentration of absorbing atoms. The relationship between the amount of light absorbed and the concentration of the analyte present in known standards can be used to determine unknown concentrations by measuring the amount of light they absorb.

3.1.3.2 Procedure

One gram of the soymilk powder was placed in a 100 mL volumetric flask, and 10 mL of acid mixture (HNO_3 and HClO_4 (9:4)) was added and the contents were mixed by swirling. The flask was placed on a hot plate in the fume hood and heated; starting at 80–90 °C and then raised to about 150–200 °C. Heating continued until the production of red NO_2 fumes ceased. The contents were further heated until the volume was reduced to 3–4 mL and become colourless, but not dried. After cooling the contents, the volume was made up with the distilled water and filtered through No. 1 filter paper. This filtrate was then used for the zinc and iron estimation. In analyzing the sample, a series of calibration solutions containing known amount of zinc and iron were used to plot a calibration curves and the concentration determined.

3.1.4 MICROBIAL ASSESSMENT OF SAFETY OF THE SOYMILK POWDER

Under aseptic conditions, 25 g of the soymilk powder was transferred into a sterile stomacher bag containing 225 mL phosphate buffered saline and punched for 30 seconds in the stomacher. The 10^{-1} (1:10) dilution was obtained using a sterile pipette to transfer 1 mL of the aliquot into 9 ml of sterile phosphate buffered saline in a

capped dilution bottle and mixed well. Serial dilutions of 10^{-2} and 10^{-3} were subsequently prepared. Inoculation was done by transferring 1 mL of each dilution into a petri dish with 20 mL of the appropriate agar (Oxoid Limited, United Kingdom) prepared according to manufacturer's manual (Vanderzant and Splittstosser, 1992). The petri dish contents were mixed thoroughly by swirling and allowed to set. Plates were then inverted and incubated at 27°C for 5 days. Incubated plates were examined for the presence of moulds, yeasts colonies or total plate count which was done using a colony counter. The results were recorded as the number of colonies formed per gram and the microbial value obtained by multiplying the number of colonies at each dilution by the dilution factor and the results recorded.

3.2 BASELINE CROSS SECTIONAL STUDY

3.2.1 Study design

A Prospective study (Non-randomized controlled trial) was conducted between July 2011 and June 2013 at the Pediatric Oncology Unit (POU), Komfo Anokye Teaching Hospital, and (KATH) Ghana. The baseline was cross-sectional study to compare the nutritional status of children with cancer and their apparently healthy non-cancer counterparts.

3.2.1.1 Study site

The study site was Komfo Anokye Teaching Hospital (KATH), Pediatric Oncology Unit (POU) where children with cancer were recruited and Bonwire a village located 20 km north east of Kumasi where the control children were recruited.

3.2.1.2 Ethical approval

Ethical approval for the study was obtained from the Committee on Human Research, Publications and Ethics of KNUST and KATH with the approval ID (CHRPE/ KNUST/ KATH/ 17/11).

3.2.1.3 Sample size calculation

The sample size (n=96) was calculated using the formula below

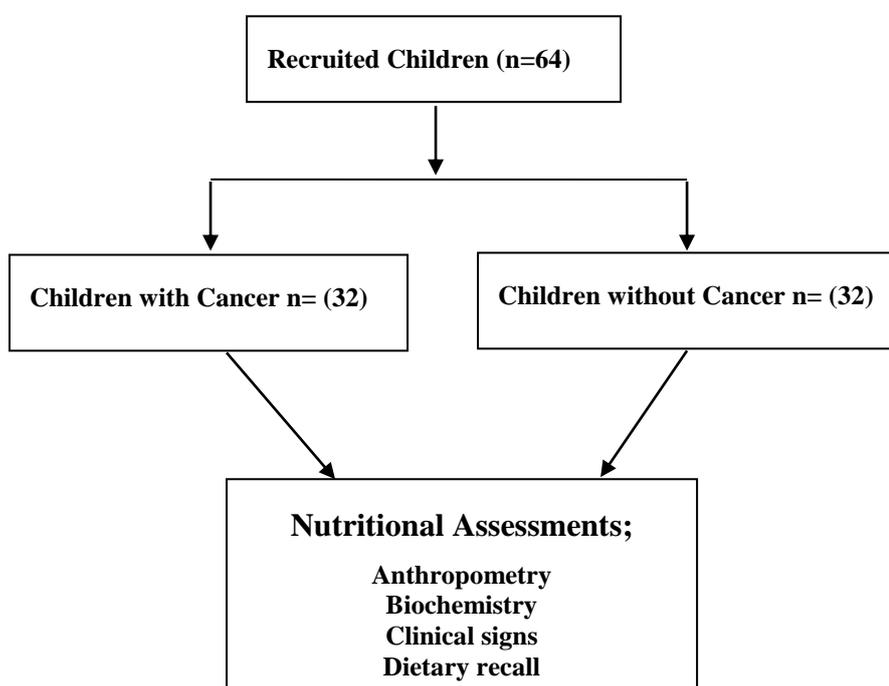
$$n = 2SD^2 \frac{(Z_{\beta} + Z_{\alpha/2})^2}{(d)^2} \quad (\text{Charan and Biswas, 2013})$$

For 80% power, $Z_{\beta} = 0.84$ (Desired power)

For 0.05 significance level, $Z_{\alpha} = 1.96$ (Desired level of statistical significance)

Difference = 10.0 (Effect size or difference in means of BMI between cancer and control group).

3.2.1.4 Flow chart for Cross-sectional study



3.2.2 SUBJECTS

3.2.2.1 Inclusion criteria

The eligible participants were children who were clinically/ histological diagnosed of Burkitt's lymphoma (BL), Wilms Tumour (WT) by a physician and were without any complications. They were also commencing chemotherapy treatment at the time of recruitment without any nutritional supplement. For the control group children whose parents gave consent and fell within the age group 1-15 yrs were recruited.

3.2.2.2 Exclusion criteria

Children were excluded from selection if the parents refused to give consent, or if the cancer fell out of the two groups of the childhood cancers used for the study. Also for the control group children who have had no history of any childhood cancer were recruited.

3.2.2.3 Study population and Recruitment

Cancer group

The study population was hospital out-patient children undergoing chemotherapy at the Paediatric Oncology Unit (POU) of the Komfo Anokye Teaching Hospital (KATH). Children between the ages of 1 and 15 years were recruited for the study since this is the age range for children with this condition and they formed the experimental group. Recruitment was done between June, 2012 to July, 2013. These were children who had a clinical or histological diagnosis of Wilms tumour or Burkitt's lymphoma. Parental consent was sought from parents of participants after the purpose of research was explained to them. The parents filled consent form and the children also filled the participant's information form. Assent by children who

were old enough to express themselves were also obtained for those who accepted to be recruited. Once recruited, data on the study subject as well as their family and household were obtained from the parents. A structured pretested questionnaire was used to collect socio-demographic information on each child and his/her parent. Other information collected includes dietary assessment, anthropometric measurements, blood samples for biochemical analyses for specific nutrients and physical examination for signs of malnutrition.

Control Group

The control group were recruited from Bonwire Assemblies of God church. This is a farming community on the outskirts of Kumasi metropolis and it was selected since most of the children in the cancer were found to be coming from rural farming communities where conditions would be similar or close to that of the control group community. A church based selection was done to get children within all age and sex group. In collaboration with the church elders and opinion leaders of the community the purpose of the study was explained to them in language they understood. Children between the age of 1 and 15 who had no medical history of any childhood cancer were recruited to serve as a control group for the study.

Consent was sought from parents whose children agreed to participate in the studies as well as assent from the children who could express themselves. Anthropometric measurements and blood samples for biochemical analysis were collected from all participants. All biochemical analyses were performed without knowledge of subject's clinical status by means of code numbering. Clinical signs of malnutrition as well as dietary recall were taken from these children with the help of their parents.

3.2.3 NUTRITIONAL ASSESSMENTS

3.2.3.1 Anthropometric assessments

Anthropometric measurements were taken in duplicates by trained personnel at baseline, 3 months and 6 months afterwards and this included height to the nearest centimeter without shoes and weight to the nearest 0.1 kg in light clothing. Height measurements without shoes on as described by (Cogill, 2003) were done using a wooden minimeter attached to a straight wall calibrated to the nearest 0.1 cm. Children less than two years were made to lie down (recumbent) and their length was measured. Subjects were weighed on a sultan scale (BR9012, Zhongshan Camry Electronic Co. Ltd, Guangdong, China). Weight and height was measured twice and the mean was recorded on each occasion. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m²).

In the measurement of the mid upper arm circumference, the children were made to sit on a chair and for the younger ones on their mothers lap. The midpoint of the child's left upper arm was located. This was done by locating the tip of the child's shoulder and the elbow when placed at a right angle. The length between the tip of the shoulder and the elbow was measured to the nearest centimetre and then divided by two to estimate the mid-point. The child's arm was then straightened and the tape was wrapped around the arm at the midpoint initially located, the mid upper arm was then recorded to the nearest centimetres in duplicates (WHO, 2005).

The triceps skin fold was measured with the children upright and weight evenly distributed on both feet, the shoulders relaxed, and the arms hanging loosely at the sides. Using the thumb and index finger, a fold of the skin and subcutaneous adipose tissue was grasped at 2.0 cm above the mid-arm circumference mark; the tip of the

jaw of the caliper was placed over the complete skinfold. The caliper handle was then released to exert full tension on the skinfold and measurement taken after 3 seconds when the needle on the caliper dial had settled. The thickness of the skin fold was read to the nearest 0.1 mm (Lohman *et al.*, 1988).



A

B

Figure 3.2 Measurement of Tricep Skin Fold (A) and MUAC (B)

Muscle arm circumference (MAC) was also calculated using the formula:

$$\text{MAC} = \text{MUAC} 0.1 (3.14 \times \text{TSF}) \text{ (Symreng, 1982).}$$

3.2.3.2 Biochemical assessments

Venous blood samples (5 mL from each child) were collected and dispensed into vacutainer ® plain tubes with 1 mL dispensed into EDTA anticoagulated tubes. The samples in the vacutainer plain tube were centrifuged at 5000 rpm for 15 minutes. The serum was used for other biochemical assays (zinc, pre-albumin and glutathione). The anticoagulated whole blood was used for the Hb assay. The haemoglobin assay was performed at the clinical analysis laboratory of the Department of Biochemistry and Biotechnology-KNUST whiles the zinc, pre-albumin and the glutathione assay was done at the Kumasi Centre for Collaborative Research (KCCR)-KNUST.

3.2.3.2.1 Blood haemoglobin assay

Principle

Blood is diluted in a solution containing sodium bicarbonate, potassium cyanide and potassium ferri-cyanide (Drabkin's solution). Hb is oxidized to methaemoglobin by potassium ferri-cyanide, and methaemoglobin in turn combines with potassium cyanide to form cyanmethaemoglobin. The absorbance of the solution is measured in a spectrophotometer at wave length 546 nm against Drabkin's solution as a blank.

Procedure

In the Hb determination, 20 μ L of anticoagulated whole blood was pipetted in 5 mL of Drabkin's solution. This was mixed uniformly and incubated for 15 mins at room temperature. After which, the absorbance of standard and samples were measured against reagent blank at 546 nm using Humalyzer Junior (United Kingdom) and calculations were done as follows.

Calculation:

$$\text{Hb (g/dl)} = \frac{\text{Absorption of sample}}{\text{Absorption of Standard}} \times \text{Concentration of standard}$$

3.2.3.2.2 Pre-albumin Assay

Principle

Prealbumin Human (Enzyme-Linked Immunosorbent Assay) ELISA kit is designed for detection of human prealbumin in plasma, serum, urine and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures prealbumin in 3.5 hours. A polyclonal antibody specific for prealbumin has been pre-coated onto a microplate. Prealbumin in standards and

samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for prealbumin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The colour development is stopped and the intensity of the colour is measured.

Preparation of Reagents

All reagents were stored at -20°C prior to the use at room temperature. Any crystals formed in the concentrate, were mixed gently until the crystals have completely dissolved. The diluent was dilute to 1:10 with reagent grade water. The biotinylated human prealbumin antibody was prepared by diluting 60 μL of the antibody with 5,940 μL of the diluent. The standard was also prepared by adding 2000 μL of diluent to 250 ng of lyophilized human prealbumin to generate a stock solution of 125 ng/mL. Triplicate standard points were generated by diluting the standard stock (125 ng/mL) 1:4 with diluent to produce the standard solution of 31.25, 7.82, 1.95, 0.49, 0.12, 0.03 ng/mL. Diluent was used as the zero standard (0 ng/mL). The wash buffer concentrate was also diluted with reagent grade water in the ratio of 1:20. The Streptavidin-Peroxidase Conjugate (SP conjugate) was also diluted with the diluent in a ratio of 1:100.

Procedure

All reagents, working standards and samples were prepared as stated above after they have been brought to room temperature. 50 μL of sample and standard were added to each well in duplicate in a 96 well microplate. The wells were then covered with a sealing tape and incubated for two hours at room temperature. The plate was then washed five times with a 200 μL of wash buffer and in each washing the plate was

inverted to decant its content and then tapped 4-5 times on adsorbent paper towel to completely remove the liquid. 50 μL of biotinylated human prealbumin antibody was added to each well and incubated for one hour. The plate was washed again as described above. Then, 50 μL of Streptavidin-Peroxidase Conjugate (SP conjugate) was added to each well and incubated for 30 minutes again. The microplate was re-washed and thereafter 50 μL of chromogen was added to each well and incubated for 10 mins for the optimal blue colour density to develop. This was then followed by the addition of 50 μL of the stop solution to each well for to change from blue to yellow. The Optical density (OD) was measured at 450 nm using the Sunrise Tecan Elisa (Austria) concentration computed.

3.2.3.2.3 Zinc Assay

Principle

At room temperature, zinc ion in sample combines with (2-(5-Bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl) amino] phenol, disodium salt, dihydrate) 5-Br PAPS in reagent to produce coloured complex. In the range of $\leq 400\mu\text{g/dL}$, its color intensity appears direct proportion with zinc ion concentration.

Preparation of Reagents

The zinc reaction mix was prepared by adding 4 parts of zinc reagent 1 to 1 part of zinc reagent 2. In the preparation of the standard, 10 μL of the 100 mM zinc standard was added to 990 μL of distilled water and mixed properly to generate a stock of 0.5 mM. Then, 0, 2, 4, 6, 8 and 10 μL of standard prepared above was pipetted into a series of well in duplicates. The volumes were then adjusted to 50 μL with distilled water to generate a concentration of 0, 1, 2, 3, 4, 5 nmol/well zinc standard.

Procedure

In the sample preparation, 50 μL of the sample was added to 50 μL of Trichloroacetic acid (TCA) and the mixture was centrifuged at 14000 rpm for 5 mins using the eppendorf centrifuge 5415 C. The clear supernatant was then taken. The sample was then diluted by adding 20 μL of the sample to 30 μL of distilled water (dilution factor of 2.5: 1.25) and then carefully pipetted into each well of the 96 well plate in duplicate. Then, 200 μL of the zinc reaction mixture was added to each well and incubated at 10 mins at room temperature. The optical density was measured at 560 nm using the Spectra Max 190 micro plate reader (USA). The actual concentration of zinc sample was then extrapolated from the standard curve drawn.

Calculation

$$C = S_a / S_v \quad \text{nmol/ } \mu\text{L} \text{ or mM}$$

Where S_a is the sample amount (In nmole) from the standard curve

S_v is the sample volume (μL) added to the wells.

3.2.3.2.4 Glutathione Assay

Principle

QuantiChrom Glutathione Assay Kit has been designed to accurately measure reduced glutathione in biological samples. The improved 5, 5'-dithiobis -2-nitrobenzoic acid (DTNB) method combines deproteination and detection into one reagent. DTNB reacts with reduced glutathione to form a yellow product. The optical density, measured at 412 nm, is directly proportional to the glutathione concentration in the sample (Hu *et al.*, 2003).

Preparation of Reagents

The sample was prepared by adding 240 μL of serum to 240 μL of reagent A and the tubes were vortex to aid proper mixing of the content. This was then centrifuge at 14000 rpm (Eppendorf centrifuge 5415 C, Germany) for 5 mins and the supernatant was then used for the assay. The standard was also prepared by adding 800 μL of distilled water to 400 μL of calibrator (100 μM glutathione) to obtain an initial concentration of 33.33 μM . The solution was serially diluted by adding half of the volume of the calibrator to an equal amount of distilled water to obtain concentrations of 16.65, 8.32, 4.16, 2.08, 1.04, 0.58 and a blank of 0 μM .

Procedure

Two hundred micro litres of the supernatant from the serum and the reagent. A mixture was pipetted into each well of the 96 well plate in duplicate. After, 100 μL of Reagent B was added to both the sample and standard and tapped to mix properly. This was incubated at room temperature for 25 mins and the optical density read at 412 nm using Spectra Max 190 micro plate reader (USA). The actual concentration was determined.

