BIOCHEMICAL INDICES OF BURKITT'S LYMPHOMA: CLINICAL RELEVANCE IN PROGNOSIS AND MANAGEMENT

by

Lawrence Owusu
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DECLARATION

I certify that this submission is my own work and has neither been submitted to any University for the award of any degree nor does it contain any material previously published by another person, except where due acknowledgement has been made in the text.

Lawrence Owusu	…….	…….
(Student)	Signature	Date

Certified by:

Dr. F. K. N. Arthur	…….	…….
(Supervisor)	Signature	Date
Dr. F. A. Yeboah	…….	…….
(Supervisor)	Signature	Date
Mrs. F. O. Mensah	…….	…….
(Head of Department)	Signature	Date

DEDICATION

To my dear mother, Mad. Margaret Abena Frempomaa

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Almighty never forget any of the above-mentioned individuals in their quest for the ultimate in this life and beyond. Amen.

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ABBREVIATIONS

AASS:	Ann Arbor staging system
ADH:	Antidiuretic hormone
AIDS:	Acquired immune deficiency syndrome
BL:	Burkitt's lymphoma
CD:	Cluster of differentiation
CIDR 1α:	Cistern rich interdomain region 1 alpha
CNS:	Central nervous system
Crt:	Creatinine
Crtcat:	Creatinine category
CTL:	Cytotoxic T-lymphocyte
DLBCL:	Diffuse large B-cell lymphoma
EBER:	Epstein-Barr encoded ribonucleic acid
eBL:	Endemic Burkitt's lymphoma
EBNA:	Epstein-Barr nuclear antigen
EBV:	Epstein-Barr virus
ECF:	Extracellular fluid
FL:	Follicular lymphoma
HIV:	Human immune deficiency virus
ICF:	Intracellular fluid
Ig:	Immunoglogulin
IgH:	Immunoglobulin heavy chain
IL:	Interleukin
IM:	Infectious mononucleosis
IPI:	International Prognostic Index

K:	Potassium
KATH:	Komfo Anokye Teaching Hospital
KNUST:	Kwame Nkrumah University of Science and Technology
LDH:	Lactate dehydrogenase
LDHcat:	Lactate dehydrogenase category
LMP:	Latent membrane protein
Na:	Sodium
NHL:	non-Hodgkin's lymphoma
NHLs:	non-Hodgkin's lumphomas
OS:	Overall survival
POU:	Paediatric Oncology Unit
sBL:	Sporadic Burkitt's lymphoma
SUA:	Serum uric acid
TNF:	Tumour necrosis factor
UA:	Uric acid
WHO:	World Health Organization

ABSTRACT

Endemic Burkitt's lymphoma (eBL) is a juvenile malignant neoplasm of B-lymphocyte origin, markedly affected by climate, vegetation and geographical location. All out-patient clinical records of patients histologically and/or clinically diagnosed of BL from January, 2000 to December, 2007 at the Komfo Anokye Teaching Hospital, Ghana, a country within the malaria and lymphoma belts of the world, were reviewed in a real country-based cross-sectional retrospective study. The effect of cyclical chemotherapy (Cyclophophamide, Vincristine, Methotrexate and Prednisolone) on serum lactate dehydrogenase (LDH), creatinine (Crt), sodium (Na), potassium (K), phosphorus (Phos), calcium (Ca), chloride (Cl) and uric acid (UA) in relation to treatment outcome in 76 newly diagnosed BL patients was also studied in a longitudinal prospective study . A mean age of 6.9 2.7 (mode: 7; range: 1-16) was observed. Males generally dominated in incidence (M: F= 1.43:1, $p< 0.001$) and significantly with facial presentation ($p<0.05$). Females weakly dominated in abdominal presentations ($p>0.05$). Age 4-8 years was the high risk range ($p< 0.001$) for both sexes. Males were affected early in life (4-7 years) compared to their female counterparts (6- 11 years). Of the 551 cases reviewed, 48.3%, 32.7%, 15.8% and 3.3% were tumour presentation(s) involving the face, abdomen, combined facial and abdominal and either facial or abdominal with central nervous system (CNS) involvement (usually paraplegia) respectively. An intriguing observation was evident between facial and combined facial and abdominal cases which exhibited direct reverse trends in incidence. Three regions within the forest zone individually showcased significantly higher ($p< 0.001$) incidences compared to their seven cohorts that constitute the coastal and savannah agro-ecological zones of Ghana. No region was explicitly associated with any particular clinical presentation. In addition to LDH and tumour stage which are known prognostic factors for high-grade

non-Hodgkin's lymphomas (NHLs), Crt ($p< 0.001$) and Cl ($p= 0.039$) were also identified as independent prognostic factors for eBL with respect to overall survival (OS) and were subsequently used in hazard modelling. LDH, Na and Ca showed significant ($p<0.05$) changes during cyclical chemotherapy within treatment time points and in comparism with healthy age-sex matched controls. Post intensive-treatment outcome was found to the associated with the trend of serum LDH, UA, Na, K and Ca after a three-month monitoring period. This study has shown that though BL can present with demographic patterns in prevalence within a given geographical location, no clinical characterisation can necessarily be found associated with such patterns. However, serum LDH, Crt, Cl and tumour stage can serve as important prognostic factors before chemotherapy, and serum LDH, UA, Na and K can be used for monitoring of cyclical chemotherapy to enhance OS in eBL.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 INTRODUCTION

Dennis Burkitt, an Irish surgeon, in 1958 identified a form of sarcoma among several Ugandan children (Burkitt, 1958). This observation aroused his interest to critically examine these tumours that were presented at various parts on these children. In contrast to what earlier missionaries had defined about the tumour (Hutt, 1970), Burkitt concluded that these tumours are of the same type even though they present at different parts of the body. This tumour was commonly named "Burkitt's tumour" by his Ugandan colleagues. The more specific term "lymphoma" was adopted to replace the general term "tumour" at an international cancer conference in Paris (Roulet, 1964) which was among other things to pathologically define the disease.

Burkitt's lymphoma (BL) forms over 74% of childhood malignancies in equatorial Africa (Magrath, 1990). The disease usually presents as painless mass at specific anatomical sites without prior symptoms in more than 75% of the population (http://www.oncologychannel.com/nonhodgkins/symptoms.shmtl, October, 2007). Children between the ages of two to fourteen years are the most predisposed to develop this cancer with a peak age around seven years (Wackenhut and Barnwell, 1979; Sandlund *et al*. 1996). This lymphoma is the first human cancer to have an etiologic link with a viral particle, Epstein-Barr virus (EBV) which belongs to the gamma-herpes family (Epstein and

Barr, 1964). This virus is now known to be associated with several lymphomas (Pagano, 2002). Also, this cancer pioneers its cohorts in terms of association with chromosomal translocation that activates an oncogene and finally the potential of been cured successfully with chemotherapy alone (de Thé, 1985a, 1985b).

1.2 PROBLEM STATEMENT

In Ghana, there are only two major treatment centres for this cancer, Korle-Bu Teaching Hospital in the Greater Accra region and Komfo Anokye Teaching Hospital, Kumasi in Ashanti region. At the Korle-Bu Burkitt's lymphoma Project Centre, about 45-50 new cases are recorded annually according to the paediatric oncologist, Dr. Lorna Renna (personal communication, July 2007), with 3-5 HIV-related cases. Most of the cases that are reported at the centre early are successfully treated (>50% in complete remission) but cases that are diagnosed at the advanced stage are most frequently treated with little remission hence chances of survival of patient are low. Komfo Anokye Teaching Hospital (KATH) presents a different scenario with an average of 70 new cases annually. Ashanti region is relatively central in geographical location among its cohorts such that a significant number of patients are also referred from the other regions and few from neighbouring countries like Togo and Burkina Faso for treatment at the Paediatric Oncology Unit (POU) of KATH. Surprisingly, about 35% of these cases are treated successfully with chemotherapy, the only mode of treatment administered by the hospital. It has been revealed at KATH that

most patients report with advance stages of the disease and also majority default during treatment which may account for the relatively lower treatment success compared to its sister centre in Accra (Dr. Theresa Rettig, personal communication, January, 2008).

Current diagnostic procedures for BL involve general clinical symptoms, presence of a tumour mass and histological analysis. It is therefore imperative to compliment diagnosis with efficient biochemical indices which can routinely be carried out during treatment and remission periods of patients to enhance survival.

1.3 JUSTIFICATION

In order to effectively contain and manage this disease, a good local epidemiology of this cancer is imperative. This information is absent in literature. Secondly, early detection of the disease, either primary or in the event of a relapse is paramount if chemotherapy is going to be effective. Generally, non-Hodgkin's lymphoma (NHL) does not present symptoms except painless "lumps" at nodal sites. Therefore specific biochemical indices that would compliment the other diagnostic procedures would serve as a first line invasive means of early diagnosis and prognosis. Also, due to the high toxicity of the drug regimens used in treating the cancer, there is the need for means to monitor the general well-being and response of patients to treatment. Identifying biochemical indices that can give fair idea(s) about

treatment progress would be of great help to physicians in making decisions about best drug regimens with minimal adverse effects on patients.

In the light of these clinical issues, this research sought to contribute to the epidemiology, prognosis and management of this paediatric cancer by drawing inferences from specific biochemical indices.

1.4 RESEARCH HYPOTHESIS

This study thus, hypothesize that the analysis of certain biochemical indices would provide an accurate means of prognosis and monitoring for better management of eBL among children.

1.5 GENERAL OBJECTIVE

The collective purpose of this study was to demographically define the disease using patients who reported at KATH Paediatric Oncology Unit (POU) as a case study and to determine applicable biochemical indices of clinical importance for diagnosis and management of Burkitt's lymphoma.

1.6 SPECIFIC OBJECTIVES

1. To conduct a real-country based epidemiology of the cancer using data from KATH treatment centre.
2. To develop a prognostic model specifically for Burkitt's lymphoma using accumulated database.

3. To study the trend of certain biochemical indices with tumour burden or disease during chemotherapy.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BURKITT'S LYMPHOMA AS A DISEASE

Burkitt's lymphoma is a malignant neoplasm of the lymphatic system which commonly affects children less than fifteen years old and occasionally adults. It is a monoclonal B-cell lymphoma and the most rapidly growing human malignancy with tumour doubling time of less than 24 hours (Iversen *et al.,* 1974; Forestier and Schmiegelow, 2005; Oxford Concise Medical Dictionary, 2007). This cytokinetic exhibited by the cancer rightly qualifies it as a high grade malignancy of the non-Hodgkin's class of lymphomas. The disease posses much burden on the sufferer, family (guardian/ dependant) and health workers. Precise diagnosis is paramount since its treatment is quite different from other non-Hodgkin's lymphomas (NHLs) such that improper diagnosis leads to very poor prognosis and therefore survival of the patient. Nurses and physicians must have a very good monitoring protocol in place in order to provide expeditious response to any reaction during or after treatment to minimize or treat disease relapse (Wackenhut and Barnwell, 1979). There are obvious aspects of both economic and emotional burden imposed on the family/ guardian of the patient. In Ghana, it cost a minimum of five hundred cedis (GH₵ 500.00) which is approximately six hundred US dollars ($600.00) to treat the disease.

2.2 EPIDEMIOLOGY

The cancer displays varied distribution in its presentation, preferred age group and incidence rate across the globe. In Europe and the United States, BL is estimated to represent only 3% of all childhood malignant neoplasms as compared to approximately 50% and 95% for North and East Africa respectively (de Thé, 1985a). Warmth and humidity have been identified as climatic factors dominating in endemic areas of this neoplasm (Haddow, 1963; Booth *et al.,* 1967). Therefore the significant increase in percentage of BL incidence from North Africa, which is relatively dry, towards the tropical regions of Africa demonstrates the strong climatic dependence of the disease. Within latitude 10^{o} North and South of the equator, described as the lymphoma belt (Figure 1) (Viral cancers, WHO, 2005), over 50% of all NHLs are BL (Pedrosa *et al.,* 2007).

Figure 1. The lymphoma belt: Stretches from about 10^{o} North to 10^{o} South of the Equator

According to available current cancer registries in Africa, Uganda presents the highest BL incidence with an age-standardized rate (ASR) of 4.7 for boys and 3.0 for girls per 100,000 children. Malawi follows with 2.8 for boys and 0.6 for girls and Mali, Nigeria, Congo and The Gambia showing relatively lower but significant rates as compared to Europe and United States (Orem *et al.,* 2007). Ghana, a West African country, is described as one of the countries in Africa with a high incidence of the disease, probably similar to Uganda (East Africa), but there is little literature to buttress this observation except a 1985 report by Nkrumah and Olweny using the Korle-Bu BL Project Centre as a case study (Nkrumah and Olweny, 1985). At the Burkitt's Tumour Project Centre, Korle-Bu, Accra, over 430 BL cases were recorded within an eleven-year period from 1968 – 1978 with male to female ratio of 1.7:1. The incidence age was found to range between 2- 20 with the peak age around 8 years. Currently, the Centre records about 50 new cases annually while the new treatment centre established in 1999 at KATH also records between 70-80 new cases annually (Dr. Renner and Dr. Rettig, personal communication, July 2007 and January 2008 respectively)

2.3 GENERAL SYMPTOMS

Other than the presentation of a neoplasm which is painless in most cases, patients generally demonstrates fever (>38^{o}C), drenching night sweats, drastic weight loss (> 10% in weight within six month preceding tumour manifestation), loss of appetite, severe itching and chronic fatigue as systemic symptoms. These symptoms are collectively called B-symptoms.

2.4 PATHOGENESIS OF THE DISEASE

Albeit the specific course and mechanism underlying the pathogenesis of the cancer are not thoroughly known, the tumour is rarely seen in areas with yearly mean temperature below 15.5^{o}C, annual rainfall less than 50cm and of high altitude (Burkitt, 1962a, 1962b).

2.4.1 Proposed Mechanisms of the disease

Generally, the disease involves the translocation of an oncogene, *c-myc*, from a non-immunoglobulin chromosome to a promoter region of an immunoglobulin gene on a different chromosome. This translocation leads to the deregulation of the *c-myc* gene which is involved in several important cellular processes such as cell growth, cellular metabolism and apoptosis (Li *et al.,* 2003; Hecht and Aster, 2000) through its effect on the expression of other genes. Some mechanisms have been put forward by several researchers to provide insight into the chronological order of malignant development (Thompson and Kurzrock, 2004; Young and Rickinson, 2004; Pelengaris *et al.,* 2002). Central to all these theories is the general acceptance of the post-germinal centre origin of the B-lymphocyte(s) involved in BL (Küppers *et al.,* 1999). All these mechanisms put forward the argument that certain environmental (etiologic) agents adversely influence the B-cell compartment during the early developmental stages of a child (especially Africans) who later in life develop BL. The emerging hypothesis is that these etiologic factors induce, immortalize and/or drives hyper-cellular proliferation (polyclonal activation) of naïve B-cells undergoing development and

maturation (Young and Rickinson, 2004). This expanded B-cell pool is kept under surveillance by the immune system unless the integrity of the immune system is compromised due to the effects of additional environmental/ etiologic factor(s) which makes these B-cell clones highly susceptible to chromosomal mutations. Continual stimulation of these cells, either from internal and/ or external "antigens" propels successful cells into germinal centre reactions within secondary lymphoid organs where somatic hypermutation, V(D)J recombination and class-switching occur (Küppers *et al.,* 1999) to yield a malignant monoclonal B-cell harbouring a specific chromosomal translocation. Thus, the final process in this tumourigenesis brings the *c-myc* gene under the control of an immunoglobulin gene which ultimately results in the deregulation of all this important gene (Sandlund *et al.,* 1996; Hecht and Aster, 2000, Pelengaris *et al.,* 2002)

2.5 AETIOLOGIC FACTORS

Holoendemic malaria by *Plasmodium falciparum,* Epstein-Barr virus (EBV) infection and Human Immunodeficiency Virus (HIV) are some etiologic factors which have been implicated in the pathogenesis of the disease. Proposition of the disease having a polymicrobial mechanism (Rochford *et al*. 2005) is gaining much support among the scientific community as more evidence comes to light.

2.5.1 Epstein-Barr virus

Epstein-Barr virus (EBV) is a gamma-herpes virus which infects greater than 90% of the world's population and possibly 100% of all Africans. It was first discovered from a Burkitt's lymphoma tumour biopsy sample of African origin in 1964 by Epstein and colleague (Epstein and Barr, 1964). Most African children are seropositive by their third birthday and remain asymptomatic but if the age of primary infection is delayed, it can result in infectious mononucleosis (IM) as observed in most developed countries (Henke *et al.,* 1973; Straus *et al.,* 1993). The main transmission mode is through saliva (Thompson and Kurzrock, 2004). The virus infects naïve B-lymphocytes in the oropharyngeal epithelium and enters the lymphoid tissue where it expresses about nine latent proteins namely Epstein-Barr Nuclear Antigen (EBNA)-1, 2, 3A, 3B, Latent Membrane Protein (LMP)-1 and 2, and Epstein- Barr Encoded Ribonucleic acids (EBERs) (Cohen, 2000). These proteins drive the B-cells through proliferation and germinal centre reactions independent of other akin antigens (Caldwell *et al.,* 1998; Kilger *et al.,* 1998; Liebowitz, 1998). After primary infection, the virus enters into a latent phase (Rickinson and Kieff, 2001) where it shuts down the expression of all the antigens except EBNA-1 and EBERs which are required to maintain the viral genome in the B-cell and being non-immunogenic, a survival advantage to the virus. This crafted mechanism of the virus enables it to evade immune recognition and hence elimination, when these latently infected memory B-cells are in circulation. The virus persists asymptomatically until there is an immune imbalance between viral latency, replication and specific immune

control. The virus has been associated with a number of important neoplastic diseases such as nasopharyngeal carcinoma, Hodgkin's disease, T-cell lymphoma, gastric carcinoma of the stomach and endemic Burkitt's lymphoma (eBL) (Cohen, 2000; Pagano, 2002).The etiological role of EBV in some of these lymphomas has been empirically demonstrated (Liu *et al.,* 2006). Nevertheless, others still remain elusive requiring further investigations to establish their causal relationship.

Several models have been put forward to explain EBV and BL lymphomagenesis. One of such models suggests that the EBER products prevent apoptosis and promote cell survival which permits *c-myc* to express its oncogenic potential in EBV-infected cells (Niller *et al.,* 2003, 2004). Another model explains that EBV, through a specific pattern of protein expression, only acts as a potentiator with little contribution to tumour maintenance (Hecht and Aster, 2000). Yet, an integrated model takes into account all these possible mechanisms and proposes that the EBV gene products initially drive a polyclonal proliferation pool of B-cells from which a malignant clone which has acquired a *c-myc* translocation emerges. This clone, due to *c-myc* deregulation, alters the EBV gene product pattern which ultimately abolishes EBNA2 (previously required for internal cellular stimulation) dependence and causes changes in morphologic and cellular protein expressions that may further act to shield transformed cells from cytotoxic T-cell immune surveillance (Hecht and Aster, 2000; Brady *et al.,* 2007). Even though the International Agency for Research on Cancer (IARC) in 1997 concluded that there was significant evidence for the carcinogenicity

of EBV in the pathogenesis of BL (IARC, 1997), Thorley-Lawson and Gross (2004) state that there is no satisfactory explanation, or a credible evidence linking EBV to the pathogenesis of BL as compared to proposed *c-myc* pathogenic models.

2.5.2 *Plasmodium falciparum* Malaria

Dalldorf in 1962 (Dalldorf, 1962), originally suggested that malaria endemicity might be associated with tumour endemicity and since then repeated and protracted episodes of *P. falciparum* malaria have strongly been implicated with a form of BL. This is further established by a conspicuous epidemiology or geographical overlap of these two diseases among children (Morrow, 1985; Epstein, 1984). Pathophysiological processes of *P. falciparum* result from the destruction of erythrocytes, liberation of parasites and erythrocyte material into the intravascular system vis-à-vis the host reaction to these materials and/or events (White and Ho, 1992). These parasite products at schizont rapture induce activation of cytokine cascades in much the same way as bacteria endotoxins (Kwiatkowski, *et al.,* 1989). The flooding of the host's vascular system with these cytokines results in a profound change in the behaviour and responses of cells involved in immunity. These changes occur through the activity or action of both pro- and anti-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukins (IL)-4, 6, 8 and 1β (Grau *et al*., 1989, Grau and De Kossodo, 1994, Kern *et al*., 1989) which results in either Th-1 or Th-2 response. During malaria episodes, the frequency, specific type and absolute numbers of T-

lymphocytes, involved in cellular immunity, are found to be different compared to a baseline control among *P. falciparum* malaria endemic populations (Hviid *et al.,* 1996, 2000, 2001). Recently, Futagbi *et al* (2007), have demonstrated a similar change in cellular immune repertoire of auto-regulatory T-cells among eBL children. As in malaria infection, they found out that there is a loss in homeostatic T-cell surveillance; some of which (subset) specifically recognized and eliminated activated or malignant B-cells (Hacker, *et al.,* 1992).