3.2.3.3 Clinical Assessment of Malnutrition

Respondents were asked through their parents if they have been sick for the past month apart from the cancer condition. The occurrence of vomiting which is one of the common side effects of the chemotherapy was also enquired. It usually happens within 24 hrs of the chemotherapy. Therefore any other vomiting after 24 hrs could not be attributed to the chemotherapy. Diarrhoea which is another side effect of chemotherapy and has nutritional implication was investigated. Intestinal obstruction which is a partial or complete blockage of the bowel and usually said to prevent the

content of the intestine from passing through was also investigated. This was done by physical examination for bloatedness, tenderness and hernia in the abdomen. Children were also asked about the level of appetite. Other clinical signs of malnutrition were also assessed. This included wasting, oedema, dermatitis, apathy and anemia. Wasting is the gradual atrophy (loss) of body tissue associated malnutrition or chronic illness. Oedema, also termed dropsy, is the retention of fluid in the body and has been found as one of the symptoms in Kwashiorkor in children i.e. dietary protein deficiency. Dermatitis which is also one of the symptoms of protein energy malnutrition was looked for.

3.2.3.4 Dietary assessment

Dietary assessment was done using food frequency questionnaire and repeated 24 hour dietary recall. The food frequency asked how often common local food items were consumed from a fixed set of responses, ranging from never or less than once a month to more than 2 times per day. A factor was then applied to each response and to produce an estimated mean daily frequency of intake of each item. The 24-hour dietary recall was done by recording food intake of subjects for 2 weekdays and a weekend as recommended by (Jackson *et al.*, 2008). This was done nonconsecutive during the visit to hospital for review and the recall was done with the help of the parents or children and in some cases both. However, for the control group, their recall was done from their respective homes by the researcher. The 24-hour dietary recall was done for three days (2 weekdays and a weekend) each time point. Using the weights/handy measures of the Dietetic group of the Dreyfus Health Foundation, Ghana (1998) the respective masses of the food taken by subjects were recorded after identifying with a measure. Composite foods such as stew, soups, drinks whose weights/handy measures were not found in the handy measure tables were weighed

using the electronic balance (Camry, China, Max weight 20 kg). The mean mass of the food intakes were analyzed into their nutrient components using food composition table. The average nutrient intake for the 3 days recall were then calculated.

3.3 INTERVENTION STUDY

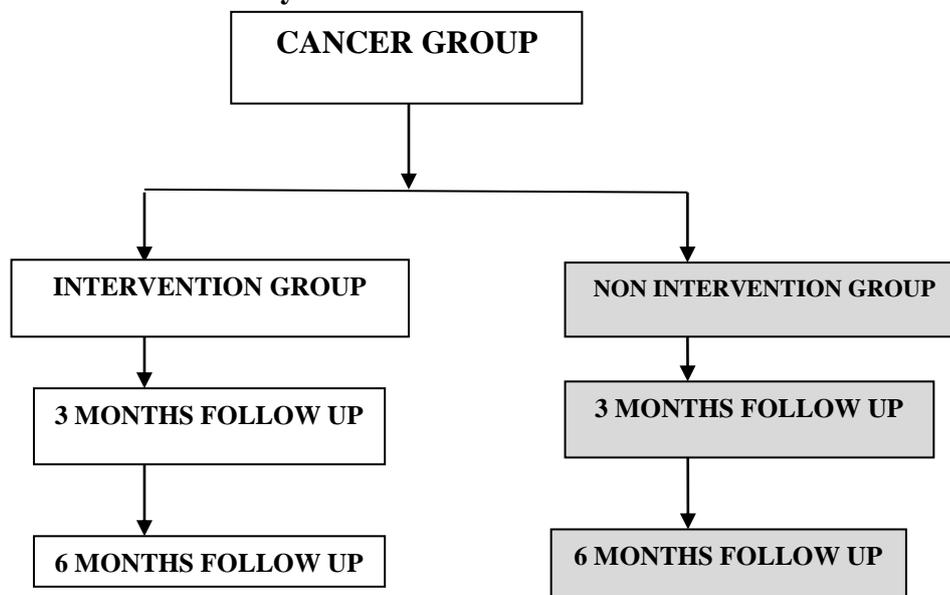
3.3.1 Study design

This was done to investigate the impact of a Soymilk powder (SMP) on nutritional status of children undergoing chemotherapy due to cancer. The nutritional status of children with cancer undergoing chemotherapy with SMP intervention and those with cancer undergoing chemotherapy without SMP intervention was investigated. A Prospective study (Non-randomized controlled trial) was used.

3.3.2 Study Site

The study site was the Pediatric Oncology Unit (POU) of the Komfo Anokye Teaching Hospital, Kumasi-Ghana.

3.3.3 Flow Chart of Study



3.3.4 Subjects

The subject used for the intervention studies were children who were clinically/histological diagnosed of either Burkitt's Lymphoma or Wilm's tumour by a physician and in this study, children whose nutritional status were determined at the baseline cross sectional study were used as the interventional group. These children were given soymilk powder supplement and then followed-up for six months. For the non-intervention group, another 32 children were recruited and their nutrition status also followed for six months to compare with the 32 in the intervention group.

3.3.4.1 Inclusion Criteria

The eligible participants were children who were clinically/ histological diagnosed of Burkitt's lymphoma (BL), Wilms Tumour (WT) by a physician

3.3.4.2 Exclusion Criteria

The exclusion criteria were any of the following: any participant whose parents refused to give consent, any participant whose disease condition fell out of the three groups of the childhood cancers used for the study, patients who were allergic to soybean, and patients who were either not allowed to take in any food or water or both due to their medical condition.

3.3.5 Study population and Recruitment

In all 64 children were recruited for the interventional studies, 32 children from the baseline cross sectional studies who had cancer and were undergoing chemotherapy and 32 children with cancer undergoing chemotherapy but without soymilk powder intervention. By default all the 32 children with cancer whose nutritional status were determined at the baseline cross sectional study were recruited for the interventional

group. The other 32 children without the soymilk intervention were recruited after the intervention. The recruitment period was between July, 2012 to July, 2013 for the interventional group and March, 2013 to June, 2014 for the non-interventional group. This mode of recruitment was necessitated by the fact that the children with cancer were mostly visiting the hospital on out-patient basis and also the cases were not forthcoming. Therefore, once a child was recruited for the cross sectional studies, baseline nutritional markers were taken and then were given soymilk powder to begin the intervention. The recruitment of the non-interventional group followed up after the each child in the interventional group have been followed-up for 6 months from the time of recruitment.

3.3.5.1 Cancer Patients with Soymilk powder (SMP) Intervention

For the intervention group, once recruited and baseline nutritional status determined, a well packaged soymilk powder was given to the parents after educating them on the need to give the food samples to their wards. They were also educated extensively on how to administer the food sample.

The SMP was packaged as 500 g pack with a caloric value of 397.1 kcal per 100 g. In terms of nutritional value, each pack contained the following; fat per 100 g was 6.3 g, carbohydrate per 100 g was 35.8, Dietary fibre 2.1 g per 100 g, ash 4.7 g per 100 g and protein 49.3 g per 100 gram. Two pack was given as 2-weekly rations since most of them usually came for review after two weeks and the amount to take by each child was calculated based on the age and gender of the child to provide 80% RDA for protein. It was to be added to liquid breakfast such as porridge and to be consumed by the child alone. In cases where the next review date exceeded two weeks, an additional one or two was added depending on the next review date. All participants

were contacted on phone twice (a weekday and a weekend) to monitor their progress and compliance. To follow up on compliance, parents were asked if they had given the SMP to their children whenever they came for review before the next package was given. A record book was also provided at the ward to aid nurses on duty when to give participants packages of soybean when they were in for review. The record book also contained the date of recruitment, date for 3 month follow up and that for 6 months follow up. This made it easier to follow up on participants as well as to reduce the incidence of drop outs of the study since participants were recruited at different periods. After the 3 months, dietary assessment, anthropometric measurements, blood samples for biochemical analyses for specific nutrients and physical examination for signs of malnutrition were taken. This was repeated sixth months from the time of recruiting each participant.

3.3.5.2 Cancer patients without Soymilk powder (SMP) Intervention

Children in this group also had BL or WT and were undergoing chemotherapy at the POU of the KATH. However, they were not given any soymilk powder as intervention. They were recruited in the same way as that of the intervention group at different points after the intervention study. Their baseline nutritional markers were taken and each child was followed at the third and the sixth months from the date of recruitment.

3.3.6 Secondary outcomes

Death and recovery which were the secondary outcome of the study apart from nutritional status was also investigated one year from the time of recruiting each child. All recruited children were therefore classified into 3 major groups (recovered, not recovered and died).

3.4 CANCER ANTI-PROLIFERATIVE STUDY.

3.4.1 Extraction of Isoflavones from Soybean (SB) and Soymilk powder (SMP).

Isoflavones were isolated from SB and SMP by the method described by Zhang *et al.* (2007) with slight modifications. This was done by weighing 30 g of SB and SMP and mixed with 300 ml of 70% ethanol (1:10). The resulting mixture was heated to 50⁰C for 24 h using a shaking water bath (Clifton Shaking Bath, England). The extract was then separated from insoluble fractions by centrifugation at 2000 rpm (Mistral 3000 E UK) for 15 mins. The extract (filtrate) was hydrolyzed with 37% hydrochloric acid and the pH of resulting mixture was adjusted to 1.5. The mixture was heated to 50⁰C for 12 h on the Clifton shaking bath. The hydrolyzed product was then mixed with distilled water at the volume ratio of 1:5 (v/v) and stirred constantly at room temperature to precipitate isoflavone crystals which were separated by centrifuging at 2000 rpm for 1 hour (Mistral 3000 E UK). The solid crystals deposited were then collected and stored at 4⁰C for the HPLC and anti-proliferative assay.

3.4.2 HPLC Analysis

Isoflavone contents in the extract isolated from SMP and SB was analyzed using a reverse-phase C18 column (VP-ODS, 5 µm, 150×4.6 mm i.d.) on the Shimadzu Prominence liquid chromatography: LC -20AB Pump, DGU-20A3 Degasser, CTO-19ASVP Oven column, SIL-ACHT Autosampler, and SPD-M20A PDA Detector. The sample injection volume of 20 µL was used. The mobile phase of 0.1% phosphoric acid (A) and methanol (B) was used. A linear gradient elution from 10% to 90% B starting from 0 to 30 mins at a flow rate of 1.0 mL/min was employed. The temperature of the column was maintained at 40 °C, and the detection wavelength was set at 300 nm. The identification and quantification of the peaks were done by

comparing the retention times and peak areas with the two standards; genistein and daidzein.

Table 3.1 HPLC Solvent Gradient with time

Time(Min)	%B	%A
0.01	10	90
10	25	75
15	50	50
20	90	10
25	90	10
28	10	90
30	10	90

3.4.3 MTT Assay

3.4.3.1 Reagents and cancer cell lines

All reagents used for extraction such as ethanol, petroleum ether, chloroform, ethyl acetate and methanol were of analytical grade. Rosewell Park Memorial Institute (RPMI)-1640 culture medium, fetal Bovine serum (FBS), penicillin streptomycin L-glutamine (PSG), curcumin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich company limited, UK. The T-Lymphoblast-like leukemia cell line (CEM) and normal prostate cell (PNT2) were obtained from the Clinical Pathology Department, Noguchi Memorial Institute for Medical Research (NMIMR), UG, Accra while the Lymphoma cell line (DG 75) was kindly provided by Keith Anderson (George Klein group, Department of Tumour biology, Karolinska Institute, Stockholm, Sweden).

3.4.3.2 Cell culture

Cells were cultured as described by Ham et al. (2012) with slight modifications. The CEM, DG 75 and PNT2 cells were cultured in RPMI 1640 medium. All culture media were supplemented with 1% PSG and 10% FBS. The cells were maintained in an

incubator with 5% CO₂ concentration at 37°C and passaged on reaching about 90% confluence.

3.4.3.3 Principle

The assay is based on the capacity of the cellular mitochondrial reductase enzyme in living cells to reduce the yellow water-soluble substrate 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a purple formazan crystals which is soluble in acidified isopropanol. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The colour change from yellow to purple can be measured at 570 nm using a spectrophotometer.

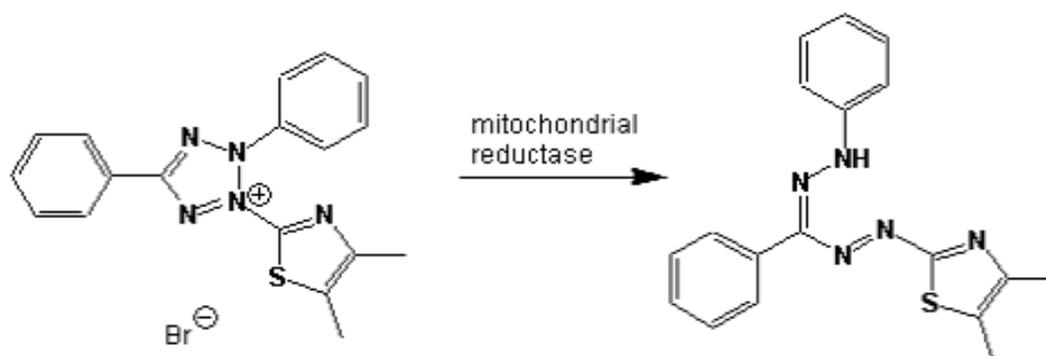


Fig. 3.3 Reduction of Tetrazolium bromide into a purple formazan crystals by mitochondrial reductase

3.4.3.4 Cell viability Assay

The tetrazolium-based colorimetric assay (MTT) was adopted for the measurement of cell growth and viability of daidzein, genistein, SB and SMP on the cancer and normal cell lines. This was done by adopting the method proposed by Ayisi *et al.* (2011).

For the SB and SMP, dilution of the 50 mg/mL in 50% ethanol of stock extract was made and 20 μ L of this sample was mixed with 80 μ L of medium to obtain 10 mg/mL concentration. Subsequently, 50 μ L of the 10 mg/mL was added to 50 μ L of medium and 10% ethanol to obtain 5 mg/mL concentration. Further serial dilution was done to obtain concentrations of 2.5 mg/mL, 1.25 mg/mL and 0.625 mg/mL. The two isoflavanoid standard were also prepared by taking 10 μ L of 10 mM of either genistein or daidzein and adding it to 90 μ L of media and 10% DMSO to obtain 1 mM concentration. Further serial dilution was done to obtain concentrations of 0.5 mM, 0.25 mM, 0.125 mM and 0.0625 mM. A hemocytometer was used to count the viable cells and the cell suspension was diluted with a media containing 5% FBS in order to obtain final density of 1×10^5 cells/mL. 96-well plates at plating density of 10,000 cells/well were seeded with 100 μ L per well of cell suspension and incubated for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. The cells were incubated for 24 hrs for all of them to attach. After this, 10 μ L of each extract and standard dilutions with 1% DMSO were added to the cells in triplicate. This was also followed by 72 hours (3 days) of incubation. Curcumin was used as a positive control in all assays. Afterwards, a volume of 20 μ L of 2.5 mg/mL MTT solution was added to each well on the 96-well plate and incubated in a humidified 5% CO₂ incubator at 37°C for further four hours. Acidified isopropanol, 150 μ L, was added to each well to stop the reaction and then incubated in the dark at room temperature overnight, before reading the absorbance at 570 nm using microplate spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria). A colour control plate was also setup for each extract including the positive control, curcumin. This was made up of 10 μ L of sample in 100 μ L of media. The plate was incubated as

describe above and absorbance read at 570 nm using microplate spectrophotometer. The percentage cell viability was determined from the formula;

$$\% \text{ Cell Viability} = \frac{A_0 - A_1}{A_0} \times 100\%$$

The mean cell percentage viability obtained from triplicate determinations at each concentration was plotted as a dose response curve using Microsoft Excel and the inhibition concentration at 50% (IC₅₀) values, that is, concentration of isoflavones in extracts (SB and SMP) or standard (Daidzein and Genistein) inducing 50% inhibition of cancer cells, determined from the dose response curve by nonlinear regression analysis.

The selectivity index (SI) was determined using the formula below. Samples with an SI greater than 2 were considered to have a high selectivity towards cancerous cells.

$$SI = \frac{\text{IC}_{50} \text{ of the extract on normal human cells}}{\text{IC}_{50} \text{ of the extract on cancer cells}}$$

3.5 STATISTICAL ANALYSIS

Data was entered in Microsoft office 2010 excel spread sheet and was also used in drawing the percentage inhibitory curves. Normality of all continuous variables was tested. All nonparametric variables were normalized by log transformation before analysis and results converted by antilog where appropriate. Continuous variables were expressed as their mean \pm SEM, whereas categorical variables were expressed as proportion. Comparisons of the general characteristics of the cancer group against the non-cancer group were performed using unpaired t tests, chi (χ^2) tests, or Fisher exact tests where appropriate. Comparison of mean parameters within groups was also

done with repeated measure ANOVA while between groups was done with one way ANOVA. For comparing difference between intervention and non-intervention parameters at baseline, 3-month and 6-month, paired t-test was used. GraphPad Prism version 5.00 for windows was used for these statistical analyses (GraphPad software, San Diego California USA, www.graphpad.com). For the anthropometric analysis, WHO Anthro software v3.2.2 and WHO AnthroPlus v 1.0.4 were used for the analyses. The prevalence of the various parameters was determined using their various cut-off points. The relationship between anthropometric and biochemical measures were investigated using age and gender-adjusted Pearson correlations. Correlation was significant at 0.001 and 0.005 (2-tailed). This analysis was performed by using SPSS for windows version 20. As a secondary analysis, the discriminative abilities of anthropometric and biochemical markers for identifying malnutrition in cancer was done by means of ROC curve analysis. The ROC area under the curve (AUC) estimated the discriminative capabilities of those anthropometric and biochemical measures associated with malnutrition in cancer. This analysis was done using Medcalc version 12.1.4.0, www.medcalc.org. A p-value < 0.05 was used to ascertain statistical significance.