A polyclonal activator, cysteine-rich interdomain region 1α (CIDR1α), from *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) has been identified to mitogenically induce B-cell in a T-cell independent pattern (Donati *et al.,* 2004). Some schools of thought propose that once the immune system is suppressed, as in the case of protracted malaria, the potential for the development of malignant B-cell clones in the presence of mitogens, such as the CIDR-1α, is high (Donati, 2005) Cytotoxic T-cell surveillance on EBV infection and replication is compromised under this condition of polyclonal B-cell expansion and hence the development of mutations which yield malignant clones (Klein, 1979).

A second model about the etiologic role of *P. falciparum* malaria in BL tumourigenesis states that after the characteristic BL chromosomal translocation(s) has occurred during chromosomal rearrangements in the maturation process of B-cells, intense immunological stimulation from

holoendemic malaria would give rise to a large pool of B-cells which would in turn increase the probability of emergence of a monoclonal malignant cell. The theory is that once the translocation involving *c-myc* has developed, the next plausible step would be the infection and immortalization of this malignant clone(s) by EBV (Klein, 1979), thereby maintaining a monoclonal malignant cell in circulation which eventually become a neoplasm after lodging at a suitable site in the body.

Notwithstanding these seemingly laudable explanations as to why BL is fairly high in holoendemic malaria regions and the possible role the parasite might play, it is worth to note that the "immunosuppression state" induced by malaria infection does not provide strong evidence for the pathogenesis of BL. This statement is supported by the observation that BL is rarely seen, if any at all, among patients/ people taking immunosuppressive drugs; for example during and after tissue or organ transplant. Secondly, in the case of HIV-AIDS patients, BL occurs (if it does) quite early when the individual is not highly immunosuppressed, thus, still showing high $CD4^+$ T-cell count (Dinati, 2005). Therefore, one can suggest that there is more to than just either the malaria or EBV contribution to the immunosuppression or immortalization of B-cells by *P. falciparum* and EBV respectively.

2.5.3 Emerging factors

Other etiologic factors, even though there are little empirical proofs for them are arboviruses (Haddow, 1964; Williams *et al.,* 1982) and certain plant

species and/ or their extracts such as the sap of milk bush (*Euphorbia tirucalli*) and *Croton* species (Mizuno *et al.,* 1983; Osato *et al.,* 1987; Baumann *et al.,* 1998; MacNeil *et al.,* 2003). In this model, it is suggested that the *c-myc*-Ig chromosomal translocation occurs during the viral infesting stage (arbovirus and EBV) and that their oncogenic potential/ action is potentiated by plant tumour promoters (Aya and Kinoshita, 1991; van den Bosch, 2004).

2.6 TYPES OF BURKITT'S LYMPHOMA

There are three general forms or clinical subtypes of the cancer designated as endemic or African, sporadic and human immunodeficiency virus (HIV)-related Burkitt's lymphoma. Each form has its unique characteristics in terms of epidemiology, preferred site of tumour presentation, putative etiologic agents/factors and their susceptibility to chemotherapy.

2.6.1 Endemic (eBL) or African Burkitt's lymphoma

This form of the disease is rightly named because of its relative exclusivity in Africa, especially, the tropical zones (15^0 North and South of the Equator). Papua New Guinea which is of tropical climate also exhibits high incidence of this type of lymphoma compared to other regions of the world (Rochford *et al*. 2005). Epstein-Barr virus is found to be present in over 90% of all tumour biopsies of this subtype. Notwithstanding, this form of the cancer infrequently occur all over the globe.

2.6.1.1 Incidence and Primary Sites of Presentation of eBL

The incidence rate according to Rochford *et al.* (2005) is 1-20 for every 100,000 children less than age 15 living in equatorial Africa and Papua New Guinea. This form of the disease has a preference for extranodal sites such as the jaw with infrequent involvement of the abdomen at initial tumour presentation. The central nervous system (CNS) is the third most common primary infection site for eBL but more frequently associated with other non-Hodgkin's lymphomas (NHLs) (Wackenhut and Barnwell, 1979, Bishop, *et al.,* 1999) with about 10% bone marrow involvement.

2.6.1.2 Aetiology of eBL

Holoendemic malaria and Epstein-Barr virus (EBV) have long been implicated in the aetiology of the cancer. Epstein-Barr virus (EBV) has been found to be present in over 90% of eBL biopsy samples (Viral cancers, WHO, 2005). This virus is now accepted as an invariable aetiological factor in eBL. High antibody titres for some Epstien-Barr viral replicative proteins are detected about six years before the onset of the lymphoma (de Thé, 1997, Klein, 1972) which remain fairly constant after the onset of the tumour. A cohort study with other neoplastic diseases such as lympho- and myeloproliferative malignancies did not demonstrate such significant correlation with EBV antibody titre (Klein, 1972). This phenomenon suggests a strong link of the virus with this form of the cancer.

2.6.1.2.1 The role of malaria in the pathogenesis of eBL

Intense and chronic *P. falciparum* malaria (holoendemic) is noted to contribute to the cancer through its proliferative effect on the B-cell compartment (Donati *et al.,* 2004), by the action of cytokines, such as IL-4 and IL-6, as well as suppression of cytotoxic T lymphocytes (CTLs) that are responsible for the control of EBV replication. This was confirmed when Donati *et al.* (2006) observed very high EBV loads in the blood of Ugandan children who were experiencing uncomplicated malaria episodes. The viral loads became virtually negative fourteen days later after treatment of the malaria. There have also been reports of lower incidence of the cancer among sickle cell carriers (Burkitt, 1971) who are better protected from chronic *P. falciparum* malaria. The final stage in this model would be the *c-myc* translocation and the development of a malignant clone (Klein, 1979).

2.6.1.3 Molecular Signature of eBL

Cytogenetically, the disease is characterized by a specific chromosomal aberration involving the translocation of an oncogene, *c-myc*, from chromosome 8 to an immunoglobulin (Ig) promoter region on chromosome 14 (Ig heavy chain or IgH) (Dave *et al*. 2006, Hecht and Aster, 2000). This translocation is designated as t(8:14) and other less common or minor translocations that may be detected with the major are t(8:22) and t(2:8).

2.6.2 Sporadic Burkitt's lymphoma (sBL)

Even though this form can be identified among all ages, it has a decrease incidence towards adulthood (Hecht and Aster, 2000) with males being more susceptible than females. As the name rightly suggest, it is an infrequent subtype of the cancer which occurs worldwide with United States and Europe been the most hit areas. Also this subtype exhibits a higher resistance to chemotherapy as compared to eBL (de Thé, 1985a).

2.6.2.1 Incidence and Primary Sites of Presentation of sBL

Incidence rate is about 1 in 10,000,000 people (Rochford *et al*., 2005) with about 10-15% EBV association (Hecht & Aster, 2000, Viral cancers, WHO, 2005). The abdomen is the most preferred site of presentation (Sandlund *et al.,* 1996) with approximately 30% bone marrow involvement.

2.6.2.2 Aetiology of sBL

Its aetiology is even less understood compared to eBL and no specific etiologic factors have been identified yet (Rochford *et al*. 2005). For example, it occurs in areas where there is no evidence of *P. falciparum* malaria.

2.6.2.3 Molecular Signature of sBL

This subtype is characterised by *c-myc* translocations of t(8:14), t(2:8) and t(8:22). The incidence of the two latter translocations is higher in this subtype (~15% and ~5% respectively) as compared to eBL (Hecht and Aster, 2000).

2.6.3 Human Immune deficiency Virus (HIV) -Related Burkitt's Lymphoma (HIV-BL)

This is a non-Hodgkin's lymphoma occasionally associated with HIV-AIDS patients. It is second to Kaposi sarcoma, the only two malignancies firmly established with HIV, and accounts for about 3% of all AIDS-related malignancies worldwide (Tirelli *et al.,* 1994). The cancer usually presents before the individual becomes highly immunosuppressed in the presence of high $CD4^+$ cell counts and has a variable incidence rate among all ages.

2.6.3.1 Aetiology of HIV-BL

In fact, BL has frequently been reported as a common neoplasm in HIV-infected patients (Carbone, 1995). However, it is not known why BL is fairly common in people living with HIV-AIDS (PLWHA) but not found in other situations of immunosuppression such as the use of immunosuppressive drugs after surgical transplantation. These lymphomas, which are now better listed as "AIDS- related BL", usually display an activation of *c-myc* by chromosome translocations that show structural similarities to those found in patients with sporadic BL (Subar, 1988). Nonetheless, most AIDS-related BLs in Western countries is EBV negative (Subar, 1988) whereas in Africa they are strongly associated with EBV (Lazzi, 1998). Therefore, the role of EBV in the pathogenesis of HIV-BL is least understood currently (Thorley-Lawson and Gross, 2004).

2.6.3.2 Incidence and Primary Sites of Presentation of HIV-BL

It accounts for about 24-35% of all NHLs among HIV-patients worldwide who normally have $CD4^+$ counts greater than 200/μL. Males are more predisposed compared to their female cohorts with primary tumour presentation site being predominantly extranodal with greater than 25% bone marrow involvement (Hecht and Aster, 2000).

2.7 TUMOUR STAGING

The stage of a cancer refers to the extent of spread within the patient. Staging is based on the size of the primary lesion, extent of spread to regional lymph nodes and the presence or absence of blood-borne metastases. Clinically, staging has proved to be of value by serving as a preliminary insight about the burden of the disease on the patient. There are several staging models available with modifications where appropriate as part of the working formulae at various treatment centres across the globe. According to the Ann Arbor Staging System (AASS) for NHL in children, the tumour burden on the patient can be classified into four stages depending on site(s) of appearance and extent of spread as presented in table 1.

Table 1. Burkitt's lymphoma staging (Ann Arbor classification)

STAGE	DESCRIPTION OF TUMOUR
I or A	Involves a single extra-abdominal site or region
II or B	Involves two or more lymph node regions or a localized extranodal organ and one or more lymph node(s) regions on the same side of the diaphragm
III or C	Involves intra-abdominal site(s) with or without facial or lymph node regions on both sides of the diaphragm
IV or D	Diffuse or disseminated involvement of one or more distant extranodal site(s) with central nervous system (CNS) involvement

Source: Wackenhut and Barnwell, 1979, Sandlund *et al*. 1996

2.8 DISEASE TREATMENT

Due to the very high proliferative rate of the lymphoma, expeditious treatment is essential. Relatively, the success of treatment inversely correlates with the stage magnitude and sub-type of the disease (Sandlund *et al.,* 1996). Late stage tumours (Stages III and IV) as well as sBL and AIDS-related BL

generally respond poorly to chemotherapy (Wackenhut and Barnwell, 1979). Chemotherapeutic regimens can be effectively and safely administered to patients between 2-8 months. Since the disease is haematogenous, systemic treatment is mostly applied with surgery playing little role unless complete resection of the tumour is necessary.

The specific combination of drugs for treatment takes into consideration several factors such as non-overlapping toxic effect, drug synergy and proven activity against relapse (Sandlund *et al.,* 1996). Intensive cyclophosphamide (CTX) combination-based systemic chemotherapy is the preferred regimen for treatment. In cases where the central nervous system is involved at diagnosis, more aggressive intrathecal chemotherapy is administered (Sandlund *et al.,* 1996, Wackenhut and Barnwell, 1979). Less intensive regimens are giving as patients respond to treatment based on tumour restaging report.

At the Paediatric Oncology Unit (POU) of Komfo Anokye Teaching Hospital (KATH), patients undergo a six-cycle intensive chemotherapy consisting of Cyclophosphamide (CTX), vincristine (VCR), methotrexate (MTX) with or without doxorubicin (Doxo) at 21- 28 days intervals (Induction period). The induction period is followed by a one-and-half year of less intensive maintenance chemotherapy period. CTX and VCR are administered intra-venously (IV) while MTX is administered by intra-thecae (IT) injection. Doxorubicin is added to the IV infusion in either relapse or CNS-involvement

cases only. Prednisolone and Allopurinol (to manage tumour lysis syndrome) are given to patients as oral drugs. Treatment at any cycle is not given when a patients has his or her haemoglobin (Hb) level below 6 (g/dL) or platelet level below 70 $x10^3/\mu L$ and/ or weight of 12 kg and below. Such a patient is admitted until these indices normalize before any chemotherapy is administered.

Even though patients who do not respond to treatment usually expire within three months, the overall survival rate of patients with Stage I and II, III, and IV tumour has been estimated to be (in the approximations of) 67%, 50% and 15% respectively (Wackenhut and Barnwell, 1979). From this data, it is obvious that the later the stage, the more life threatening the disease, especially when the central nervous system (CNS) is involved.

2.9 BIOCHEMICAL INDICES

The human body, though complex, must maintain a dynamic equilibrium between its components both intra and extra-cellularly to remain functional. This homeostatic state within and about cells, tissues, organs and organ systems is what ultimately manifest as the health or well-being of an individual. Therefore, the body is normal and optimum when all biological and chemical constituents at all organizational levels are within normal limits. Thus, by determining or measuring the plasma levels of such constituents directly or indirectly, one is able to have a clinical insight. This principle has advanced from the theoretical to the practical level in clinical medicine such

that biochemical or laboratory (in general) investigations remain core in disease screening and diagnosis, treatment monitoring and prognosis.

All neoplasms are essentially parasites and deprive the body of essential nutrients to various degrees. This is reflected by the magnitude to which the body's homeostasis has been shifted from equilibrium with respect to vital biological and/ or chemical constituents or indices. Certain biochemical indices have proved very useful in diagnosis, treatment monitoring and prognosis of some specific types of adult cancers but none has been identified for paediatric cancers. Follicular lymphoma (FL) (Federico *et al.,* 2000) and diffuse large B-cell lymphoma (DLBCL) (Yang *et al.,* 2005) are some of the cancer types whose prognosis have been specifically modelled using biochemical indices (with other demographic indices). An International Prognostic Index (IPI) for cancer was developed in 1993 to predict treatment outcome for adult cancer (The NHL Prognostic Factors Project, 1993). The IPI seem reasonably convincing for the few high-grade NHLs that have been tested against it but have been inconsistent with other low-grade NHLs yielding conflicting results (Lopez-Guillermo *et al.,* 1994; Cameron *et al.,* 1993; Leonard *et al.,* 1991). Since no prognostic index has been defined for paediatric cancers, of which BL is of great importance, it has not been possible for models to be developed and therefore compared to the IPI. Some of the biochemical indices that are of great importance to clinicians during the diagnostic, treatment and monitoring phases of BL are lactate dehydrogenase

(LDH), serum electrolytes, serum calcium, phosphorus and uric acid (Sandlund *et al.,* 1996, Wackenhut and Barnwell, 1979).

2.9.1 Serum Lactate dehydrogenase (LDH)

This is the major enzyme involved in the reversible conversion of lactate to pyruvate under anaerobic (hypoxic) conditions. It is an intracellular enzyme found in many body tissues and hence used to support diagnosis and/ or disease involvement of any of these tissues. There are five isoforms (LDH 1-5) of the parent enzyme which are distributed with varied predominance in various tissues/organs such as liver, heart, red blood cell (RBC), skeletal muscle, brain, kidney, etc. In a normal individual, LDH-1 accounts for about 17%-27%; LDH-2: 27%-37%; LDH-3: 18%-25%; LDH-4: 3%-8% and LDH-5 account for 0%- 5% of total serum LDH. Damage to any tissue thus results in a higher than normal level of the enzyme or more specifically the predominant isoenzyme(s) of the tissue in the blood. The deregulation of *c-myc* due to its translocation affects the metabolic activity of this enzyme by activation of its transcription. This is reflected by the elevation of all LDH isoenzymes (total LDH) in the serum of BL patients. The high level of serum LDH correlates with the tendency for tumour cells to produce high levels of lactic acid under low oxygen conditions (Hecht and Aster, 2000) known as the Warburg effect. This has lead to the use of serum LDH as an important index for tumour burden and invasive potential during the diagnosis of cancers and other systemic infections.

2.9.2 Serum Creatinine

This is a catabolic end product of a high-energy reserve molecule, creatine phosphate. The production of creatinine from creatine, found predominantly in the skeletal muscles and entirely excreted by the kidneys, is relatively constant with respect to a person's muscle mass. Therefore the measurement of serum creatinine provides a means to monitor renal excretory function. Generally, elevated levels suggest chronic renal impairment while decreased level suggests debilitation. Monitoring the kidney excretory function is of great importance for and during chemotherapy since the cytotoxics as well as the cellular constituents of the tumour cells upon lyses must be excreted (Gaw *et al.*, 1999).

2.9.3 Serum Uric Acid

It is a nitrogenous catabolic product of purine; a building block of deoxyribonucleic acid (DNA). Uric acid is made primarily in the liver and excreted by the kidney hence the serum level is affected by both the rate of synthesis and excretion. Other than its association with gout (periarticular deposition of uric acid salt) and tophi (soft-tissue deposits of uric acid salt) inferred from hyperuricemia, elevated levels are also expected in cancers because of the high DNA turnover in these cancerous cells. At the genetic level, *c-myc* influence genes involved in nucleotide synthesis (Hecht and Aster, 2000). Cancer chemotherapy meant for the destruction of rapidly growing tumours result in the spill of nucleic acids into the bloodstream which are then carried to the liver for conversion into uric acid. Hence the

monitoring of this index would be important in the assessment of tumour lyses tolerance and renal excretion capacity.

2.9.4 Serum Calcium

Serum calcium determination is mainly used to diagnose parathyroid function and calcium metabolism since it is an important cation for many physiological enzymatic reactions. Second to hyperparathyroidism, elevated serum calcium (hypercalcemia) is associated with malignancies. This is seen in two main ways; tumour metastasis extending to the bone leading to their destruction, hence calcium reabsorption which in turn reflects in the blood and secondly, the possibility of the cancer to produce a parathyroid hormone-like substance to cause hypercalcemia (Gaw *et al.*, 1999). About half of the total calcium in the body exist in a protein-bound form (albumin predominantly) hence hypocalcemia is mostly associated with lower levels of blood albumin- an index of nutritional status.

2.9.5 Serum Electrolytes

Electrolytes are positively and negatively charged ions which are in solution in all body fluids. These include sodium, potassium, bicarbonate, phosphate and chloride. Some of these are predominantly found in the intracellular fluid (ICF) of cells whiles others are within the extracellular fluid (ECF). There exist a constant movement in both directions across the cell membrane to maintain a balance of these electrolytes within and about cells. Electrolytes function, among other things, to maintain cellular electrical potential, acid-

base equilibrium and body fluid (especially water) balance. Hence this test is used to evaluate and monitor fluid and electrolyte balance. The high rate of tumour growth as well as tumour lyses, upon chemotherapy, which characterizes BL affect the level of these electrolytes. For example, in a state of massive tumour lyses, serum potassium becomes highly elevated and this can result in alkalosis in the patient (Gaw *et al.*, 1999).

2.9.5.1 Serum Potassium

This is the principal intracellular cation and the most important determinant in maintaining membrane electrical potential. Even though its extracellular concentration is very small and does not very appreciably with water loss or retention, factors which yield small changes in extracellular concentration have major consequences (Gaw *et al.*, 1999). In addition to maintaining cellular electrical neutrality, potassium also performs important functions during protein synthesis and maintenance of normal osmotic pressure. Of particular importance is the inverse relationship between potassium and hydrogen ions. When intracellular hydrogen ion concentration increases during acidosis, due to a disease or treatment, potassium ions are released from the cell into the extracellular environment to maintain electrical neutrality. Changes in plasma concentration also affect the response of excitable cells, such as nerves and muscles, to stimuli and this may have serious consequences. It is primarily excreted by the kidney hence can be used in association with relevant indices to assess renal filtration function (Gaw *et al.*, 1999).