CHAPTER FOUR

4.0 RESULTS

4.1 NUTRIENT, MICROBIAL AND QUALITY ASSESMENT OF SOYMILK POWDER

The results of the proximate, microbial, and mineral analysis of the soymilk powder (SMP) is presented in Tables 4.1.1, 4.1.2 and 4.1.3.

4.1.1 Proximate Analysis of soymilk powder

Table 4.1.1 indicates the results of proximate analysis of the soymilk powder used as supplement. Analysis was done to determine the proximate composition of moisture, crude fat, crude protein, total ash, crude fibre and carbohydrate. The crude protein content was found to be highest (49.62%) and crude fibre the least (2.08%). About a third of the SMP was carbohydrate (34%).

Table 4.1.1. Proximate Composition (%) of Soymilk powder

COMPONENT	% WEIGHT
Moisture	3.27
Crude Protein	49.62
Total Ash	4.68
Crude fat	6.22
Crude Fiber	2.08
Carbohydrate	34.13

4.1.2 Mineral Analysis of Soymilk powder

Table 4.1.2 indicates the zinc and iron concentration of the soymilk powder. The mean concentration of zinc was 0.24 ± 0.012 mg/g dry weight while that of iron was 0.15 ± 0.010 mg/g of dry weight.

Table 4.1.2 Zinc and Iron composition of the Soymilk powder

Component	Concentration (mg/g)
Zinc	0.24±0.012
Iron	0.15±0.010

4.1.3 Microbial Analysis of Soymilk powder

This was done to assess the microbial safety of the soymilk powder and the investigation was done to detect the presence and quantify the amount of mould, yeast, *E. coli*, coliform salmonella. The results are presented in Table 4.1.3. There was no *E. coli*, coliform or *Salmonella* found in the sample. The load of mould enumerated was 1.2×10^2 cfu/g, yeast was 1.1×10^2 cfu/g and total plate count 2.4×10^2 cfu/g. For presence of mould and yeast the acceptable load is <1000 cfu/g. The coliform present should be < 100 cfu/ g to be within acceptable range. That of *E. coli* is 3 MPN/g while standard plate count should be < 100,000 cfu/g.

Table 4.1-3 Microbial load assessment of Soymilk powder

Test	Microbial Load (cfu/g)
Presence of Mould	1.2×10^2
Presence of Yeast	1.1×10^2
Total Plate Count	2.4×10^2
<i>E. coli</i>	None Seen
Coliform Test	None Seen
Presence of <i>Salmonella</i>	None Seen

4.2 BASELINE CROSS SECTIONAL STUDY**4.2.1 Basic socio-demographic characteristics of the baseline study population.**

The demographic characteristics of children recruited for the baseline study is shown in Table 4.2-1 with that of their parents. The mean age of children used for the study was 6.3 ± 3.1 years with the cancer group having the same mean age of 6.3 ± 3.2 . In terms of disease segregation, abdominal Burkitt's was the highest (46.9%) followed by jaw Burkitt's (34.4%) and Wilm's tumour (18.8%). Majority of the children recruited for

this study also resided in rural areas (81.3%) compared to their urban counterparts (18.7%). A smaller percentage (12.5%) of the study population were of the Islamic religion with the rest being of the Christian faith (87.5%). Some biodata of parents of these children were also collected and a large number (84.4%) of parents were married with those of the control constituting the largest number of 90.6%. Two (6.3%) of the parents of children with cancer were divorced. A large population of (60.4%) of these parents have had only basic education which includes primary and junior high school. About 32.3% of the parents had no formal education at all with just 7.3% attaining secondary education. The children were classified into 4 major ethnic groups Akans, Ewes, Ga-Adangme and the Mole-Dagbani. Under this classification, the Akan formed the largest (73.4%) ethnic group followed by the Mole-Dagbani (18.8%). Only 7.8% were Ewes with no Ga-Adangme. The study also looked at the occupation of the parents and this was classified under three major groups; farming, Trading, Others. The others group captured occupation such as hairdressing, welding, tailoring, Mason, teaching and journalism. From Table 4.2-1, 39.1% of these parents were farmers followed by (37.5%) being traders and 23.4% in the other category explained above.

Table 4.2-1: Demographic of the Children recruited for the baseline study

Parameters	Cancer	Control	p-Value
Childs Biodata			
n	32(100)	32(100)	NA
Age	6.3±3.2	6.3±3.2	0.843
Gender			
Male	19 (59.4)	19(59.4)	1
Female	13(40.6)	13(40.6)	
Type of Diseases			
Jaw Burkitt's	11(34.4)	NA	NA
Abdominal Burkitt's	15(46.9)	NA	
Wilms Tumour	6(18.8)	NA	
Location			
Rural	20(62.5)	32(100.0)	0.0001
Urban	12(37.5)	0(0.0)	
Religion			
Islam	8(25.0)	0(0.0)	0.005
Christian	24(75.0)	32(100.0)	
Parents Biodata			
Marital Status			
Married	25(78.1)	29(90.6)	0.329
Single	4(12.5)	3(9.4)	
Widowed	1(3.1)	0(0.0)	
Divorced	2(6.3)	0(0.0)	
Educational Level			
None	14(43.8)	7(21.9)	0.305
Basic	17(53.1)	21(65.6)	
Secondary	1(3.1)	4(12.5)	
Tribe			
Akan	18(56.2)	29(90.6)	0.001
Ewe	2(6.3)	3(9.4)	
Mole-Dagbani	12(37.5)	0(0.0)	
Occupation			
Farming	17(53.1)	8(25.0)	0.069
Trading	9(28.1)	15(46.9)	
Others	6(18.8)	9(28.1)	

Continuous data were presented as mean ± standard error of mean (SEM). Categorical data are presented as frequency with percentages in parenthesis. Continuous data were compared using t-test whilst categorical data were compared using Chi-square or Fischer's exact test where appropriate. Other in occupation includes Hairdressing, Tailoring, Mason, and Welder.

4.2.2 Anthropometric and Biochemical Characteristics of Intervention, Non-Intervention and Control children at Baseline

Table 4.2-2 compared the anthropometry and biochemical parameters of the cancer and the control (non-cancer) groups of children recruited for the baseline study. The mean anthropometry as well as biochemical parameters for the cancer and control group have been represented in the Table 4.2-2. For the anthropometry, the highest mean height of 118.40 ± 3.81 was observed for the control group with the cancer group (111.80 ± 3.30). The mean weight and BMI of the controls was significantly higher than the mean weight and BMI of the cancer ($p=0.001$). Mean TSF for the control was higher than that of the cancer group and significant (0.037) when the two groups were compared. The mean MUAC and MAC for the cancer and control groups were also significant ($p<0.0001$) when the two groups were compared. For the biochemical/hematological markers, Hb and prealbumin also had significant difference ($p<0.0001$) when the two groups were compared. The mean reduced glutathione also differed significantly ($p=0.0003$) comparing the cancer and the control group. Zinc was the only exception where the mean concentration of serum zinc in the cancer group (0.0060 ± 0.00058) was higher than that of the control group (0.0059 ± 0.004) though this difference was not significant ($p=0.8793$).

Table 4.2-2 Anthropometry and Biochemistry parameters for Cancer and Control children at baseline

Parameter	Cancer (n=32)	Control (n=32)	p-value
Age (years)	6.3±3.2	6.3±3.2	0.8428
Height (cm)	111.80±3.30	118.40±3.81	0.097
Weight (kg)	18.68±1.19	27.36±2.40	< 0.0001
BMI (kg/m ²)	14.55±1.05	18.36±0.66	< 0.0001
TSF (cm)	4.93±0.31	6.12±0.47	0.033
MUAC (cm)	14.05±0.36	17.80±0.54	< 0.0001
MAC (cm)	12.44±0.36	15.79±0.43	< 0.0001
Hb (g/dl)	7.74±0.18	13.20±0.27	< 0.0001
Zinc (mM)	0.601±0.00058	0.59±0.004	0.041
Red. Glut (µM)	13.80±1.28	62.37±1.23	< 0.0001
PreAlb. (ng/ml)	369.10±11.76	590.30±14.96	< 0.0001

Data were presented as mean ± SEM and the differences between the cancer and non-cancer group was done using unpaired t-test. BMI: Body Mass Index, TSF: Triceps Skin Fold, MUAC: Mid Upper Arm Circumference, MAC: Muscle Arm Circumference, Hb: Haemoglobin, Red. Glut: Reduced glutathione.

4.2.3 Prevalence of Malnutrition among the study population at the Baseline

Table 4.2-3 summarizes prevalence of malnutrition among the study group at the baseline, using the cut-off for various parameters. This was classified into three different categories of malnutrition; severely malnourished for values less than -3 Standard deviation (SD), moderately malnourished between -3SD and -2SD and normal above -2SD. The numbers under each category were identified with their percentages (in brackets) and their p-values were computed using Fisher exact test or chi-square where appropriate. Using BMI-for-age as indicator, the cancer group had the largest percentage of malnutrition (50%) compared to the control (3.7%). A total of 50% of the children recruited for the study were stunted (Low height-for-age). The cancer group had the largest percentage (53.1%) of children in the normal range using height-for-age as an indicator. Low weight-for-height which is an indicator of wasting was also used to measure the level of malnourishment in children aged less than 5yrs for the three category of children at the baseline. From Table 4.2-3, about 19 % of the children were wasted or too thin for their weight. Weight-for-age reference data are not available beyond age 10 because this indicator does not distinguish between

height and body mass in an age period where many children are experiencing the pubertal growth spurt and may appear as having excess weight (by weight-for-age) when in fact they are just tall. Low weight-for-age which also a measure of underweight was used to determine the number of children who were either moderately or severely malnourished. In all 20 % of the children were either severely or moderately underweight with the cancer group contributing the largest percentage (34.4%).

Mid-upper arm circumference (MUAC) was another parameter used and 5% of the children had MUAC < 115 (Severely malnourished) with 8% having MUAC between 115 and 125 (moderately malnourished). The data for tricep skin fold (TSF) and muscle arm circumference (MAC) were divided into three parts to classify them into lower, middle and upper third in Table 4.2-3, the largest proportion of children had MAC in the middle and lower third with the cancer group having the highest (88%) and the control group (81%). For the TSF, the upper third predominated with 44% with the cancer group having the highest percentage (47%) in the middle third. Using the WHO cut-off point for haemoglobin level in blood (Hb) of 11g/dL, 62.5% of the children had Hb level less than 11 g/dL and were classified anaemic. For the cancer group all 32 children had Hb < 11 g/dL with the control group having only 1%. Majority (86%) of the children recruited for the study were also Zinc deficient (<10.09 $\mu\text{m/L}$). The level of reduced glutathione in blood was also measured and 14% of the study population were deficient (4.5 μM). There were 6% children without cancer who also had deficient level of reduced glutathione. Using pre-albumin as a marker, about 100% of the study population were within the normal range (>170 mg/L).

For nutritional indices such as low weight-for-age (underweight), low weight-for-height (wasting) and low height-for age (stunting) and low BMI-for-age, the WHO cut off of less than -3 Standard deviation (SD) for severe malnutrition, between -2 and -3 SD for moderate malnutrition and above -2 SD for normal were used in the classification. Children with mid-upper arm circumference of less than 115 mm were classified as severely malnourished, between 115 mm and 125 mm as moderately malnourished and above 125 mm as normal (WHO, 2004). The data for muscle arm circumference and tricep skin fold were divided into three thirds and values classified as lower third, middle third and upper third. Prealbumin values above 170 mg/L was classified as normal, between 100 mg/L and as moderately malnourished and less than 100 mg/L as severely malnourished (Godwill *et al.*, 2013, Spiekeman, 1995). For zinc, values less than 10.09 $\mu\text{mol/L}$ were classified deficient (Ndeezi *et al.*, 2010). A cut-off point of 11g/dl was also used for hemoglobin (WHO, 2011) to determine children who were anemic. Children who were deficient in reduced glutathione had concentration $< 4.5 \mu\text{M}$ (Kidd, 1997).

Table 4.2-3. Prevalence of Malnutrition among cancer and control children

Parameter	Cancer (32)	Control (32)	p-Value
BMI for Age			
Severely malnourished	7(21.9)	0(0)	0.0001
Moderately malnourished	9(28.1)	1(3.1)	
Normal	16(50)	31(96.9)	
Height for Age			
Severely malnourished	4(12.5)	3(10.0)	0.884
Moderately malnourished	11(34.4)	12(40.0)	
Normal	17(53.1)	15(50.0)	
Weight for Height			
Severely malnourished	1(3.1)	0(0)	0.451
Moderately malnourished	3(9.4)	1(3.1)	
Normal	9(28.1)	12(37.5)	
Weight for Age			
Severely malnourished	5(15.6)	0(0)	0.031
Moderately malnourished	6(18.8)	2(6.3)	
Normal	21(65.6)	25(78.1)	
MUAC			
Severely malnourished	3 (9.4)	0 (0)	0.066
Moderately malnourished	4 (12.5)	1 (3.1)	
Normal	25 (78.1)	31 (96.9)	
MAC			
Lower third	7 (21.9)	15 (46.9)	0.040
Middle third	21 (65.6)	11 (34.4)	
Upper third	4 (12.5)	6 (18.8)	
TSF			
Lower third	8 (25.0)	2 (6.3)	0.020
Middle third	15 (46.9)	11 (34.4)	
Upper third	9 (28.1)	19 (59.4)	
Hb			
Anaemia	32 (100)	1 (3.1)	< 0.0001
Normal	0 (0)	31 (96.9)	
Zinc			
Normal	4 (12.5)	5 (15.6)	0.719
Deficiency	28 (87.5)	27 (84.4)	
Glutathione			
Normal	25 (78.1)	30 (93.7)	0.072
Deficiency	7(21.9)	2 (6.3)	
PreAlbumin			
Normal	32 (100)	32 (100)	0.364
Deficiency	0 (0)	0 (0)	

Data represented proportions with the corresponding percentage in parenthesis. The proportions were compared using X^2 and fisher exact test, where appropriate. BMI: Body Mass Index, MUAC: Mid upper arm circumference, MAC: Muscle arm circumference, TSF: Tricep skin fold, Hb: Haemoglobin

4.2.4 Clinical manifestation of malnutrition among children

Some clinical signs of malnutrition in the children were also observed and the results presented in Table 4.2-4.

Table 4.2-4 Clinical information on children with cancer and control at baseline

Parameter	Cancer (32)	Control (32)	p-value
Clinical Signs of Malnutrition			
Sickness for past month excluding cancer	16 (50.0)	8 (25.0)	0.042
Diarrhoea	8 (25.0)	4 (12.5)	0.220
Appetite	25 (78.1)	29 (90.6)	0.564
Wasting	14 (43.8)	0 (0.0)	< 0.0001
Oedema	5 (15.6)	0 (0.0)	0.008
Anaemia	14 (43.8)	2 (6.2)	< 0.0001
Flaky paints dermatitis	4 (12.5)	0 (0.0)	0.029

Table 4.2-4 represents the clinical signs of malnutrition. They are represented as numbers with the percentage in parenthesis as well as their p-value calculated using Fisher exact test.

From Table 4.2-4, 37.5% of these children had other disease conditions in the past month apart from cancer and cited malaria, fever and stomach upsets as some other illness they have experienced. As indicated in Table 4.2-4, 12.5 % of the control group had diarrhoea with the cancer group registering 25%. Majority of the respondents also had high appetite (84.4%) when asked about their appetite level. For physical signs of wasting, 25 children from the cancer group were identified. For other clinical signs of malnutrition, 5 (7.8%) were observed to have oedema, 14 (21.9%) had anaemia as observed from the paleness of the sclera of the eye and 4(6.2) with flaky paints dermatitis.

4.2.5 Dietary Intake at Baseline

The results of dietary intake of children at baseline has been presented in the Table 4.2.5. This was obtained from a three 24 hr dietary recalls for each child (2 weekdays and 1 weekend). The average nutrients intake at baseline for the control, intervention and non-intervention have been presented in Table 4.2.5. From the recall the difference in nutrient intake between the cancer and non-cancer control were not significant at $p < 0.05$.