2.9.5.2 Serum Sodium

Sodium is the major extracellular cation and it exhibits a significant relationship with water balance in an individual. Physiologically, sodium and water are closely related in the observation that as free body water is increased, serum sodium becomes diluted and vice versa. Under normal physiological conditions with proper kidney function, these changes are compensated for appropriately to maintain homeostasis. The serum (or blood) concentration of sodium is a reflection of dietary intake vis-à-vis renal excretion in addition to minimal non-renal losses. Traumas or shocks due to disease or treatment may cause increased levels as a result of decreased renal blood flow. The hormone, aldosterone, also stimulates increased renal reabsorption of sodium thereby affecting its serum concentration. Natriuretic hormone and antidiuretic hormone (ADH) inversely affect high sodium level in the serum by decreasing renal reabsorption and increasing water reabsorption. By measuring and keeping track of the sodium level of a patient (in association with other indices), especially during treatment of any systemic disease, provides a means of monitoring the fluid and solute balance of the patient in response to treatment (Gaw *et al.*, 1999).

2.9.5.3 Serum Chloride

Chloride, though being the predominant anion in the extracellular fluid (ECF), does not provide much clinical information by itself but when analysed with other electrolytes it serves to give insight about the hydration and acid-base status of an individual. Because it is a major anion in the ECF,

it flows along with the major ECF cation (sodium) to maintain electrical neutrality and also effects water balance (Gaw *et al.*, 1999). Chloride ions, to some extent, serves to neutralize the effect of increased hydrogen ions (acidity) in the ECF by shifting into cells to make room for bicarbonate (anion) which moves from the ICF to buffer the extracellular acidity. Metabolic and renal tubular acidosis, general kidney dysfunction and hyperparathyroidism are some factors that can contribute to increased serum chloride level (hyperchloremia) while overhydration, metabolic alkalosis and chronic diarrhoea may result in hypochloremia.

2.9.6 Serum Phosphorus

Phosphorus in the body is in a form of a phosphate and an important intracellular and extracellular anion. It is relatively abundant in the body and much of it is covalently attached to organic molecules such as proteins, lipids, high-energy compounds such as adenosine triphosphate (ATP), and nucleic acids. Most of the body's inorganic phosphorus is in the bone in combination with calcium. There exist an inverse relationship between calcium and phosphorus hence its serum level is significantly affected by calcium metabolism (as phosphate levels decrease, calcium levels rise in the blood) (Gaw *et al.*, 1999). Advanced lymphoma and haemolysis may cause elevated blood phosphate (hyperphosphatemia) due to the release of intracellular phosphorus or phosphate into the blood stream when cells lyse. Other indications for hyperphosphatemia include renal failure due to excretion impairment, hypocalcemia and acidosis (phosphate moves into the ECF to

buffer hydrogen ions). Hypophosphatemia, though rare, may lead to respiratory impairment and factors that contribute to this status include hyperparathyroidism, malnutrition, alkalosis and oncogenic effect (this result in severe hypophosphatemia but the causative factor induced by the tumour is yet to be defined).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

This study has a retrospective and a prospective component.

3.2 RETROSPECTIVE STUDY

This study reviewed the clinical records of all Burkitt's lymphoma cases from January, 2000 to December, 2007 at the Paediatric Oncology Unit (POU) of Komfo Anokye Teaching Hospital (KATH). The purpose was to develop a local epidemiology and define prognostic factors using the extracted data. The inclusion criteria for this work were;

(a) Patients should have at least three of the following biochemical tests carried out at diagnosis;

i. Serum lactate dehydrogenase (LDH)
ii. Serum Creatinine
iii. Serum potassium
iv. Serum chloride
v. Serum sodium
vi. Serum uric acid
vii. Serum phosphate
viii. Serum calcium

(b) Stage or thorough description of the tumour should be indicated at diagnosis

(c) Complete record of patient's treatment till an event. i.e. either death or relapse

Anything less of the above criteria was excluded from the study

3.2.1 Sample Size

All cases of eight years in retrospect that met the inclusion criteria were considered. A total of 569 cases were retrieved from the 2000- 2007 records. Parameters such as age, gender, region of origin, stage of tumour and site of primary tumour presentation were retrieved for epidemiological analysis. Out of the 569 cases, 180 patients had some of the previously outlined biochemical indices of interest carried out before the commencement of chemotherapy. This group of patients were selected and used for overall survival (OS) analysis in prognostic index modelling.

3.2.2 Data Analysis

The demographic and clinical information obtained from the records of patients (569) were used in descriptive and chi-square (χ^2) - analysis for the epidemiological study. Initial Data Analysis (IDA) was performed on each set of pre-treatment biochemical index obtained from the 180 patients. Each biochemical index was categorized according to the degree of change relative to either the upper or lower limit of the normal range of the particular test. The categories considered were "below lower limit of normal range", "within normal range" and "greater than one fold of normal upper limit". Early death, treatment failure, relapse and partial remission were collectively classified as

"treatment event" and all other cases were considered not to have experienced the "treatment event" (censored), including complete remission (survival of two years or more) in the overall survival (OS) model development. Kaplan-Meier analysis was carried out on each biochemical index to identify univariates that influence the disease and its outcome (event). These independent prognostic factors were plug into the Cox Hazard Regression model, a multivariate hazard estimator, to design a prognostic model.

3.3 PROSPECTIVE STUDY

Newly diagnosed Burkitt's lymphoma patients were voluntarily recruited into this study to investigate the effect of cyclical chemotherapy and/ or disease on some biochemical indices. The trends of these biochemical indices with respect to treatment outcome were also investigated.

3.3.1 Patients recruitment

Recruitment was strictly voluntary among children who were cytologically and/ or clinically diagnosed to have developed Burkitt's lymphoma. Informed consent was sought from the patients (depending on age and clinical state) or their guardians after thoroughly explaining the rationale behind the study, what would be required of them and the risk and benefits if a patient voluntarily enrol.

3.3.2 Study subjects

This comprised of 76 newly diagnosed Burkitt's lymphoma patients referred to the Paediatric Oncology Unit (POU), KATH from all over Ghana. The patients were initially examined by consultant paediatricians at the Child's Health Department of KATH and cytological examination of tumour aspirate conducted by pathologist. Ultrasound scans and X–rays were also conducted as part of the diagnostic procedures. Twenty healthy age-sex-match children were also recruited as control subjects.

3.3.3 Blood Sampling

With the assistance of phlebotomist, about 5 ml of venous blood were taken from subjects into 10 ml sterile tubes using sterile needles and strings. Samples were immediately taken to the Clinical Biochemistry Dept. of KATH for processing and analysis. All samples were analysed within 12 hours of collection. This procedure was carried out on each patient three times at forty two days interval.

3.3.4 Biochemical Indices

The following biochemical tests were considered under this study.

Table 2: Biochemical indices of interest and their normal range values

BIOCHEMICAL TEST	NORMAL RANGE
Lactate dehydrogenate (LDH)	M: 80-285 U/L F: 103- 227 U/L
Creatinine	53-123.8 µmol/L
Uric Acid	154-357 µmol/L
Serum Calcium	2.12-2.6 mmol/L
Serum Potassium	3.6-5.5 mmol/L
Serum Sodium	134-149 mmol/L
Serum Phosphorus	0.81-1.55 mmol/L
Serum Chloride	94-112 mmol/L

3.3.5 Biochemical analysis and Equipment

An automated blood chemistry analyzer (ATAC 8000, Japan) was used for all the selected biochemical indices of each patient. Each sample was analysed three times for the same indices.

3.3.6 Data analysis

The differences in the indices between active patients receiving chemotherapy were compared to a control group using Kruskal-Wallis test at 5%

significance. Student-Newman-Keuls (SNK) multiple range test was used to detect the exact time point(s) of significant differences within biochemical indices that showed general significance during cyclical chemotherapy. To test for relationship between indices and treatment outcome after completion of chemotherapy, one factor repeated measures analysis of variance (One way-ANOVA) was used at 95% confidence interval. Scheffe's multiple range test was used to compare the controls to outcome groups.

3.4 ETHICAL CONSIDERATION

The study was approved (CHRPE/ KNUST/ KATH/ 11_05_08) by the Committee on Human Research and Ethical Publications of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST) and Komfo Anokye Teaching Hospital (KATH).

3.5 STATISTICAL TOOL

Statistical Package for Social Scientist version 13 (SPSS Inc., Chicago, IL) and Microsoft Excel with KADD-Stat modification (Microsoft Office Package, 2003) were used for all IDEs, descriptive statistics and univariate analysis. Statgraphics XV.I (Centurion) was used for multivariate analysis, except otherwise stated.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Retrospective Study

4.1.1.1 Epidemiological

A total of 569 cases were retrieved from the registry with 6.94 2.7 years as the mean age of patients (modal age = 7). These patients ranged in age from 1 to 16 years as illustrated by figure 2. Although the disease was fairly present in both sexes, the male sex dominated with 58.8% (334/568, $p < 0.001$) and the remaining were females, hence an overall male-female ratio of 1.42:1. The proportion for common sites of primary clinical presentation(s) of the 553 cases for which data was available is illustrated in figure 3. The lowest incidence of the disease was observed within the age ranges of 1-3 and 13-16 years (figure 4) with general male dominance at all ages except the range of 9-11 years where female dominance emerged ($p = 0.725$).

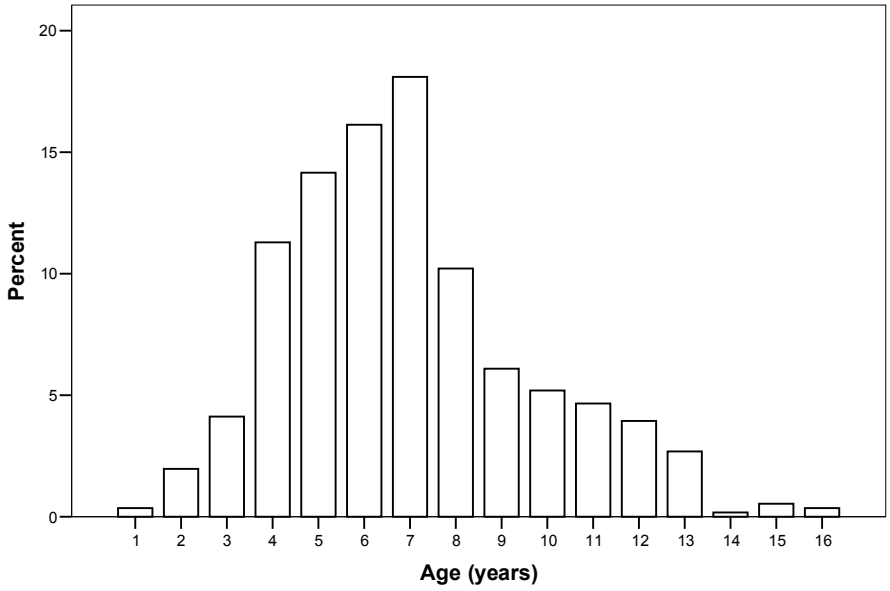
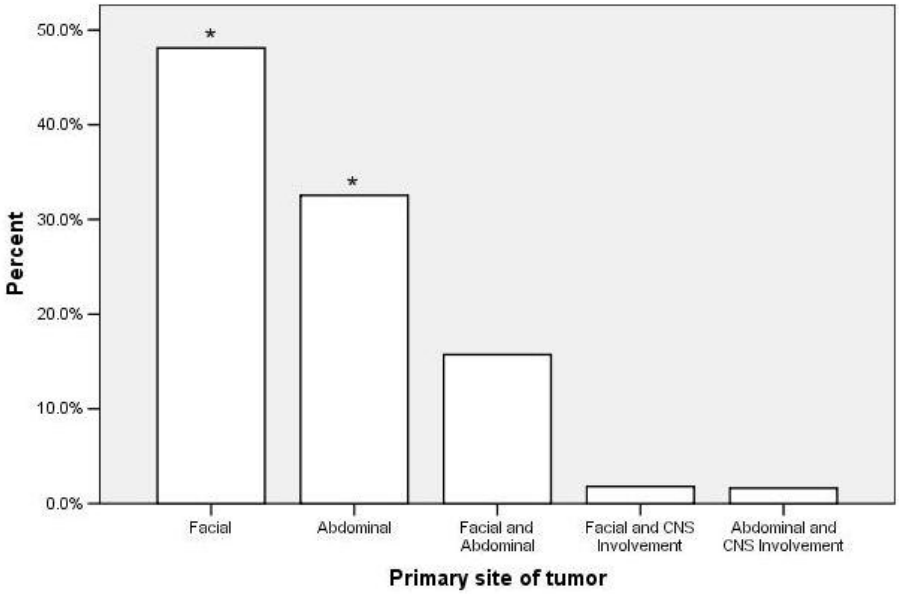
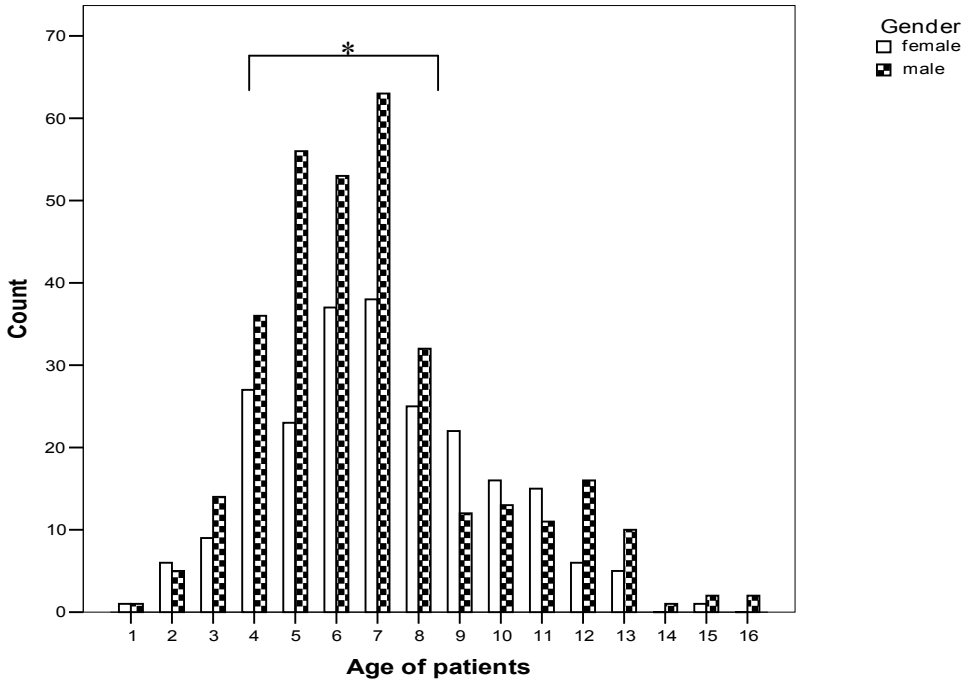


Figure 2: Overall age distribution of Burkitt's lymphoma patients (KATH, 2000-2007)



** Significant: p< 0.05*

Figure 3: Common sites of primary tumour presentation(s)



** Significant age range: p< 0.001 at 95% CI*

Figure 4: Age and gender distribution of cases

Albeit no significant correlation was found between site of tumour presentation and gender, more males presented with facial involvement (55.2%, M:F= 2.21:1) while abdominal involvement dominated among their female cohort (42.1%, M:F= 1:1.14). The other anatomical sites of primary involvement demonstrated varied percentages as shown in figure 5.

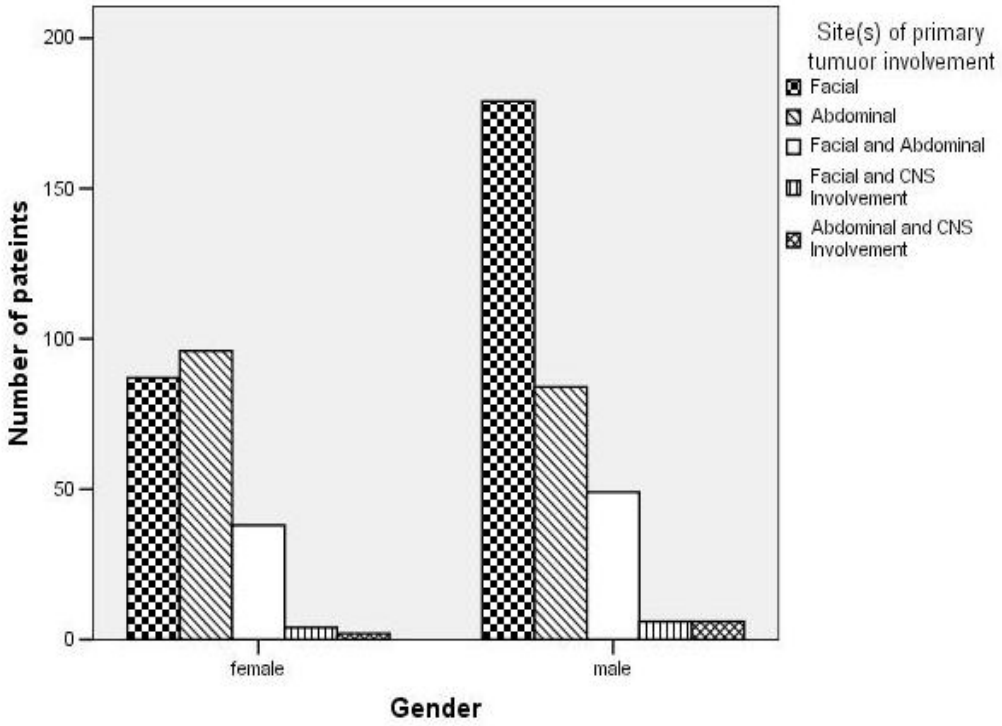


Figure 5: Site(s) of primary tumour presentation stratified by gender

Facial involvement was the most observed clinical presentation throughout the study period with highest incidence occurring in 2003 and 2005. The minimum incidence of facial presentation was observed in 2006 which sharply reverted within the preceding year. Interestingly, as the incidence of facial involvement either rises or falls at any time point, combined facial and abdominal presentation demonstrated a direct opposite trend as shown in Figure 6. Even though both facial (only) and abdominal (only) hiked in 2005, abdominal (like combined facial and abdominal cases) assumed a downward trend afterwards. Facial cases, on the other hand, assumed an upward trend while cases involving CNS virtually plateaued.

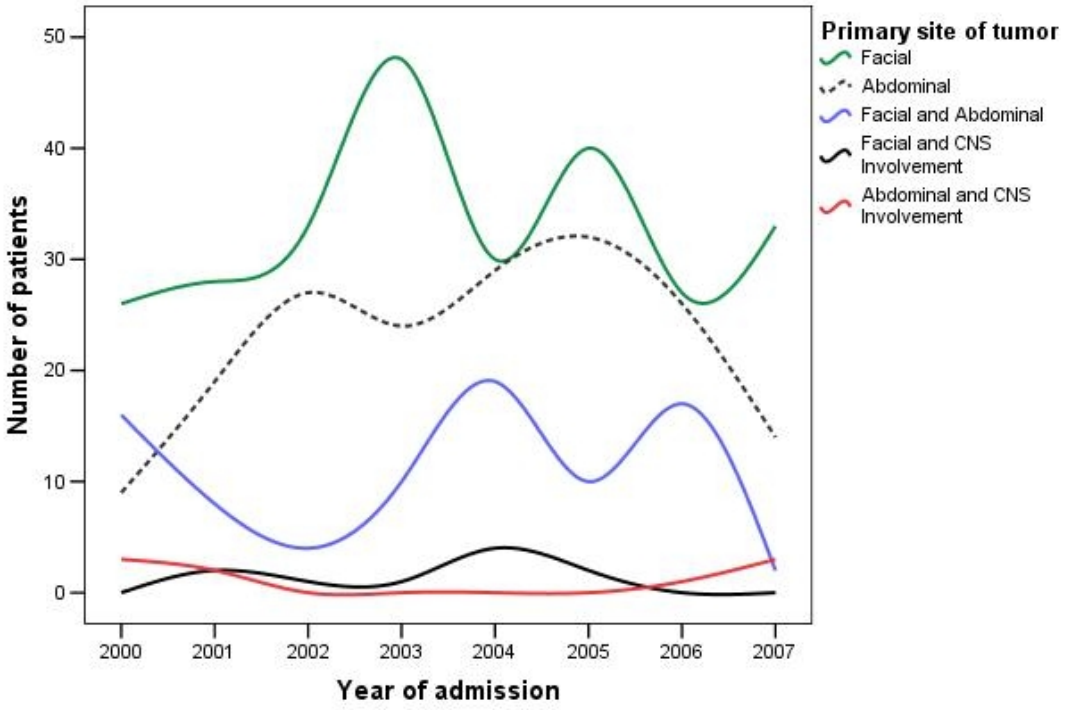


Figure 6: Trend of sites of primary tumour presentations (2000- 2007)

The frequency of facial involvement increased to a peak at 4 years, fell at 5 years to rise again to a highest peak at 7 years and decreased thereafter. There was a small rise from age 10 years to a minor peak at 12 years (Figure 7). Abdominal cases, as well as, combined facial and abdominal presentations demonstrated their highest incidence (11.5% and 7% increase from an average of 13 cases respectively) at around age six.