The control group had the highest caloric intake (1436 ± 255.30) with the cancer group having the least (1340 ± 70.53). However, these difference in caloric intake between the two groups were not statistically significant at $p < 0.05$.

Table 4.2.5 Nutrient intake at baseline for Intervention, Non-intervention and Control children.

FOOD NUTRIENT	CANCER	CONTROL	p-Value
Calorie (Kcal)	1340.0±70.53	1436.0±255.30	0.736
Protein (g/d)	40.8±4.28	33.9±4.59	0.333
Fats (g/d)	38.2±6.12	49.5±14.41	0.509
Carbohydrate (g/d)	209.8±1.66	219.6±28.06	0.786
Calcium (mg/d)	209.5±20.84	202.9±9.31	0.786
Iron (mg/d)	7.2±0.52	7.5±1.30	0.858
Vit A (mcg/d)	128.7±18.98	127.1±35.67	0.333
Thiamine (mg/d)	0.6±0.03	0.6±0.13	1.000
Riboflavin (mg/d)	0.47±0.07	0.5±0.11	0.795
Niacin (mg/d)	9.3±1.63	9.4±2.18	0.991
Pantothenic acid (mg/d)	3.2±0.20	2.8±0.50	0.499
Vit B 12 (mcg/d)	1.4±0.27	0.8±0.03	0.118
Vit C (mg/d)	105.9±36.40	105.6±48.30	0.660
Vit E (mg/d)	4.0±0.83	3.8±0.00	1.000
Vit K (mcg/d)	15.2±4.83	26.6±6.27	0.225

4.3 INTERVENTION STUDY

The results of the SMP intervention for six months has been presented under this section. The intervention group were supplemented with SMP for six months while the non-intervention group were not.

4.3.1. Differences in Anthropometric variables at baseline, 3 months and 6 months for intervention and non-intervention group.

Table 4.3-1 presents the differences in anthropometric and biochemical indices at baseline, 3-month and 6-month. For the intervention group, with the exception of height which increased significantly from baseline to the 6-month, all other parameters either increased or in some cases decreased though these differences were not statistically significant at $p < 0.05$. The various parameters in the intervention group increased from baseline to 3-month except that of Zinc which decreased from 0.601 ± 0.05 to 0.213 ± 0.017 at the end of the 6-month. The mean weight increased by a difference of 2.2 ± 0.5 and this was statistically significant at $p < 0.001$. TSF, MUAC, MAC, Hb, reduced glutathione and prealbumin all increased from the baseline to the 6-month and this increment was statistically significant when all three groups were compared. However, for Zinc, the level of increase was not statistically significant.

Table 4.3.1 Differences in Anthropometric indices for Non-intervention and intervention group at baseline, 3 months and 6 months.

PARAMETER	Baseline	3 Months	6-Months
Non-Intervention			
Height (cm)	103.1±28.8 ^{***}	105±28.8 ^{\$\$\$}	106.8±28.9 ^{###}
Weight (kg)	16.8±6.5	17.4±6.5	17.7±6.4
BMI (kg/m ²)	14.1±3.4	13.9±3.2	14.2±3.4
TSF(cm)	4.8±1.7	5.0±1.6	5.1±1.6
MUAC (cm)	13.8±4.1	13.9±3.6	14.2±3.5
MAC (cm)	12.3±3.6	12.4±3.2	12.7±3.0
Hb (g/dl)	8.9±2.6	8.9±2.4	9.4±2.2
Zinc (Mm)	0.056±0.21	0.103±0.43	0.16±0.65
Red. Glut (µM)	13.8±1.3	10.1±1.6	12.9±1.8
PreAlb. (ng/ml)	309.1±16.12	300.2±10.66	300.9±14.04
Intervention			
Height (cm)	105.6±30.7 ^{***}	107.0±30.9 ^{\$\$\$\$}	109.0±31.4 ^{####}
Weight (kg)	18.7±1.1 ^{***}	19.5±6.6	19.5±6.7 ^{###}
BMI (kg/m ²)	14.6±1.0 ^{**}	15.2±3.9	15.1±4.6
TSF (cm)	4.9±0.3 ^{***}	6.1±1.4 ^{\$\$\$\$}	6.8±1.2 ^{###}
MUAC (cm)	12.4±0.4 ^{***}	15.4±3.8 ^{\$\$\$\$}	16.4±3.8 ^{###}
MAC (cm)	12.4±3.1 ^{**}	13.4±3.4 ^{\$\$\$\$}	14.3±3.2 ^{##}
Hb (g/dl)	7.8±0.18 ^{***}	9.9±2.4 ^{\$\$\$\$}	11.6±2.4 ^{###}
Zinc (Mm)	0.601±0.05	0.213±0.2	0.213±0.17
Red. Glut (µM)	13.8±1.3 [*]	46.8±11.2 ^{\$\$\$}	100.1±21.9 ^{##}
PreAlb. (ng/ml)	369.1±11.8 ^{***}	421.4±27.2 ^{\$\$\$}	539.2±34.4 ^{###}

Data were presented as mean ± SEM and the differences between the baseline, 3-month and 6-month anthropometry and biochemistry were determined using one-way paired Anova with Tukey multiple comparison test as post hoc. * was used to represent mean significance when baseline and 3-months were compared, # for baseline and 6-month and \$ for 3-month and 6-month. The number of *, # or \$ also represent the level of significance; One is p≤0.05, two is p≤ 0.01, three is p≤0.001 and four p≤ 0.0001. BMI: Body Mass Index, TSF: Triceps Skin Fold, MUAC: Mid Upper Arm Circumference, MAC: Muscle Arm Circumference, Hb: Haemoglobin, Red. Glut: Reduced glutathione.

4.3.2. Comparison of Anthropometric and Biochemical indices between the intervention and non-intervention group.

The difference between intervention and non-intervention indices at baseline, 3-month and 6-month were also compared and the results presented in Table 4.3-2. The differences in mean height and weight between intervention and non-intervention group were not statistically significant during the 6-month period of study. The mean BMI at baseline for intervention and non-intervention was not statistically different, however, comparing mean BMI for intervention and non-intervention at the 3-month, the difference was significant ($p=0.013$). The difference at the sixth month was also not significant when compared. The differences in mean TSF, MUAC and MAC at baseline between intervention and non-intervention were not different statistically ($p=0.943, 0.952, 0.973$) respectively. At the third and sixth month, they differed significantly ($p<0.05$). In terms of biochemical markers, the differences in mean Hb and pre-albumin were significant only at baseline and at 6 month. In most instances, the means of the intervention group were higher than that of the non-intervention group with reduced glutathione being the only exception at baseline. For reduced glutathione, the mean for the intervention group was significantly higher only at the sixth month.

Table 4.3- 2 Differences between intervention and Non-intervention indices at baseline, 3-months and 6-months

Parameters	Baseline			Third Month			Sixth Month		
	Intervention	Non-Intervention	p-value	Intervention	Non-Intervention	p-value	Intervention	Non-Intervention	p-value
n	n=32	n=32		n=26	n=26		n=19	n=22	
Height	111.8±3.3	107.8±3.1	0.376	113.8±3.5	110.3±3.1	0.457	114.0±3.9	111.0±3.5	0.572
Weight	18.7±1.2	17.0±0.9	0.288	20.7±1.1	17.8±1.0	0.057	20.5±1.2	18.2±1.2	0.171
BMI	14.9±0.8	14.4±0.3	0.555	15.9±0.4	14.5±0.4	0.013	15.8±0.6	14.6±0.4	0.092
TSF	4.9±0.3	4.9±0.3	0.943	6.6±0.3	5.1±0.3	0.001	7.1±0.2	5.2±0.3	0.0001
MUAC	14.1±0.4	14.0±0.5	0.952	15.9±0.3	14.1±0.5	0.003	16.9±0.4	14.7±0.5	0.002
MAC	12.4±0.4	12.5±0.4	0.973	13.8±0.4	12.5±0.4	0.026	14.6±0.4	13.1±0.4	0.011
Zinc	0.04±0.02	0.0078±0.0007	0.347	0.13±0.05	0.0081±0.001	0.021	0.06±0.05	0.02±0.010	0.402
Red. Glut	13.8±1.3	53.0±15.2	0.018	68.8±14.0	47.2±10.7	0.211	136.1±21.0	59.6±14.9	0.005
Hb	7.8±0.2	9.3±0.3	<0.0001	10.2±0.3	9.1±0.3	0.023	11.9±0.3	9.9±0.3	<0.0001
PreAlb	369.1±11.76	308.3±15.1	0.002	611±16.8	295.8±13.5	0.058	561.1±14.8	300.9±14.04	<0.0001

Data has been presented as Mean± SEM and comparison between intervention and non-intervention parameters at baseline, 3-month and 6 month was done using Unpaired T-test.

4.3.3 Prevalence of Malnutrition at baseline, 3 –month and 6-month for intervention, Non-intervention.

Table 4.3-3 represents the percentage of malnutrition using anthropometric indices at baseline, 3-month, and 6-month for intervention and non-intervention group. The number of malnourished children reduced by 50% after six months of SMP intervention whilst for the non-intervention group it reduced by 10.1% when BMI-for-age was used to assess the percentage of malnutrition. For height-for-age there was an increase in number of normal children from 53.1% to 63.2% for intervention group and 59.5% to 90.9% for non-intervention group at month 6. There were 28.1% normal children for both intervention and non-intervention as baseline using weight -for-height and this decreased to 18.8% for intervention and 25.0% for non-intervention at the end of the six month intervention. Weight-for-age also reduced the number of malnourished children to 22.2% at the end of the six month for both intervention and non-intervention group. Using MUAC as a measure, 21% of the children were malnourished at the baseline and these reduced to 0% after the six months intervention. The decrease in the non-intervention group was from 43.7% to 13.6%. Majority of the study population had their MAC and TSF in the lower and middle third as presented in Table 4.3-3.

The prevalence of malnutrition using the various biochemical markers has also been presented in Table 4.3-4. All the 32(100%) of children recruited for the intervention study had Hb level less than 11.0 g/dL thus anaemic, however, after the six months intervention, 84.2% of these children had their Hb level rise above 11.0 g/dL. For the non-intervention group, the baseline percentage of anaemic children was 84.4% and this reduced to 72.7%. Majority of the children recruited were deficient in serum zinc concentration. However, the number that was deficient in the intervention group

(52.6%) was less than the non-intervention group (68.2%). The percentage of children deficient in reduced glutathione reduced by 21.9% in the intervention group compared to 20.5% of non-intervention group. For prealbumin concentration, 3.1% were deficient at the non-intervention baseline. This increased to 7.8% at the third month and back to 1.45% at the sixth month. Similar trend was observed for the intervention group, where no child was deficient at baseline, and one child at the third month and then again to no child at the sixth month.

Table 4.3-3 Prevalence of Malnutrition using anthropometry markers at baseline, 3 –month and 6-month for intervention and Non-intervention.

Parameters	Baseline	3-Months	6-Months	p-value
Non-Intervention				
N				
BMI for Age	N=32	N= 26	N= 22	
Severely malnourished	2(6.3)	2(7.7)	1(4.5)	0.895
Moderately malnourished	7(21.9)	6(23)	3(13.6)	
Normal	23(71.8)	18(69.3)	18(81.8)	
Height for Age				
Severely malnourished	4(12.5)	3(11.6)	0(0)	0.142
Moderately malnourished	9(28)	5(19.2)	2(9.1)	
Normal	19(59.5)	18(69.2)	20(90.9)	
Weight for Height				
Severely malnourished	1(3.1)	0(0.0)	1(3.1)	0.810
Moderately malnourished	2(6.3)	1(3.1)	2(6.3)	
Normal	9(28.1)	10(31.3)	8(25.0)	
Weight for Age				
Severely malnourished	10(31.3)	1(3.8)	0(0)	0.004
Moderately malnourished	5(15.6)	4(15.4)	2(9.1)	
Normal	17(53.1)	21(80.8)	20(90.9)	
MUAC				
Severely malnourished	5(15.6)	3(11.5)	2(9.1)	0.195
Moderately malnourished	9(28.1)	6(23.1)	1(4.5)	
Normal	18(56.1)	17(65.4)	19(86.4)	
MAC				
Lower third	19(59.4)	12(46.2)	5(22.7)	0.021
Middle third	11(34.4)	6(23.1)	9(40.9)	
Upper third	2(6.3)	8(30.8)	8(36.4)	
TSF				
Lower third	10(31.3)	7(26.9)	6(27.3)	0.609
Middle third	16(50)	10(38.5)	8(36.4)	
Upper third	6(18.8)	9(34.6)	8(36.4)	

INTERVENTION				
BMI for Age				
Severely malnourished	7(21.9)	3(11.5)	0(0)	0.005
Moderately malnourished	9(28.1)	3(11.5)	0(0)	
Normal	16(50)	20(76.9)	18(100)	
Height for Age				
Severely malnourished	4(12.5)	7(26.9)	1(11.1)	0.276
Moderately malnourished	11(34.4)	5(19.2)	6(31.6)	
Normal	17(53.1)	14(53.8)	12(63.2)	
Weight for Height				
Severely malnourished	1(3.1)	0(0.0)	0(0.0)	0.688
Moderately malnourished	3(13.1)	1(3.1)	1(3.1)	
Normal	9(28.1)	9(28.1)	6(18.8)	
Weight for Age				
Severely malnourished	5(15.6)	0(0)	1(11.1)	0.119
Moderately malnourished	6(18.8)	4(15.4)	1(11.1)	
Normal	21(65.6)	22(84.6)	17(89.5)	
MUAC				
Severely malnourished	3(9.4)	0(0)	0(0)	0.0286
Moderately malnourished	4(12.5)	0(0)	0(0)	
Normal	25(78.1)	26(100)	19(100)	
MAC				
Lower third	7(21.9)	5(19.2)	7(36.8)	0.261
Middle third	21(65.6)	17(65.4)	7(36.8)	
Upper third	4(12.5)	7(26.9)	5(26.3)	
TSF				
Lower third	8(56.3)	9(34.6)	6(31.6)	0.486
Middle third	15(46.9)	13(50)	6(31.6)	
Upper third	9(28.1)	4(15.4)	7(36.8)	

Table 4.3-4 Prevalence of Malnutrition using biochemical markers at baseline, 3 –month and 6-month for intervention and Non-intervention.

Parameters	Baseline n= 32	3-Months n=26	6-Months n= 22	p-value
NON-INTERVENTION				
Hb				
Anaemia	27(84.4)	22(84.6)	16(72.7)	0.485
Normal	5(15.6)	4(15.4)	6(27.3)	
Zinc				
Normal	3(9.4)	9(34.6)	7(31.8)	0.047
Deficiency	29(90.6)	17(65.4)	15(68.2)	
Reduced glutathione				
Normal	24(75)	22(84.6)	21(95.5)	0.133
Deficiency	8(25)	4(15.4)	1(4.5)	
PreAlbumin				
Normal	31(96.9)	24(92.3)	21(95.5)	0.725
Deficiency	1(3.1)	2(7.8)	1(4.5)	
INTERVENTION				
Hb				
Anaemia	32(100)	20(76.9)	3(15.8)	< 0.0001
Normal	0(0)	6(23.1)	16(84.2)	
Zinc				
Normal	4(12.5)	13(50)	9(47.4)	0.004
Deficiency	28(87.5)	13(50)	10(52.6)	
Reduced glutathione				
Normal	25(78.1)	26(100)	19(100)	0.005
Deficiency	7(21.9)	0(0)	0(0)	
PreAlbumin				
Normal	32(100)	25(96.2)	19(100)	0.370
Deficiency	0(0)	1(3.8)	0(0)	

Normal values for Hb> 11.0 g/dl, Serum Zinc> 10.09 µmol/L, Prealbumin> 170 mg/L, reduced glutathione> 4.5 µM.

4.3.4. Dietary Intake for intervention and Non-intervention group.

The results of average dietary intake of children for intervention group for the six months period of study is presented in Table 4.3.6. This was obtained from a three 24 hr dietary recalls for each child (2 weekdays and 1 weekend). The average caloric intake, protein, ash, carbohydrate, fibre, calcium, iron, potassium, sodium, zinc, manganese, vitamin C, thiamine, riboflavin, niacin, pantothenic acid, vitamin B-6, folate, and folic acid were not significantly different when the baseline, 3-month and 6-month were compared.

Table 4.3.6 Average nutrient intake for the intervention group

Food Nutrients	Baseline nutrients	3 months nutrients	6 months nutrients	p-value
Calorie	1374± 15.6	979± 12.4	899.9±10.4	0.083
Protein (g)	28.3±3.4	27.7±2.9	24.7±2.8	0.073
Total fat (g)	40.9±3.4	35.2±3.6	24.9±3.5	0.016
Ash (g)	5.6±8.8	8.9±1.1	7.6±0.9	0.189
Carbohydrates(g)	198.6±20.5	142.1±21.2	146.2±18.9	0.113
Fibre (dietary)g	15.8±1.3	12.4±2.1	11.2±1.5	0.107
Sugars (g) total	44.8±10.1	16.3±2.8	10.4±1.4	0.018
Calcium (mg)	204.8±22.2	183.1±28.6	133.2±8.8	0.117
Iron (mg)	5.4±12.1	5.8±0.7	5.2±0.7	0.312
Magnesium (mg)	254.2±24.0	167.3±20.6	165.8±18.9	0.013
Phosphorus (mg)	838± 121.5	481.6±49.9	461.7±52.2	0.029
Potassium (mg)	1712±168.6	1767±385.3	1223±195.5	0.286
Sodium (mg)	2542±253.6	1949±226.5	1965±208.0	0.170
Zinc (mg)	5.2± 0.5	4.2±0.5	3.6±0.4	0.098
Copper (mg)	1.2±0.1	0.8±0.1	0.8±0.1	0.044
Manganese (mg)	5.7±1.6	2.3±0.3	2.4±0.3	0.129
Selenium (mg)	67.9±7.1	32.1±4.0	33.9±4.7	0.0003
Vitamin C (mg)	55.3± 7.7	65.0±11.6	44.1±7.1	0.381
Thiamine (mg)	0.7±0.1	0.5±0.1	0.5±0.1	0.159
Riboflavin (mg)	1.3±0.4	0.4±0.1	0.3±0.1	0.095
Niacin (mg)	10.7±1.1	8.4±1.2	6.1±0.6	0.023
Pantothenic acid	3.4±0.3	2.3±0.2	2.2±0.2	0.126
Vit B-6	2.8±13.6	0.9±0.1	0.8±0.1	0.320
Folate (mcg)	198.4±25.3	161.4±24.2	182.1±30.1	0.650
Folic acid (mcg)	14.3±3.3	21.7±7.9	3.6±1.2	0.059

Data were presented as mean ± SDV and the differences between the baseline, 3-month and 6-months for intervention groups were determined using one-way Anova with Tukey multiple comparison test as post hoc.

Table 4.3.7 Average dietary intake for the non-intervention group

Food Nutrients	Baseline nutrients	3 months nutrients	6 months nutrients	p-value
Calorie	1279± 85.6	1179± 82.4	1045.9±105.4	0.087
protein (g)	30.4±2.6	30.3±2.2	26.4±2.9	0.466
Total fat (g)	43.2±5.2	33.92±3.59	28.26±3.45	0.368
Ash (g)	8.2±0.7	9.2±0.7	8.1±1.0	0.594
Carbohydrates(g)	251.8±12.8	174.2±16.2	146.9±15.4	0.384
Fibre (dietary)g	11.8±1.1	13.8±1.5	12.4±20.8	0.418
Sugars (g) total	13.6±3.6	18.9±2.9	32.6±12.9	0.295
Calcium (mg)	147.6±12.01	166.8±20.9	200.3±32.5	0.295
Iron (mg)	5.4±0.5	6.5±0.4	6.2±0.9	0.602
Magnesium (mg)	187.8±21.3	209.5±17.3	164.3±20.9	0.276
Phosphorus (mg)	522.3±42.0	546.7±37.3	465.1±48.1	0.333
Potassium (mg)	1360.2±189.6	1790.1±257.1	1567.8±262.8	0.541
Sodium (mg)	2079.3±222.9	2174.6±189.8	1830.5±241.0	0.522
Zinc (mg)	4.4±0.5	4.8±0.4	5.6±1.3	0.674
Copper (mg)	0.8±0.1	0.9±0.1	7.3±6.4	0.374
Manganese (mg)	2.4±0.2	2.9±0.2	2.6±0.3	0.482
Selenium (mg)	43.9±4.1	39.7±3.3	41.0±8.3	0.807
Vitamin C (mg)	47.1±6.9	59.2±9.1	76.8±24.6	0.403
Thiamine (mg)	0.5±0.1	0.7±0.1	79.1±78.6	0.374
Riboflavin (mg)	0.4±0.0	0.5±0.0	0.3±0.0	0.373
Niacin (mg)	8.0±0.9	8.9±0.9	7.2±0.9	0.411
Pantothenic acid	2.4±0.2	2.6±0.2	2.3±0.2	0.563
Vit B-6	0.9±0.1	1.1±0.1	0.9±0.1	0.485
Folate (mcg)	148.7±20.8	211.6±21.2	167.4±30.6	0.184
Folic acid (mcg)	5.1±1.8	16.9±9.5	12.6±5.6	0.456

Data were presented as Mean± SDV the p-value determined by one way anova.

Similarly, the nutrients intake in the non-intervention group was also not significant when baseline, 3-month and 6 month recall was done. This is represented in Table 4.3.7.

Table 4.3.8. Sensitivity and specificity of Cancer cases identified using ROC, Sex specific cut-off point for some Anthropometry and Biochemical markers.

Parameter	Optimal cut- off	FEMALE				MALE				p-Value
		Sensitivity (CI)	Specificity (CI)	ROC value	p- Value	Optimal cut- off	Sensitivity (CI)	Specificity (CI)	ROC value	
BMI	≤ 15.89	92.31 (64-99.8)	66.67 (38.4-88.2)	0.785	0.0013	≤ 15.77	78.95 (54.4-93.9)	84.62 (54.6-98.1)	0.854	<0.0001
TSF	≤ 4	46.15 (19.2-74.9)	80 (51.9-95.7)	0.615	0.2972	≤ 6.1	73.68 (48.8-90.9)	53.85 (25.1-80.8)	0.644	0.16
MUAC	≤ 16.85	100 (75.3-100)	66.67 (38.4-88.2)	0.856	<0.0001	≤14.7	68.42 (43.4-87.4)	100 (75.3-100)	0.887	<0.0001
MAC	≤ 14.71	100 (75.3-100)	73.33 (44.9-92.2)	0.892	<0.0001	≤13.54	73.68 (48.8-90.9)	92.31 (64.0-99.8)	0.874	< 0.0001
Hb	≤ 9.1	100(75.3-100)	100 (78.2-100)	1	0	≤9.4	100 (82.4-100)	100 (75.3-100)	1	0
Zinc	≤ 0	46.15 (19.2-74.9)	60 (32.3-83.7)	0.531	0.7518	>0	66.67 (41.0-86.7)	46.15 (19.2-74.9)	0.564	0.486
Red. Glut.	≤ 27.25	69.23 (38.6-90.9)	93.33 (68.1-99.8)	0.81	0.0007	≤ 22.33	68.42 (43.4-87.4)	84.62 (54.6-98.1)	0.781	0.001
Prealbumin	≤451.52	100 (75.3-100)	93.3 (68.1-99.8)	0.99	<0.0001	≤ 475.74	94.74 (74-99.9)	100 (75.3-100)	0.98	<0.001

ROC: Receiver Operative characteristic, BMI: Body Mass Index, MUAC: Mid upper arm circumference, MAC: Muscle arm circumference, TSF: Tricep skin fold, Hb: Haemoglobin.

4.3.5 Determination of population specific cut-off point for various

Anthropometric and Biochemical markers.

Using the receiver operator characteristic curves (ROC), area under the curve (AUC), the discriminative power of the population-specific cut-off points for identifying malnutrition was estimated using the 64 cases and 32 control recruited for the study. A level of $p < 0.05$ was considered as statistically significant for all analysis

In females, MAC (ROC-AUC: 0.892, $p < 0.0001$) was the best anthropometric measure for discriminating malnutrition in cancer (intervention and non-intervention) and in non-cancer children followed by MUAC (ROC-AUC: 0.856, $p < 0.0001$) and BMI (ROC-AUC: 0.785, $p = 0.0013$) (Table 4.3-8). However, for males, MUAC was the best anthropometric measure for discriminating malnutrition in cancer and non-cancer children (ROC-AUC: 0.887, $p < 0.0001$) followed by MAC (ROC-AUC: 0.874, $p < 0.0001$) and BMI (ROC-AUC: 0.854, $p < 0.0001$). The best biochemical measure for discriminating malnutrition in cancer and in non-cancer children for both males and females was Hb (ROC-AUC: 1, $p < 0.0000$) followed by Prealbumin (ROC-AUC=0.990, $p < 0.0001$ for female and ROC-AUC= 0.980, $p < 0.0010$ for male).

For BMI the optimal cut off point for malnutrition of $\leq 15.9 \text{ kg/m}^2$ (92.31% sensitivity and 78.95% selectivity) was identified for females and $\text{BMI} \leq 15.8 \text{ kg/m}^2$ for males (78.95% Sensitivity and 84.62% selectivity). Similarly, the optimal TSF $\leq 4.00 \text{ cm}$, MUAC $\leq 16.8 \text{ cm}$ and MAC $\leq 14.7 \text{ cm}$ was identified as best cut off point for females for the various anthropometric markers in determining malnutrition in children with or without cancer. That of males were $\text{BMI} \leq 15.8 \text{ kg/m}^2$, TSF $\leq 6.1 \text{ cm}$ MUAC $\leq 14.7 \text{ cm}$. Specificity and sensitivity of 100% were identified in both males and females for Hb with optimum cut-off point of 9.1 g/dl and 9.4 g/dl in females and

males respectively. Recommended prealbumin cut-off points identified malnutrition better in females (high sensitivity of 100%) than in male where specificity was 94.7%. The optimal cut-off was ≤ 451.5 ng/ml for females and ≤ 475.7 ng/ml for males.

4.3.6 Cure and survival rate for intervention and non-intervention

The data on percentage survival of children in intervention and non-intervention group is presented in Fig 4.1. For the intervention group, 47% of the children recovered as opposed to 16% in the non-intervention group. About 56% of the non-intervention group were not fully recovered a year after the chemotherapy. On the other hand 34% of the intervention group did not recover. The number of expired children reduced by 9% by the intervention and this difference was significant ($p < 0.0001$). Comparing between sex, the survival rate for male was less than females for both the intervention and the non-intervention group (20% vrs 17% for intervention and 43% vrs 17% for non-intervention) (Data not shown). However the figure below shows the percentage of children who recovered, died or did not recover one year after intervention

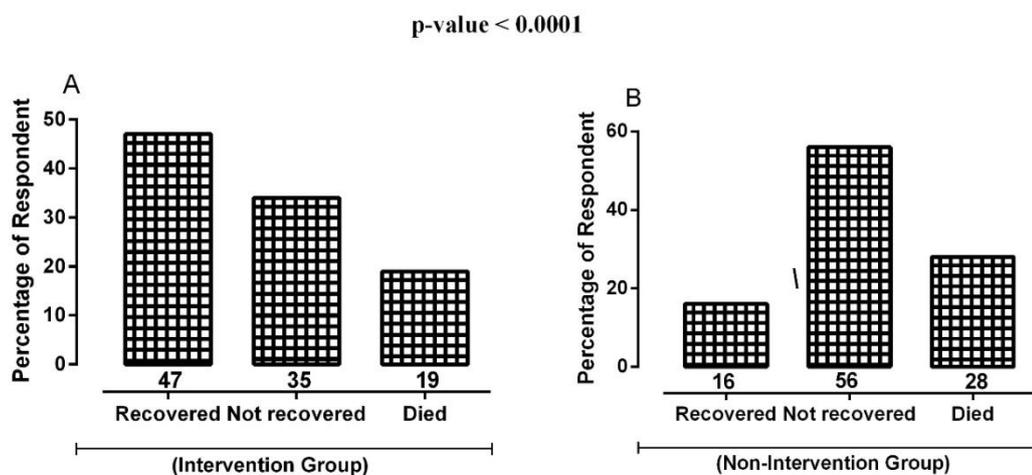


Fig 4.1 Rate of Recovery and expiry for intervention and non-intervention group.

4.4 Anti-proliferative study of isoflavanoids in soybean

4.4.1 HPLC Assay

The results of the HPLC assay to identify the presence of genistein and daidzein in SB and SMP is shown in the chromatogram in Fig 4.2 A, B and C. Fig 4.2 A and B indicates the chromatogram of the pure genistein and daidzein. A single peak was observed indicating a pure substance. Fig 4.2 C represent the chromatogram of the isoflavanoids isolated from SB/SMP. Multiple peaks were observed in both extract due to its crude nature. The concentration of daidzein and genistein was calculated and this shown in Table 4.4-1.

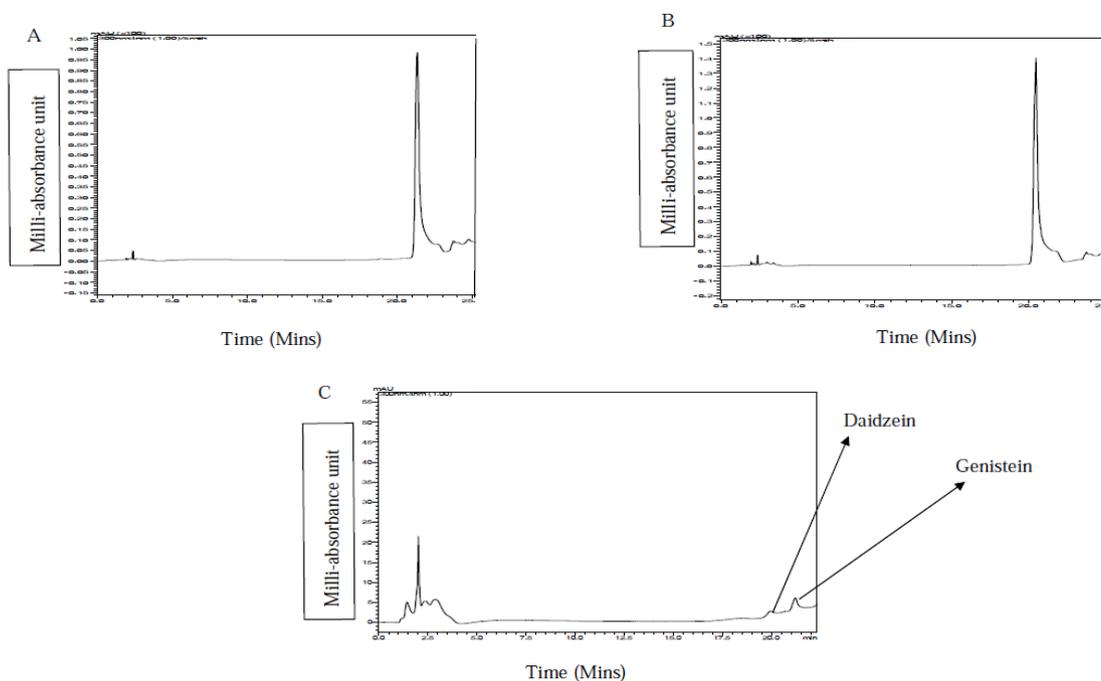


Fig 4.2 HPLC chromatogram of genistein (A), daidzein (B), SMP/SB (C)

Table 4.4.1. Concentration of Genistein, Daidzein in SB and SMP

les	Conc. (mg/ml)	Conc (ug/ml) per 100mg
Genistein		
G-SB	0.000586	0.586
G-SMP	0.005517	5.517
Daidzein		
D-SB	0.000392	0.392
D-SMP	0.006247	6.247

It was observed that the concentrations of both daidzein and genistein were higher in SMP compared to that in the SB. The highest was daidzein in SMP (6.247 µg/ml per 100 mg) while the lowest was daidzein in SB (0.392 µg/ml per 100 mg).

4.4.2 Anti-proliferative effects of isoflavanoids

Genistein inhibited the growth of DG-75 cells with IC_{50} of 298.6 mg/ml whereas daidzein showed less inhibitory effect ($IC_{50} > 2542.3$ mg/ml). The effect of SB on DG-75 was not drastic compared to SMP which was able to kill about 50% of the viable cells at 193.92 µg/ml. However, the IC_{50} for SB was greater than 1000 µg/ml as indicated in Fig 4.5 (D). The anti-proliferative effect of a standard cancer drug curcumin was also tested on the lymphoma cell lines. Curcumin was also able to clear about 50% of DG 75 at the concentration of 389.4 mg/ml as indicated in Fig 4.6 (E)

The anti-proliferative effects of genistein, daidzein, SMP, SB and curcumin on CEM are as shown in Fig 4.5. Genistein exhibited a strong inhibitory effect with IC_{50} value of 767.5 mg/ml while daidzein showed less inhibitory effect ($IC_{50} > 2542.3$ mg/ml). The effect of SMP on CEM was observed to be more profound with $IC_{50} = 54.17$ µg/ml compared to that of the SB ($IC_{50} = 858.88$ µg/ml). Curcumin was also able to inhibit the growth of the leukaemia cell lines by clearing about 50% of the viable cells at a concentration of 684.45 mg/ml.