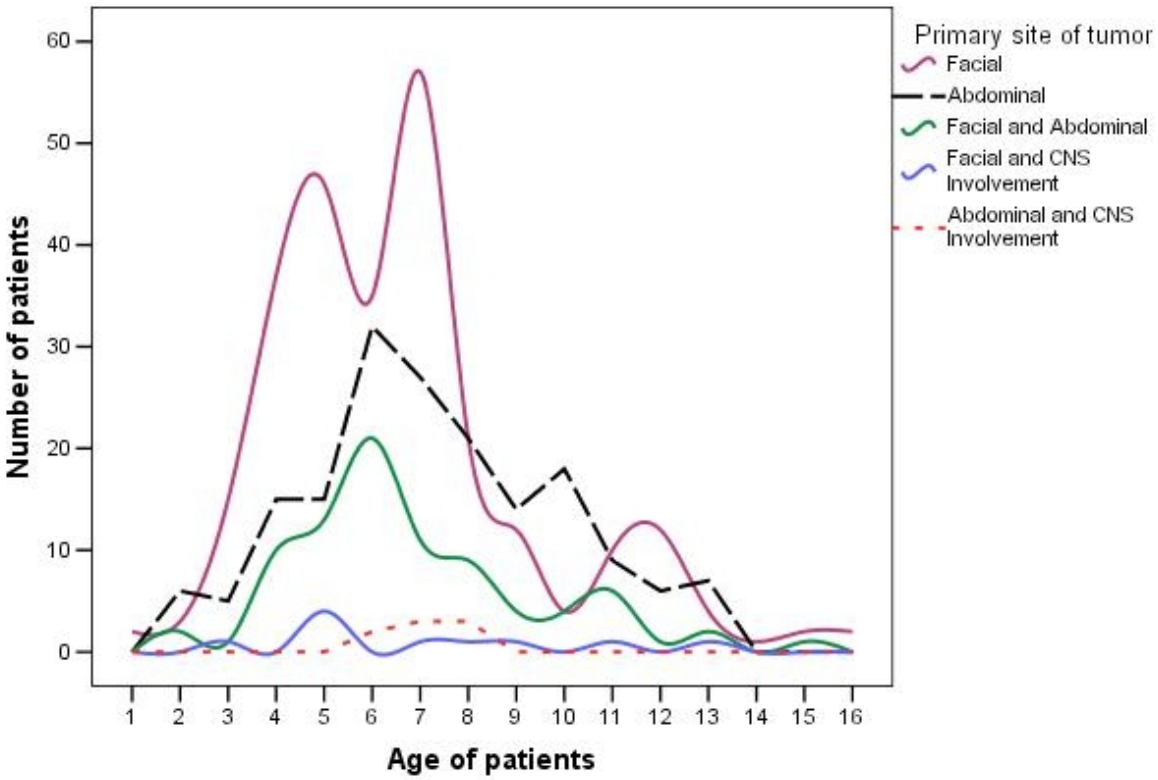


Figure 7: Trend of site(s) of primary tumour presentation(s) in relation to age of patients

To further investigate gender as a factor on the trend shown in Figure 6, the data was stratified as shown in Figure 8.

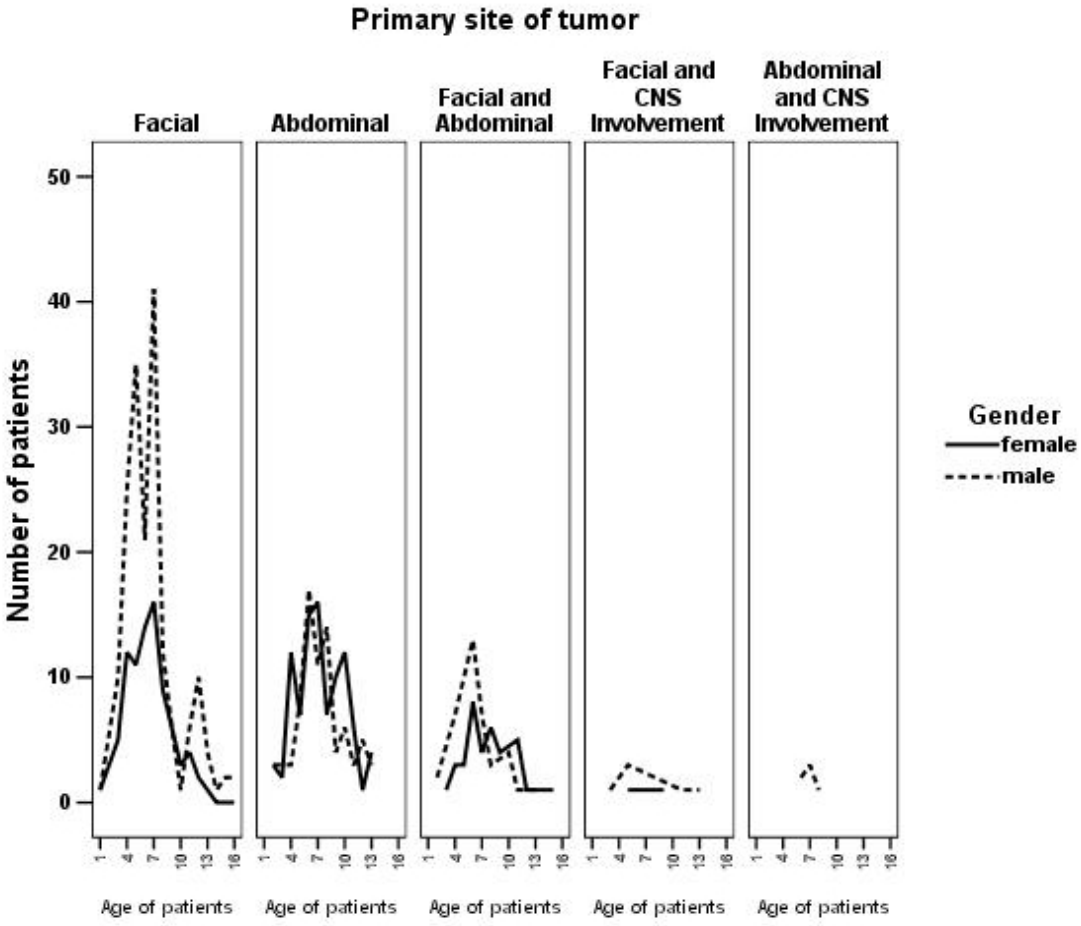


Figure 8: BL distribution with respect to gender, site of tumour presentation and age