Anti-proliferative effect of Isoflavanoid extracts of SB, SMP and Standards D, C and G

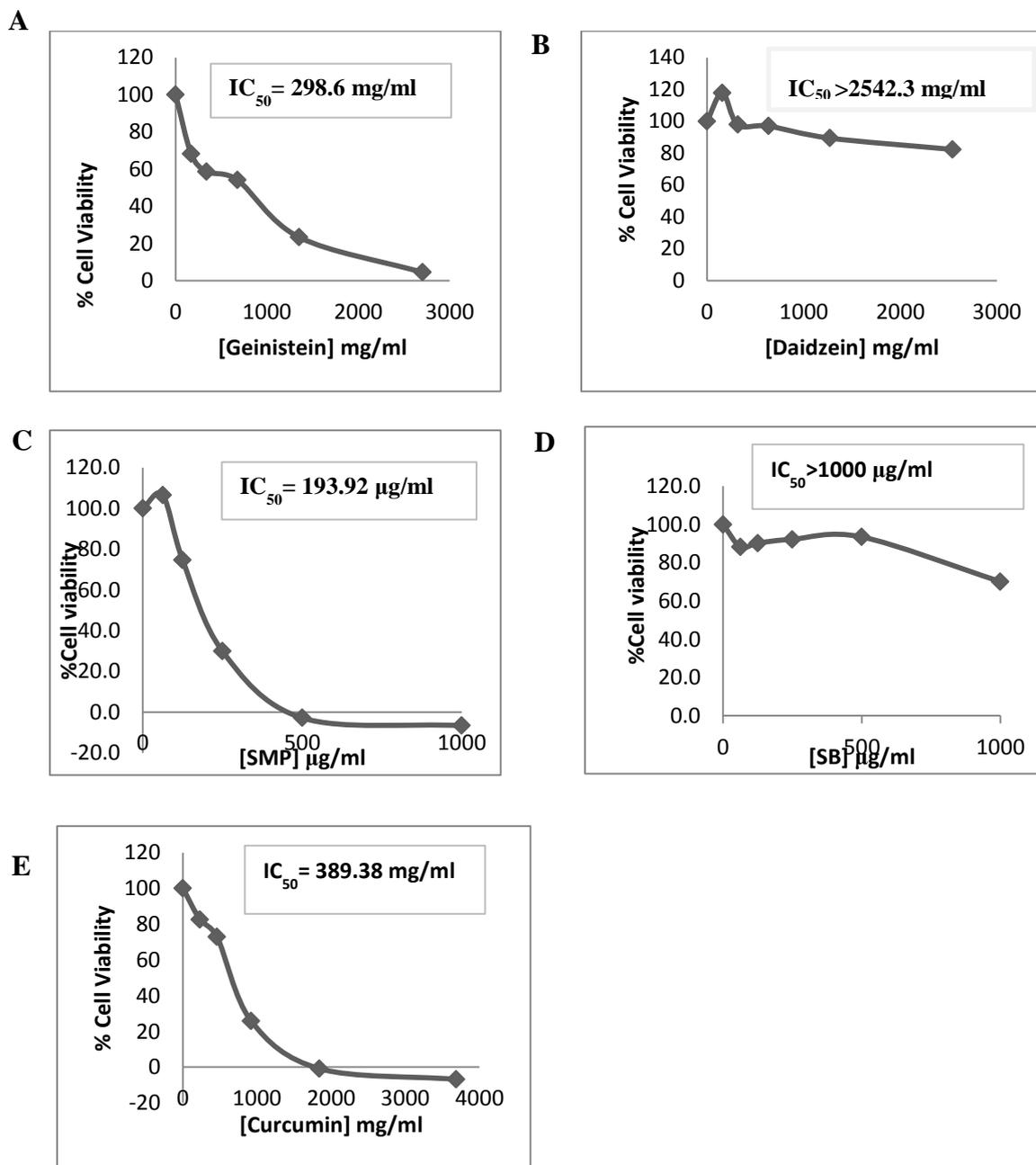


Fig. 4.5 Percentage inhibitory effect of Genistein (A), Daidzein (B), SMP (C), SB (D) and Curcumin (E) on lymphoma cell line (DG-75)

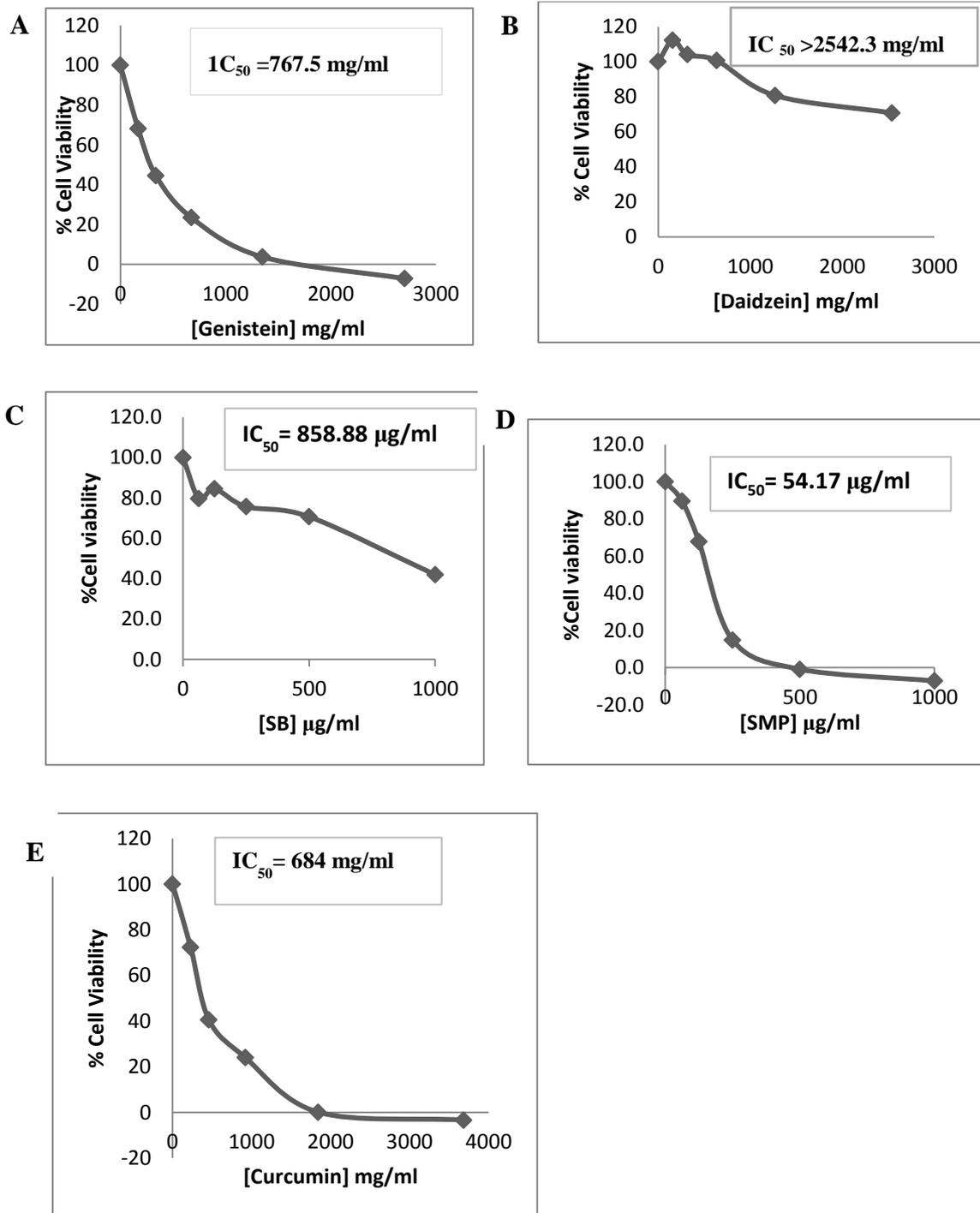


Fig 4.6 Anti-proliferative activity of genistein (A), daidzein (B), SB (C), SMP (D) and curcumin (E) on *CEM* cells.

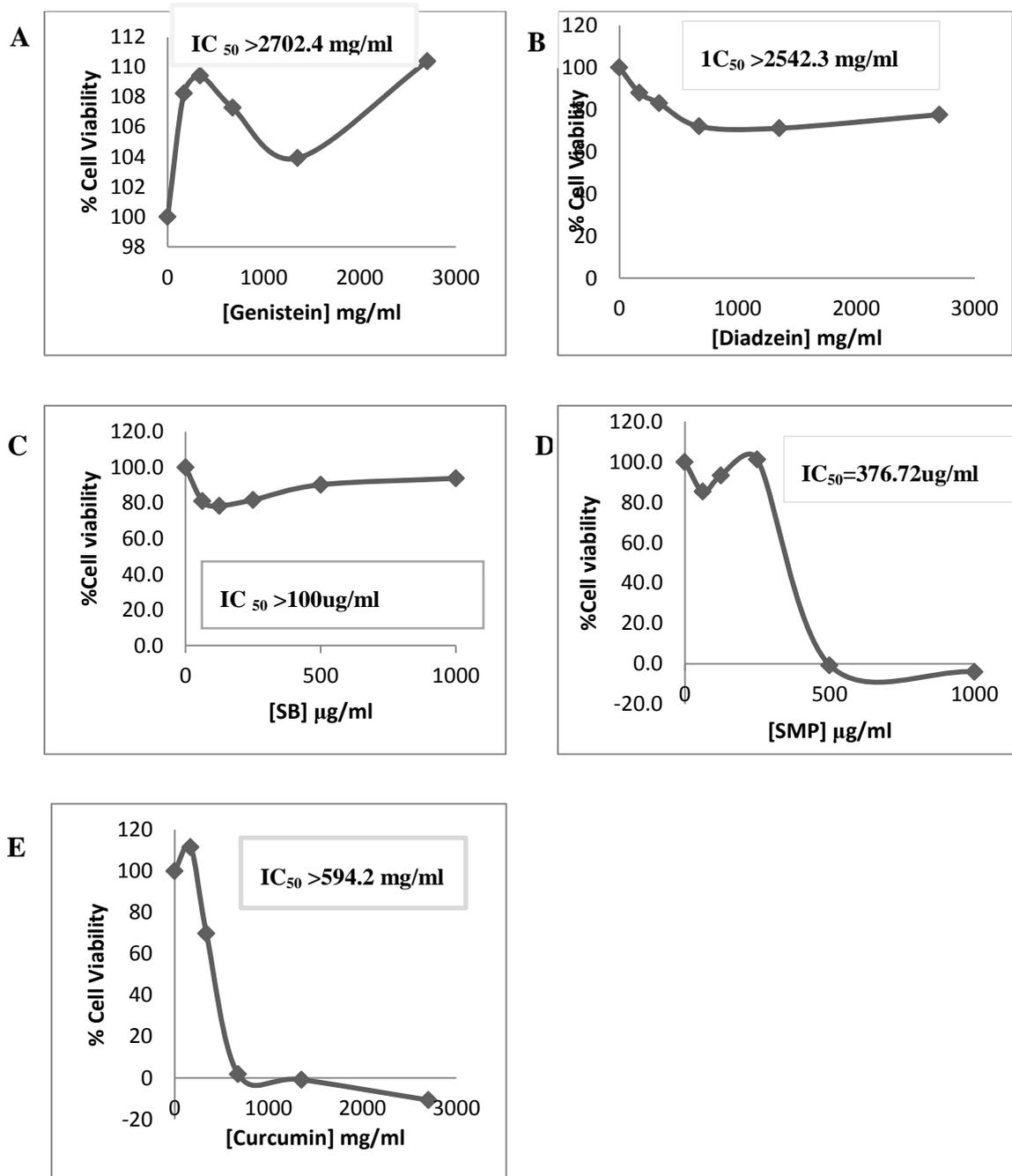


Fig. 4.7. Anti-proliferative activity of genistein (A), daidzein (B), SB (C), SMP (D) and Curcumin D on *PNT2* Cell line.

The effects of daidzein, genistein, curcumin and isoflavanoids from SB and SMP on normal *PNT2* cells have been shown in Fig 4.7. The two pure isoflavanoid standards; daidzein and genistein had minimal effect on the normal cell lines at an $IC_{50} > 2542.3 \text{ mg/ml}$ and $IC_{50} > 2702.4 \text{ mg/ml}$ respectively Fig 4.7 (A and B).

SB showed no inhibitory effect ($IC_{50} > 1000 \mu\text{g/ml}$) while SMP inhibited the growth of normal cells with ($IC_{50} > 376.72 \mu\text{g/ml}$). Curcumin also inhibited the growth of the normal *PNT2* cells ($IC_{50} > 594.20 \text{ mg/ml}$).

Table 4.4.2 Selectivity Index of genistein, daidzein, SMP, SB and curcumin.

	DG 75	CEM
Genistein	9.05	3.52
Daidzein	1	1
SB	1	1.16
SMP	1.94	6.95
Curcumin	1.52	0.86

CHAPTER FIVE

5.0 DISCUSSION

5.1 Nutritional, Mineral and Microbial analysis of soymilk powder

Soybean is a major source of high quality protein and its amino acid composition is said to be close to the composition of animal proteins (Grieshop and Fahey, 2001). Soymilk powder is an aqueous extract of whole soybean and is said to be rich in water soluble proteins (Nsofor *et al.*, 1997). Its nutritional value and comparatively low cost has made it the preferred choice of protein for most nutritional research in developing countries (Madukwe *et al.*, 2013). Analysis showed that the composition of crude protein in soymilk powder was high (49.63%) and this explains why it is the much preferred choice for intervention study especially in children undergoing treatment for cancer who usually have increased need for extra calories and protein (Marian and Roberts, 2010). The composition of protein found also corroborates with similar work done by Cheftel (1986) which estimated the composition of crude protein in soymilk powder to be 45%. The composition of carbohydrate (34.13%), fat (6.22%) as found from the analysis were also quite significant in terms of their contribution to the caloric content of the supplement. Soybean is known to be relatively rich in zinc and iron (Messina, 1999) and this was confirmed in this study which revealed Fe and Zn concentration of 0.15 ± 0.012 mg/g and 0.24 ± 0.010 mg/g respectively.

Children with cancer undergoing chemotherapy have poor nutritional status, and this can be attributed to the reduced needs due to the cancer itself and/or the effect of the chemotherapy. Hence, the additional zinc and iron should be provided in the diet of such children to improve their nutritional status. Zinc is a co-enzyme in major metabolic pathways and also play physiological role as an activator in bone formation

(Wapnir, 2000). Wapnir (2000), observed that zinc deficiency can lead to malnutrition, impaired cellular function, diarrhoea. Zinc deficiency also leads to growth retardation (Brandão-Neto *et al.*, 1995). This means that any diet or supplement rich in zinc (0.24 ± 0.010 mg/g) which contributes to the total zinc level in the body also have the capacity to help improve malnutrition. The SMP supplement we used conforms to this Zinc and Iron levels.

Moreover, the soymilk powder did not have *E. coli*, *coliform* or *salmonella* as shown in the microbial analysis and this made it safe for consumption. The acceptable level of mould and yeast load in similar studies by (McLauchlin and Little, 2007) should be < 1000 cfu/g and the levels found were less (1.2×10^2 and 1.1×10^2 respectively). Also, total plate count was found to be (2.4×10^2 cfu/g), far below than the acceptable count limit of 100,000 cfu/g. The soymilk powder was therefore safe for consumption.

5.2 Baseline Study

5.2.1 General characteristics of the study population

A baseline nutritional status assessment of the subjects was performed. A total of 64 children were recruited for the baseline study, 32 children with cancer and 32 non-cancer controls. The cancer children included children with two solid tumors (BL and WT) were used for the study and the age range was between 1-14 years. The most common cancer among the two was BL which constituted about 81.2% of the baseline study population. This reflected the higher prevalence of BL and it is in conformity with the study by Afrox (2011), which showed BL to constitute more than 50% of childhood cancers in Ghana. The mean age of children with BL in the cancer group in the study was 6.3 ± 3.0 similar to other studies conducted in Ghana and Africa between 2000 and 2007 (Owusu *et al.*, 2009a, Orem *et al.*, 2007). The mean age of the children

with WT (3.67 ± 2.94) corroborated with that of another study which reported that 78% of children with Wilm's tumour were diagnosed at 1-5 years of age with peak incidence occurring between age 3 and 4 (Zugor *et al.*, 2007). Similarly, in the Owusu *et al.* (2009a) study, males were more (58.8%) than female (41.2%) and this is consistent with the findings of this current study where the males constituted 59.38% and female 40.63%. (data not shown). Abdominal BL was predominant in the cancer group (46.9%) (Table 4.2.1).

The socioeconomic status of the study population was low as most of the recruited children (81.3%) were from the rural settings with 92.7% of the parents having either none or basic education. Orem *et al.* (2007) in their study identified low socioeconomic factors as surrogate marker for the exposure to the above mentioned identified risk factors that are believed to play an etiological role in childhood cancers. In that same studies, low socioeconomic factors was also identified to be associated with poor immune response due to poor nutrition and/ or poor hygienic conditions.

5.2.2 Anthropometry and Biochemical assessment of the study population

In determining the nutritional status of children, three main indices were used; stunting (h/s < -2 SD, -3 SD score), underweight (w/a < -2 SD, -3 SD score) and wasting (w/h < -2 SD, -3 SD score). Other anthropometric markers such as BMI-for-age, TSF, MUAC and MAC are also used in determining nutritional status. MUAC is an indicative of the amount of fat and muscle in the upper arm whiles TSF provides an estimate of the thickness of subcutaneous fat. In Table 4.2-2, the differences between the cancer and control group TSF and MUAC were significant ($p=0.037$ and <0.0001) respectively. MUAC and MAC in the control group were significantly higher

($p < 0.0001$) than the cancer group. This is indicative of the fact that at baseline the cancer group had low amount of fat and muscle in the upper arm compared with their healthy counterparts (control) who has higher amount of fat and muscle stored in their upper arm. Muscle arm circumference (MAC) which is also an indication of quantity of fat and muscle mass in the body has also been used in determining malnutrition level in children (Martorell *et al.*, 2000).

Halpern-Silveira *et al.* (2010) observed that malnutrition can result from the disease process or from the use of cancer therapies, or from both. Side effects related to chemotherapy, are key contributors in promoting the deterioration in nutritional status and this may also explain why the anthropometric measure in the controls in this study were better nourished than children with cancer. This may therefore explain why children with cancer were malnourished since they were metabolizing fats and muscle to compensate for their decreased in nutrient intake resulting in decline in TSF, MUAC and MAC.

According to WHO (2005), BMI is not a strong indicator of malnutrition in children population hence other indicators such as low BMI-for-age, low height-for-age (stunting), low weight-for-age (underweight) and low weight-for-height (wasting) are preferred anthropometric indices. Based on the WHO classification of $< -3SD$ for severe malnutrition, $-3SD$ to $-2SD$ for moderate malnutrition and above $-2SD$ for normal, the number of malnourished children (severely and moderately) for the cancer group was higher than that of the control group (Table 4.2.2).

Prealbumin also known as transthyretin, a hepatic protein and has a shorter half-life (2-4 days) compared to albumin (20 days), is a sensitive indicator of protein-energy malnourishment (Ingenbleek *et al.*, 1975). In Table 4.2.2, the mean concentration of

prealbumin, Hb and reduced glutathione for the control group were significantly higher than that of the cancer group.

The cancer group at the baseline consistently recorded lower measures of both anthropometric and biochemical/hematological markers of nutritional status assessment (Table 4.2.2). The lower anthropometry in the cancer group could be as a result of the effects of the tumour such as anorexia, cachexia, malabsorption, obstruction, diarrhoea and vomiting. These are responsible for high prevalence of undernutrition in the cancer group and this is evidenced by the observation of more clinical signs of malnutrition such as wasting, pitting oedema, anemia and flaky paints dermatitis in the cancer group compared to the control group (Table 4.2.4). These differences is likely due to the effect of the cancer by perhaps increasing nutrient requirements and utilization, nutrient loss and altered metabolism rather than inadequate nutrient intake (Katona and Katona-Apte, 2008, Halpern-Silveira *et al.*, 2010) though the nutrient intake from the 24-hr dietary recall in Table 4.2.5 were similar as no significant difference was observed at $p < 0.05$. This is consistent with the observation made by Müller *et al.* (2003) about the positive correlation between malnutrition and cancer patients. Shike (1996) observed that up to 40% of cancer patients are already malnourished before the onset of medical and surgery treatment. Bauer *et al.* (2011) also estimated about 46% of children and young adult with cancer experienced malnutrition as a result of the tumour and the treatment regime. Zinc as an essential micronutrient is known to play various biological roles in human body function and its deficiency has been linked strongly with protein deficiency (Gracey, 1996). Leung (1998) indicated that zinc is also an intrinsic constituent of superoxide dismutase which is a major scavenger of free radicals. Antioxidants are intimately involved in the prevention of cellular damage which is a common pathway for cancer

and a variety of diseases (Han and Park, 2009). However, the differences in the concentration of zinc were not significant at baseline (Table 4.2.2).

5.3 Intervention Study

5.3.1 Effect of Nutritional intervention on Nutritional status of children with cancer

For the intervention study 32 children with cancer were provided with SMP supplement to provide 80% of RDA for protein based on age and gender of the child. Other 32 cancer children not on supplement were also recruited and followed up for six months.

The various anthropometry and biochemical/hematological indices taken at the baseline (before the soymilk powder intervention) were repeated at the third month and the sixth month following. This was done to monitor the impact of the SMP on the various nutritional parameters. Generally, there was an increase in all parameters measured, 3-month and 6-month after baseline, however, the level of increase in the non-intervention group differed from the intervention group (Table 4.3.1). The supplementation was found to have little effect by the third month but this effect became prominent by this sixth month. With the exception of Hb and prealbumin which were significantly different at the baseline, the rest of the parameters were not significant when compared at the baseline. However, after the six month of intervention the differences between intervention and non-intervention mean variable were significant with the exception zinc, BMI, weight, height.

From Table 4.3.1, it was clear that irrespective of the starting point, the indices in cancer intervention group was improved and this can be attributed to the SMP intervention whereas that of the group without supplementation did not change

significantly after the 6 months follow up. The rise in the values of most parameters from the baseline to the sixth month of intervention was in most case higher in the intervention group than the non-intervention group. (weight: 2kg vrs 0.3 kg; BMI: 1.3 kg/m² vrs 0.1 kg/m², TSF; 2.3 mm vrs 0.3 mm, MUAC; 3 cm vrs 0.4 cm, MAC; 2.4 vrs 0.4). It is also worthy to note that the nutrient intake between the intervention and non-intervention groups were similar at baseline, 3 month and 6 months (Table 4.3.7 and Table 4.3.8). Therefore, the improvement in the various anthropometric parameters for the intervention group may be attributed to the SMP intervention.

The mean BMI of the intervention group increased significantly from baseline to the third month. When the comparison was done between the intervention and non-intervention group, there was a significant ($p=0.0133$) improvement by the third month (Table 4.3.2). BMI is not a good nutritional marker in children compared to their adult counterparts as the amount of body fat changes in growing children hence the BMI-for-age. It is therefore preferably to use BMI-for-age in children. From Table 4.3.3, 50% of the malnourished children became normal after the SMP intervention for six months using BMI-for-age as a marker.

Stunting or low height-for-age is usually caused by long term insufficient nutrient intake and frequent infection and is said to generally occur before age 2 and the effects are largely irreversible (WHO, 1995). This could also explain why of the number of stunted children, there was a reduction of only 4.4% in the intervention group for the six months period. Wasting or low weight for height is also a strong predictor of mortality among children under 5 years and this is said to occur as a result of acute significant food shortage and/ or diseases. Wasting is usually said to cause muscle and fat tissue to 'waste' away. WHO (2005) also observed that wasting which can result from acute malnutrition has a short duration and gets to normal

shortly after intervention. In this study, for children less than 5 years, the percentage of wasting reduced from 16.2% to 3.1% after the intervention compared to the non-intervention that remained at 9.4% for the six months period study (Table 4.3.3). Underweight also known as low weight-for-age has been associated with factors such as illness, genetics, metabolism and lack of food. MUAC is also an indicator of muscle mass and can be used as a proxy of wasting and also believed to be a good predictor of the risk of death (Trowbridge *et al.*, 1982). It is mainly measured in children aged 6 – 59 months. At baseline, the differences in MUAC between the intervention and non-intervention was not significant (Table 4.3.2) but improved significantly at the end of the sixth month SMP intervention (Table 4.3-2). The 25% of the children in the intervention group who were malnourished using MUAC as indicator at the baseline all became normal after the SMP intervention. This also gives an indication that the SMP intervention to some extent improved the muscle mass in the children undergoing the chemotherapy. Their fat reserves were also normalized with a remarkable improvement in the mean level of TSF at the sixth month of intervention as compared to the non-intervention group (4.9 ± 0.3 to 7.1 ± 0.2 for intervention vrs 4.9 ± 0.3 to 5.2 ± 0.3 for non-intervention group) (Table 4.3-2). Skin fold thickness measurement is a well-established means of assessing the thickness of subcutaneous fat at all ages including infants and represents a significant advance over body mass index (McCarthy *et al.*, 2006, Chopra *et al.*, 2002). It is also important to note that fat is usually reserved in the adipocyte when normal nutrition is attained, however, for children with cancer recovering from severe malnutrition, 6 months was not even enough for fat build up though the improvement was significant at the sixth month.

The level of haemoglobin concentration alone cannot be used to diagnose iron deficiency; it is mostly vital in indicating anaemia. Anaemia usually brings about the inability of the red blood cells to carry oxygen enough to meet the body physiological needs. The prevalence of anaemia is therefore an important health indicator (WHO, 2007). In this study, the level of Hb increased from 7.6 ± 1.9 to 11.7 ± 2.4 by the sixth month in the intervention group. (Table 4.3.1). The differences in Hb between the intervention and non-intervention group was significant throughout the study (Table 4.3.2). The dietary intake of folate which could also impact on anaemia was not significantly different ($p= 0.6503$ for intervention and $p=0.1841$ for non-intervention) from the dietary intake as indicated in Table 4.3.6 and 4.3.7. Using a cut-off of >110 g/L for non-anaemia (6-59 months of age), > 115 g/L (5-11 years of age) and > 120 g/L (12-14 years of age) adopted from WHO (2001), the intervention was associated with reduced anaemia by 84.2% compared to the 11.7% decrease in the non-intervention group at the end of 6 month (Table 4.3-4). Since the protein intake for both intervention and non-intervention did not differ for the six months period, it is likely that the SMP had an association with the improvement in red blood cell production or even survival.

Prealbumin which is an important marker for assessing protein deficiency was also measured for the six months intervention period. As indicated by Ingenbleek *et al.* (1975) prealbumin is the preferred marker for protein malnutrition as it correlates with patients outcome in a wide variety of clinical conditions. In this study, the mean level of prealbumin in the intervention group increased significantly from 338.3 ± 24.1 at baseline to 539.2 ± 34.4 (Table 4.3-1). This improvement could mean increase in protein level due to increase in protein synthesis. From Table 4.3-2, the non-intervention group had a decrease in the level of pre-albumin for the six months

period as compared to the intervention group which saw an increase from 369.1±11.76 to 611.0±16.8 for the 3 months intervention and then decreased to 561.1± 14.8 at the end of the six months intervention. This overall rise in prealbumin level could be attributed to the SMP supplement given since the level of protein intake from other food sources as indicated by the outcome of a 24 hr dietary recall on the patients in the course of the study found in Table 4.3-7 was not significant (p= 0.0726). When a cut-off of >170 mg/L (Godwill *et al.*, 2013, Spiekeman, 1995) was used in classification, one patient was deficient in the non-intervention group from baseline to the sixth month (Table 4.3-4). This increase corroborate with studies done by Ingenbleek *et al.* (1975) in which they observed that synthesis of prealbumin is increased above baseline level with 48 hrs of protein supplementation in children with severe protein calorie malnutrition and returns to normal level within eight day. It also confirms the findings by Spiekeman (1995) which indicated that the level of prealbumin should rise 2 g/dL per day with adequate nutritional support.

Zinc deficiency is usually said to be prevalent among undernourished children and is mostly associated with poor growth and development (Brown *et al.*, 2004). Using the cut off set by Brown *et al.* (2004) for zinc $\leq 9.94 \mu\text{mol/L}$ in children less than 10 years of age and $< 10.09 \mu\text{mol/L}$ for children greater than 10 years, there was a decrease of 34.9% for zinc deficiency in the intervention group with that of the non-intervention group decreasing by 22.4% by the end of the intervention (Table 4.3-4). (Lewandowski *et al.* (2007), Al-Mubarak *et al.* (2010)), also reported dermatitis and diarrhoea in children suffering from Kwashiorkor and these conditions were found to improve after the nutritional intervention with zinc rich supplement. Zinc was also found to correlate negatively with MAC (0.512) and positively with TSF (0.599) as represented in Table 4.3-5.

Baynes (1996) observed that glutathione provides reducing capacity for several reactions as well as aids in detoxification of hydrogen peroxide and other chemicals found in the human body. The total glutathione include the free and those bound to proteins and the measurement of the free glutathione in its reduced form in blood serum is essential for evaluating the redox and detoxification capacity of the cell free radical or damage tissues resulting from the cancer or the chemotherapy drugs. Similarly, when Dringen (2000) wanted to study the role of reduced glutathione in the brain he found out that patients with neurologic disorder had a low level of reduced glutathione in serum. In this study, the level of reduced glutathione was significantly lower at the baseline (Table 4.3.1). The level of reduced glutathione also increased significantly in the intervention group (30.6 ± 6.7 to 136.1 ± 21.0) but not in the non-intervention group (53.0 ± 15.2 to 59.6 ± 14.9) at the end of the study (Table 4.3-2). Using the cut-off point of $< 4.5 \mu\text{M}$ for deficiency suggested by (Look *et al.*, 1997, Rudman, 1984) there was a decrease of 21.9% in the intervention group. (Table 4.3.4). The higher the concentration of reduced glutathione, the better it is in scavenging free radicals. This is therefore an indication that the SMP could also have increased the free radical scavenging ability of the cancer cells.

Most of the cut-off used for the various anthropometric and biochemical indices were inferred from studies done elsewhere and in some cases these cut-offs were generated from healthy adults and children or children other than those with cancer (Godwill *et al.*, 2013, Spiekeman, 1995) . This suggests the need for specific cut-offs for determining malnutrition in children with cancer. The ROC-AUC curve was used to estimate possible cut-offs for various indicators. Overall, comparison of the ROC-AUC of various parameters identified Hb as the best biochemical marker in predicting malnutrition in both males and female children with cancer (Table 4.3.8). MAC was

the best anthropometric predictor of malnutrition in females whereas MUAC was the best predictor in males. Generally, cut-offs used were under different conditions and this was for assessing prevalence of malnutrition using different parameters. This could also be challenging and misleading. It is therefore not surprising that the estimated cut-offs generated were found to be different from current literature (MUAC= 11.5 cm by WHO versus 16.85 cm for females and, 14.70 cm for males in this study and Hb= 11g/dL by WHO versus 9.1 g/dL for female and 9.4 g/dL for males in this study). These suggest the need for further research to generate specific local cut-offs for various markers of malnutrition for specific diseases in general and cancer to be specific.

For a prospective study of this nature, there were challenges of children defaulting as well as some expiring in the third and sixth month follow-up. A total of 32 children each were recruited at baseline for both the intervention and non-intervention group. This number reduced to 19 in the intervention group and 22 for the non-intervention group. Three children defaulted in both groups at the third month whiles in the sixth month follow up, there were 7 defaulters in the intervention group and 5 in the non-intervention group. Some of the children also expired in the course of the sixth month study and these have been presented in fig 4.1. For the intervention group, 47% were recovered after a year of chemotherapy / radiotherapy, 35% not recovered and 19% expired. About 28% of the non-intervention group also expired in the same period whiles 16% recovered.

5.4 Anti-proliferative studies of isoflavonoids.

Soy isoflavonoids are said to be a class of bioactive polyphenols with cancer chemopreventive properties (Bronikowska *et al.*, 2010) and the three major

isoflavonoids found in soybean are daidzein, genistein, and glycetin which comprise 40%, 50%, and 10% of the soybean isoflavones, respectively (Murphy *et al.*, 1999). HPLC is used in the chemical characterization of crude extract metabolite in most plants. The polyphenols and flavonoids, being secondary metabolites, are present in several plants. Phytochemical analysis of the crude extract was assessed by HPLC analysis. Since the retention time can be shifted due to the different polarity of the extract environment, the HPLC detection was done at wavelengths after a spectrum and purity view was performed. In this study two isoflavonoid standards i.e. daidzein and genistein were compared with those isolated from soybean and soymilk powder using HPLC assay. The two chromatogram for the isoflavonoid standard had single peaks and that confirmed the purity of the standards (Fig 4.2). The elution time of the two standards corresponds with two peaks found in the chromatogram in figs 4.3 and 4.4 representing SB and SMP respectively. However, there were other peaks that could not be identified in the crude SMP and SB and this could probable be other isoflavonoids since there are about 12 isoflavonoids in soybean (Lojza *et al.*, 2012). Other phytochemicals such as phenolic and polyphenolic compounds could be present since the ethanolic crude extraction could also extract them from soybean. Soybean, which has been identified as a rich source of isoflavones, is believed to contain about 1.2-2.4 mg of total isoflavonoids per gram of sample as observed by Rostagno *et al.* (2004). In quantifying the concentration of daidzein and genistein in the two samples, it was clear that the SMP had higher concentration of both genistein and daidzein compared to their concentration in the SB (Table 4.41). This could probably be as a result of the processing of the SB into its milk powder (SMP), which is a concentrate of the former. However, the concentrations of daidzein and genistein in SB and SMP may vary due to variation in genotype, environment, location, post-harvest storage

and even assay procedure (Luthria *et al.*, 2007). Genistein has been identified as the major isoflavone constituent of soybean that is found naturally in the glycoside genistin (Merchant *et al.*, 2011) and this is consistent with this study where the concentration of genistein was higher (0.000586 mg/ml) compared to 0.000392 mg/ml for daidzein Table 4.4-1.

Numerous mechanisms for isoflavones have been suggested for their inhibition of growth of cancer cells. These include classical competitive activity to estrogen which has been considered in estrogen-related cancer prevention. Messina *et al.* (1994) have also suggested that these isoflavones play anti-cancer role by binding to the endoplasmic reticulum which in turn results in the inhibition of cell cycle. In this study, it has been shown that genistein had a stronger inhibitory effects on the growth of lymphoma cell line *DG-75* (IC_{50} = 296.6 mg/ml) compared to daidzein whose IC_{50} was greater than 2542.3 mg/ml (Fig. 4.5). Similarly the inhibitory effect of genistein on leukaemia cell line (*CEM*) was stronger (IC_{50} = 767.5 mg/ml) than that of daidzein (IC_{50} > 2542.3 mg/ml). This observation is consistent with what was reported by Matsukawa *et al.* (1993) that daidzein induced cell cycle arrest at G1, but genistein almost completely arrested the cell cycle progression at G2/M. Akiyama *et al.* (1987) also studied the inhibitory action of genistein on protein tyrosine kinase and found out that genistein selectively showed strong inhibition on the protein receptor, thus preventing signal transduction but daidzein did not. This goes to confirm that the inhibitory effect of genistein on cell growth is stronger than daidzein. The growth inhibitory effect of the crude isoflavones isolated from SB and SMP was also determined. From Fig 4.5, SMP had greater inhibitory effect on both lymphoma (IC_{50} =193.92 μ g/ml against IC_{50} >1000 μ g/ml) and leukaemia cell lines (IC_{50} =54.17 μ g/ml against IC_{50} = 858.88 μ g/ml) compared to SB. This could probably be due to

the higher concentration of isoflavone content of SMP compared SB as indicated in Table 4.4-1. Thus the higher the isoflavone content, the greater its growth inhibitory effect.

Curcumin is a major constituent of *Curcuma longa*, an Indian spice derived from the rhizomes of the plant and has a long history of use in Ayurvedic medicine as a treatment for inflammatory conditions (Jurenka, 2009, Gonzales and Orlando, 2008). Curcumin is a known potent anti-cancer agent and exerts its effect on various stages of cancer development, i.e. oncogene activation (Singh and Singh, 2009), cancer cell proliferation (Simon *et al.*, 1998), apoptosis evasion (Han *et al.*, 1999), anoikis resistance (Pongrakhananon *et al.*, 2010) and metastasis (Chen *et al.*, 2008). This was therefore used as a standard drug for the anticancer assay to compare the anti-proliferative effect of the two isoflavone standards, SMP and SB. The IC_{50} for curcumin was therefore less than all other standards and samples used for this analysis.

The selectivity index (SI) i.e. ratio of IC_{50} of on normal cell lines to IC_{50} of cancerous cell line which represent the selectivity of a drug or sample towards a cancer cell line was also evaluated. For the lymphoma cell line (*DG 75*), genistein had the highest SI (9.049) followed by SMP (1.943) and curcumin (1.517). A drug/ extract with $SI > 2$ is adjudged to have a better selectivity index for a particular cancerous cell. It can therefore be said that genistein and to some extent SMP had a best selectivity towards *DG 75*. The selectivity index of the various extract and standard were also evaluated for the leukaemia cell line (*CEM*). Genistein had the best of the selectivity index (3.520) followed by SMP (2.256) and then SB (1.179). This also clearly indicates that genistein and SMP were better anti-proliferative agents on leukaemia cell lines.

CHAPTER SIX

6.0 CONCLUSIONS

This study was aimed at assessing nutritional status of children with cancer undergoing chemotherapy compared with their non-cancer control, it also investigated the impact of a high protein based SMP on nutritional status and recovery of children undergoing chemotherapy due to cancer. The anti-proliferative effect of isoflavanoids isolated from SMP on lymphoma, leukemia and prostate cell lines were determined. The following are the conclusions obtained.

- The proximate, mineral, and microbial safety of SMP was determined.
- The study found out there is higher prevalence of malnutrition in cancer children compared to the non-cancer apparently healthy controlled children.
- There was an improvement in nutritional status, survival and recovery in children with SMP intervention compared to those who didn't receive the SMP intervention.
- An ROC-AUC curve suggested Hb as the best biochemical marker for predicting malnutrition in children with cancer. MUAC was also the best anthropometric predictor of malnutrition in children with cancer. Estimated cut-off mark for various markers also differed mostly from that in literature for various markers.
- The improved nutritional status, recovery and survival of children in the SMP intervention group can be attributed to the protein and the antiproliferative effect of the isoflavanoids found in the SMP.
- This finding has implication in the treatment of children with cancer (Burkitt's lymphoma and Wilm's tumour) as well as defining cut off-point for various anthropometric and biochemical variables in cancer cases.

6.1 RECOMMENDATIONS

- It is recommended that larger sample size and more anthropometry and biochemical parameters should be used to further predict malnutrition in cancer for diagnostic and/or prognostic purposes.
- This study was a non-randomized controlled and it is recommended that a randomized control study could also be done with BL and other childhood cancers studied in isolation to assess the impact of nutritional intervention during treatment.

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APPENDICES

APPENDIX A

1.1 Moisture Content

The moisture content of the soymilk powder was determined by the air-oven method according to the America association of serial chemist (AACC, 2000) method. In the analysis, 5.00 g of the soymilk powder was in a thermostatically controlled oven (Gallenkamp drying oven) at 105°C for 5 hrs. The plates were then removed and the placed in a desiccator to cool to room temperature. The mass was taken and the dried again for 30 min and this was cooled again and weight until a constant weight was reached. The results were the expressed as the percentage of weight lost after drying the sample and the average taken.

1.2 Crude fat content

This was also run in duplicates and the method used was that of the AACC, 2000. In the determination, 5.00 g of a dried sample was transferred into a 22 * 80 mm filter paper and was the placed in a 250 ml round bottom flask. An amount of 200 ml (petroleum spirit Bp 60-80°C) was added to the sample. This was then connected to a quickfit condenser to the Soxhlet extractor and refluxed for 6 hours on the heating mantle (Electromantle ME). The sample was then recovered after extraction by distillation and the flask was then heated for 30 mins in an oven and then cooled in a desiccator. The crude fat content was expressed as the percentage by weight.

1.3 Total Ash determination

In the total ash determination, 2.00 g of the soymilk powder was weighed and transferred into a crucible. The crucible and its content was incinerated in a muffle furnace (Gallenkamp) at 600°C for 2 hours according to the AACC, 2000 method. The crucible was then removed afterwards and cooled to room temperature. The difference in weight was expressed as the percentage total ash content of the soymilk powder

1.4 Crude Protein determination

In the crude protein determination, the Kjeldahl method as described by the AACC, 2000 was used and this involves heating the sample in conc. H₂SO₄ in the presence of

a catalyst and digesting until the carbon and hydrogen are oxidized and the protein nitrogen is reduced and transformed to into ammonium sulphate. The concentrated sodium hydroxide is added and then digest heated to drive off the liberated ammonia into a volume of the standard acid solution. Then the unreacted acid is determined and the result are transformed into a percentage of protein in the organic sample using a conversion factor of 6.25 (equivalent to 0.16 nitrogen per gram of protein).

1.4.1 Digestion

To the digestion flask, 2.00 g of the soymilk powder was added and a half of a selenium based catalyst tablet was added. A few anti bumping agents was also added with 25 ml of concentrated H₂SO₄ and the mixture shaken thoroughly. The sample was the digested until a clear solution was obtained.

1.4.2 Distillation

This was done by pouring the digested solution into a 100 ml volumetric flask and topping it up to the 100 ml mark. To three separate 250 ml conical flask, 25 ml of boric acid was measured and 2 drops mixed indicator added. Through the funnel of the steam jacket, 10 ml of the digested soymilk powder was poured into the decomposition flask and then 18 ml of 40% NaOH was added. The funnel was then cocked and distillation process allowed whiles the stopcock of the steam tap closed to force the liberated ammonia out of the digested soymilk into the collecting flask containing the boric acid. The boric acid changed to bluish green whiles the distillation was allowed for 3 mins. The end of the condenser was the washed and distilled for another 30 seconds.

1.4.3 Titration

The distillates were then titrated with 0.1 N HCl solution. The acid was added until the solution turned colorless. The volume of acid added was then recorded while a blank was run alongside. The % total nitrogen was then estimated using the formula

$$\% \text{ Total Nitrogen} = \frac{100 \times (V_a - V_b) \times N_A \times 0.01401}{W \times 10}$$

V_a = Volume in ml of standard acid used in titration

V_b = Volume in ml of standard acid used in blank

NA = Normality of acid (HCl)

W = Weight in grams of sample

% Protein = $F \times \%N$ F= Conversion factor (6.25)

1.5 Crude Fiber Determination

The sample used for the crude fat determination was transferred into 750 Erlenmeyer flask and about 0.5 g of asbestos was added. A 200 ml of boiling 1.25 % H₂SO₄ was added and then connected to the condenser. The sample was then boiled for 30 mins and after that the flask and its contents were removed and the solution was then filtered through linen cloth in the funnel. The residue was then washed with enough water until it contained no acid. The filtrate was then boiled again for 30 mins with 1.25 % NaOH solution and the filtration repeated until the solution contain no base. The residue was then washed with approximately 15 ml alcohol and then transferred into a crucible. The crucible and its contents were dried in an oven for an hour at 100°C. This was then cooled in a desiccator and weighed. The crucible and its content were incinerated for 30 mins. This was then cooled and reweighed and difference in weight before and after ignition was taken. This was then expressed as percentage crude fibre.

1.6 Total Carbohydrate

This was calculated as the differences between 100 and the sum of the moisture, crude protein, crude fat, crude fibre, total ash content.

APPENDIX B

2.1 Questionnaire used for Studies

Department of Biochemistry and Biotechnology

Kwame Nkrumah University of Science and Technology

Nutritional intervention among children undergoing chemotherapy for cancers at Komfo Anokye Teaching Hospital, Kumasi

Questionnaire for parent and child

Questionnaire should be completed at recruitment, third and sixth month for both experimental group and controls.

A. Personal/socio-demographic data

1. Date of interview _____
2. Name of Child: _____
3. Code of Child: _____
4. Date of birth
5. Age of Child: _____
6. Gender of Child:
 1. Male
 2. Female
7. Education Level of Child: _____
 1. None
 2. Primary
 3. Junior High
 4. Senior High
8. Guardian's name: _____
9. Relationship to child
 1. Mother
 2. Father
 3. Guardian
10. Maritalstatus: _____
 1. Single
 2. Married
 3. Widowed
 4. Divorced
11. Religion: _____
12. Ethnic origin: _____
 1. Ashanti
 2. Dagomba
 3. Fante
 4. Ga
 5. Other (specify)
13. Education level of Guardian: _____
 1. None
 2. Primary
 3. Junior High
 4. Senior High
 5. Tertiary
 6. Other (specify)
14. Guardian's primary occupation
 1. Farming

- 2. Trading (Business)
- 3. Office work
- 4. Professional
- 5. Hairdresser
- 6. Tailoring
- 7. Other Specify

15. Guardian's secondary occupation

- 1. Farming
- 2. Trading (Business)
- 3. Office work
- 4. Professional
- 5. Hairdresser
- 6. Tailoring
- 7. Other Specify

16. Residential Address: _____

17. Contact number : _____

18. Where is your house close to (any landmarks)?

B. Anthropometrics

15. HEIGHT/length:

- a) A cm
- b) B cm
- c) Average cm

16. WEIGHT:

- a) A Kg
- b) B Kg
- c) Average Kg

17. BMI Kg/m² (to be calculate d)

18. MID-ARM CIRCUMFERENCE

- a) A cm
- b) B cm
- c) Average cm

19. TRICEPS SKIN FOLD.....

- a. A cm
- b. B cm
- c. Average cm

C. Biochemistry

20. Pre- albumen Level

- 1. A
- 2. B
- 3. Average

21. Blood Hemoglobin level.....

- 1. A
- 2. B
- 3. Average

22. Blood glutathione level.....

- 1. A
- 2. B
- 3. Average

23. Zinc level

- 1. A
- 2. B
- 3. Average

24. Haemoglobin.....

- 1. A
- 2. B
- 3. Average

25. Haematocrit.....

- 1. A
- 2. B
- 3. Average

D. Family medical history

26. How many children has parent?

27. What is the birth order of index child

28. Has any of the siblings suffered from cancer before?

29. Has any family member suffered from cancer before?

30. Type of Diseases (Add location of Tumour if BL):.....

31. Stage of Chemotherapy:.....

E. Morbidity information

26. Has the child been ill/sick in the past month

- a) Yes
- b) No

If yes, what was it?

27. Vomiting

- a) Yes
- b) No

28. Diarrhoea.....

- a) Yes
- b) No

29. Obstruction.....

- a) Yes
- b) No

30. Malabsorption.....

- a) Yes
- b) No

31. Appetite.....

- a) Yes
- b) No

Signs of malnutrition

33. a. Wasting.....

Yes

No

b. Extent of Wasting

a) Mild

b) Moderate

c) Severe

34. Oedema.....

Yes

No

b. Extent of Oedema

a) Mild

b) Moderate

c) Severe

35. Dermatitis.....

Yes

No

36. Apathy.....

Yes

No

37. Anaemia

Yes

No

Food	Household measure	Amount (kg)
After dinner		
Dinner		
After lunch		
Lunch		
After breakfast		
Breakfast		
Before breakfast		

2.2 Consent Form used for the studies

Participant Information Leaflet and Consent Form

This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate

Title of Research:

Nutritional Intervention in Children undergoing chemotherapy for Burkitt's lymphoma at the Komfo Anokye Teaching Hospital, Ghana.

Names and affiliations of researchers:

This study is being conducted by Dr. F.K.N Arthur, Principal Investigator, and Reginald Annan. (PhD.), Dr. Alex Osei-Akoto, Co-investigators, Mr. Charles Apprey, student, Department of Biochemistry and Biotechnology, KNUST.

Background:

Chemotherapy has been part of treatment for cancers like Burkitt's lymphoma. This process however is not without problems as it sometimes prevents the patients from benefiting fully from the food they eat which then affect how fast they recover from the disease due to the drugs. This project is therefore aimed at supplementing the diet of children with Burkitt's lymphoma undergoing chemotherapy with high-protein soybean.

Purpose of research:

The purpose of the research is to provide a protein-rich soybean-based food supplement for children undergoing chemotherapies for Burkitt's lymphoma and monitor the effect of the intervention in recovery.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

Through preliminary staging according to the Children's Cancer Group system (St. Jude Children's Research Hospital), 72 participants will be put into three major groups of 36 each; 12 with soy supplement and 12 as control i.e. without soy supplement. Each participant will be required initially to provide personal information (age, sex, place of residence etc.), then venous blood shall be drawn from volunteers for biochemical analysis. Body measurements will also be taken from each participant and the food supplement shall then be given to the volunteers in each category as they are recruited.

Risks:

The drawing of blood can be painful and the phlebotomist will try as much as possible to make it less painful. In the event that one cannot bear the pain blood, he/she or the parent can decide not to participate in this study. Exposing of thighs, triceps and other parts of the body can be embarrassing and one may feel uncomfortable especially when the waist and hip circumference are measured.

Benefits:

Participants will improve their nutritional status as results of the supplement and this will in turn improve their response to the chemotherapy. Participant would also be educated on the need for a balanced nutrition as a means of surviving other similar diseased conditions.

Confidentiality:

All information collected in this study will be given reference code. No name will be recorded. Data collected cannot be linked to you in anyway. No name or identifier will be used in any publication or reports from this study. However, as part of my

responsibility to conduct this research properly, I may allow officials from ethics committees to have access to your records. After completion of the study, findings will be made available to various hospitals and the scientific community which will help them to evaluate the treatment of the cancer and modify it, if necessary.

Voluntariness:

Taking part in this study should be out of your own free will or from that of the parent. Parent or participants are not under obligation to take part in this study. Participation is entirely voluntary.

Alternatives to participation:

If a parent chooses to withdraw his/her ward or the child chooses not to participate, this will not affect your treatment in the hospital/institution in any way.

Withdrawal from the research:

You may choose to withdraw your child/ward from the research at anytime without having to give reasons. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:

There will be no consequence, loss of benefit or care to your ward if you choose to withdraw the child from the study. Please note however, that some of the information that might have been obtained from you before you withdrew might have been modified or used in analysis of results and subsequent publications. These cannot be removed anymore. I do promise comply with your wishes as much as practicable.

Contacts:

If you have any question concerning this study, please do not hesitate to contact Dr F.K.N Arthur on 0209502341, Dr. Alex Osei-Akoto on 0208168458, Dr. Reginald Annan on 0206491175 and Mr. Charles Apprey on 0243 826275.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel: 0322063248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____
and have given sufficient information, including that about risks and benefits, to
enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a
language I understand. I have also talked it over with the interviewer to my
satisfaction.

I understand that my participation is voluntary, thus. it is not compulsory.

I know enough about the purpose, methods, risks and benefits of the research study to
decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having
to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

Code Number _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

WITNESS' SIGNATURE _____

WITNESS' NAME: _____

WITNESS' SIGNATURE _____

WITNESS' NAME: _____