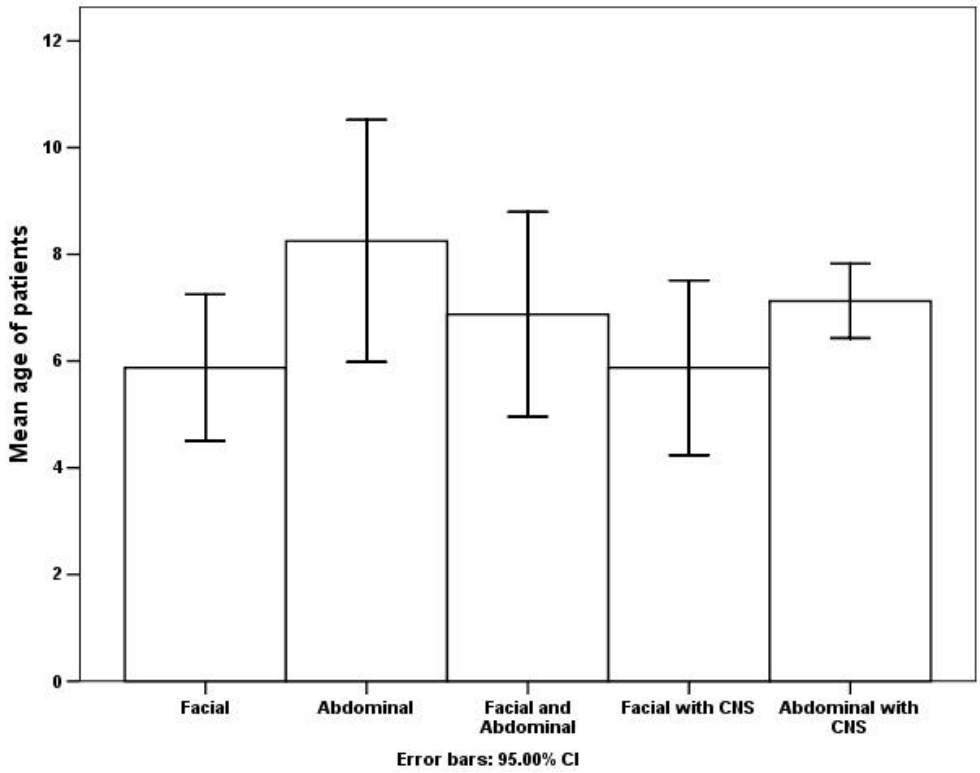


Figure 9: Mean age distribution of BL cases with site

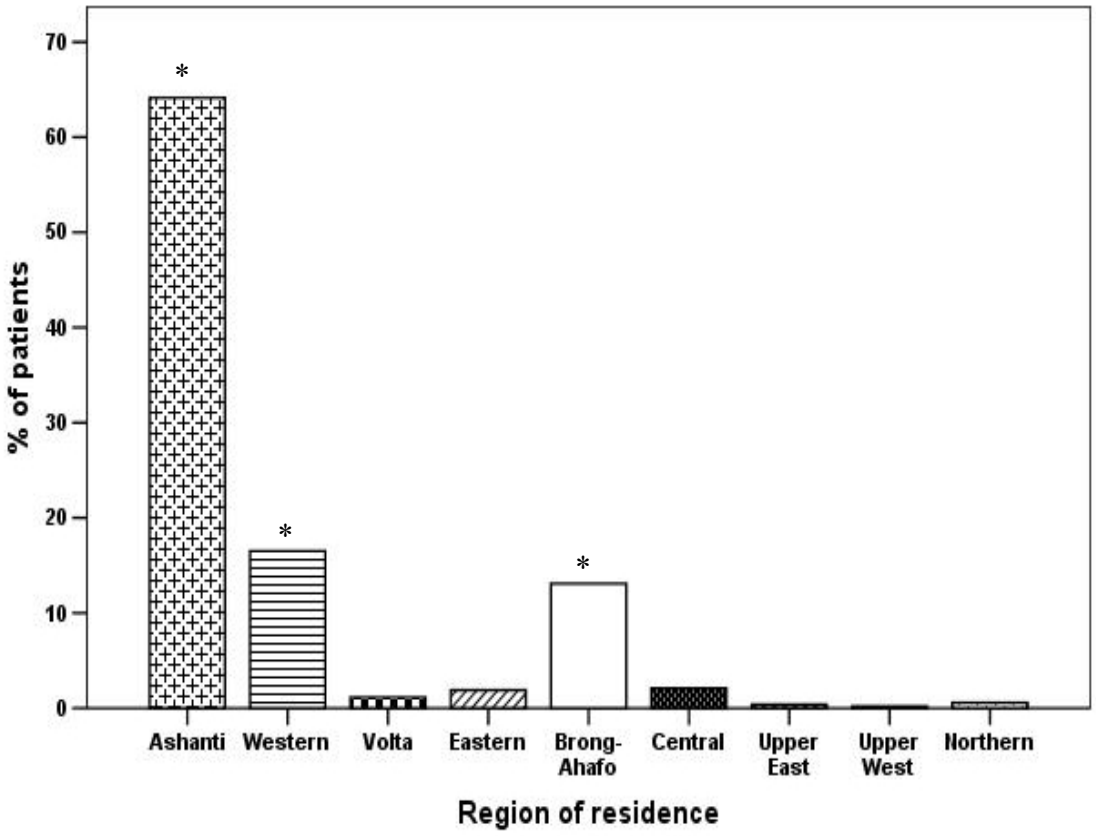
Figures 8 and 9 provide a vivid description of the clinical presentation burden of the disease on gender and age. On the average, BL cases that involves the face predominantly affect younger children (age: 6.6 2.7 years) with males being frequently involved (67.3%). Abdominal cases appear to be frequent among children of 7.3 2.6 years with equal dominance among the sexes. Cases that involves both facial and abdominal sites rightly assumes an intermediate mean age of presentation (compared to facial only and abdominal only) but with females affected later (age: 7.7 2.8 years) than their male cohort (6.2 2.2 years). Cases involving the CNS and other anatomical sites were less common. Males were the most affected (66.78%) cumulatively and early as well, indicated by table 1.

	Facial plus CNS		Abdominal plus CNS	
		(*n*)		(*n*)
Overall	7.1	3.1 (*10*)	7.1	0.8 (*8*)
Male	7.0	4.0 (*6*)	6.8	0.8 (*6*)
Female	7.3	1.7 (*4*)	8.0 (*2*)	

CNS= Central Nervous System *n*= number of cases

Table 3: Mean age and gender characteristics of BL cases involving either facial or abdominal site with CNS

Climate, vegetation and geographic location has long been associated with BL incidence (Burkitt, 1962; Haddow, 1963), therefore, the origin of all the recorded cases were analysed using the regional boundaries within Ghana as the grouping determinant. Among the ten regions of Ghana, Ashanti, Brong-Ahafo and Western (all within the forest zone) were the regions that presented significant number of cases ($p< 0.05$) as shown in Figure 10. From Figure 11 and Table 2 (Appendix A), the general trend of facial dominance followed by abdominal, facial and abdominal, abdominal and CNS and finally facial and CNS were observed among the regions. It could be inferred from this data that all the various sites of tumour presentation are common in all the regions of Ghana such that no region is denotatively associated with particular site(s).



** Significant regions: p<0.001 at 95% CI*

Figure 10: BL incidence among the ten regions of Ghana (KATH, 2000-2007)

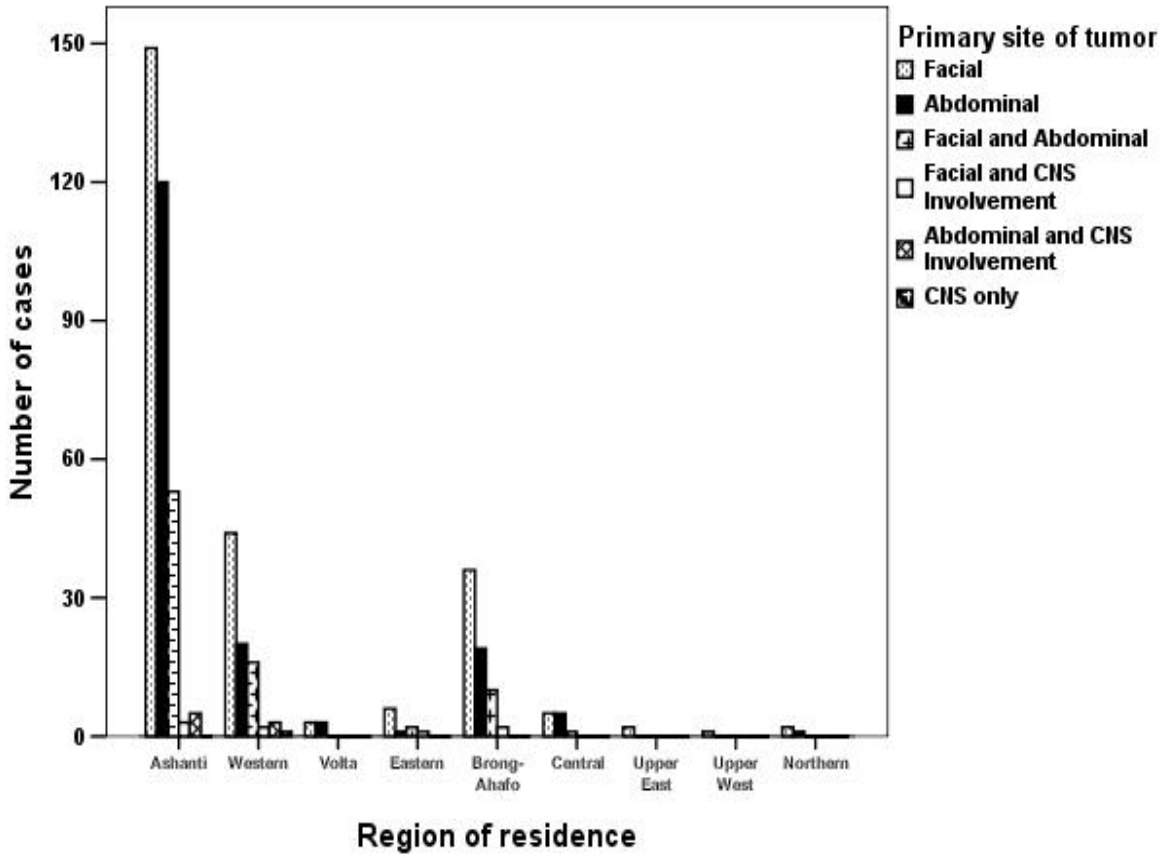


Figure 11: Sites of primary BL presentation(s) among the ten regions of Ghana

4.1.1.2 Prognostic indices modelling and overall survival (OS) analysis

A total of 180 patients had pre-treatment biochemical data in addition to their general clinical data in their records. These data were extracted and used for prognostic indices modelling and overall survival (OS) analysis. Table 4 gives a detailed distribution of the total number and the proportion which experienced "treatment event" with respect to each prognostic parameter of interest. Using the log-rank test for significance, Chloride (Cl), Creatinine (Crt), Lactate dehydrogenase (LDH) and Stage were identified as independent

prognostic factors with 95% confidence. From their survival curves (Figures 12- 15), it is evident that patients who were within the normal range for Cl, Crt, LDH and stage I at diagnosis had better overall survival (OS) than their cohorts in the other categories. Overall survival for patients who had lower than normal Crt level at diagnosis appeared to be better than the other categories but upon the removal of the category "Crt greater then normal", the difference (prognostic power) was no longer significant ($p > 0.05$).

Table 4: Summary of prognostic parameters

Categorical clinical and biochemical variables	Total No.	Events (%)	*p*-value
Age			
> 8 years	73	86.3	0.568
< 8 years	104	91.3	
Cl			
< normal	20	95.0	**0.039***
within normal	125	92.0	
Alk phos			
> normal	14	100.0	0.267
within normal	48	89.6	
Crt			
< normal	92	90.2	
within normal	53	90.6	**< 0.001***
> normal	8	100	
Gender			
M	113	89.4	0.681
F	66	89.4	
LDH			
> 1 fold normal	126	91.3	**0.003***
within normal	41	82.9	
K			
< normal	8	75.0	0.288
within normal	135	93.3	
Site			
F	69	84.1	
A	77	93.5	0.203
FA	23	87.0	
any with CNS	9	100.0	
Na			
< normal	9	100.0	0.224
within normal	134	91.8	
Stage			
I	60	81.7	
II	85	94.1	**0.018***
III-IV	33	90.9	
UA			
< normal	11	90.9	
within normal	46	91.3	0.548
> normal	4	75.0	

Cl: Choride, Alk phos: Alkaline phosphate, Crt: Creatinine, M: Male, F: Female, K: Potassium, F: Facial, A: Abdominal, CNS: Central nervous system (usually paraplegia), Na: Sodium, UA: Uric Acid

* significant at 95% Confidence interval (CI)

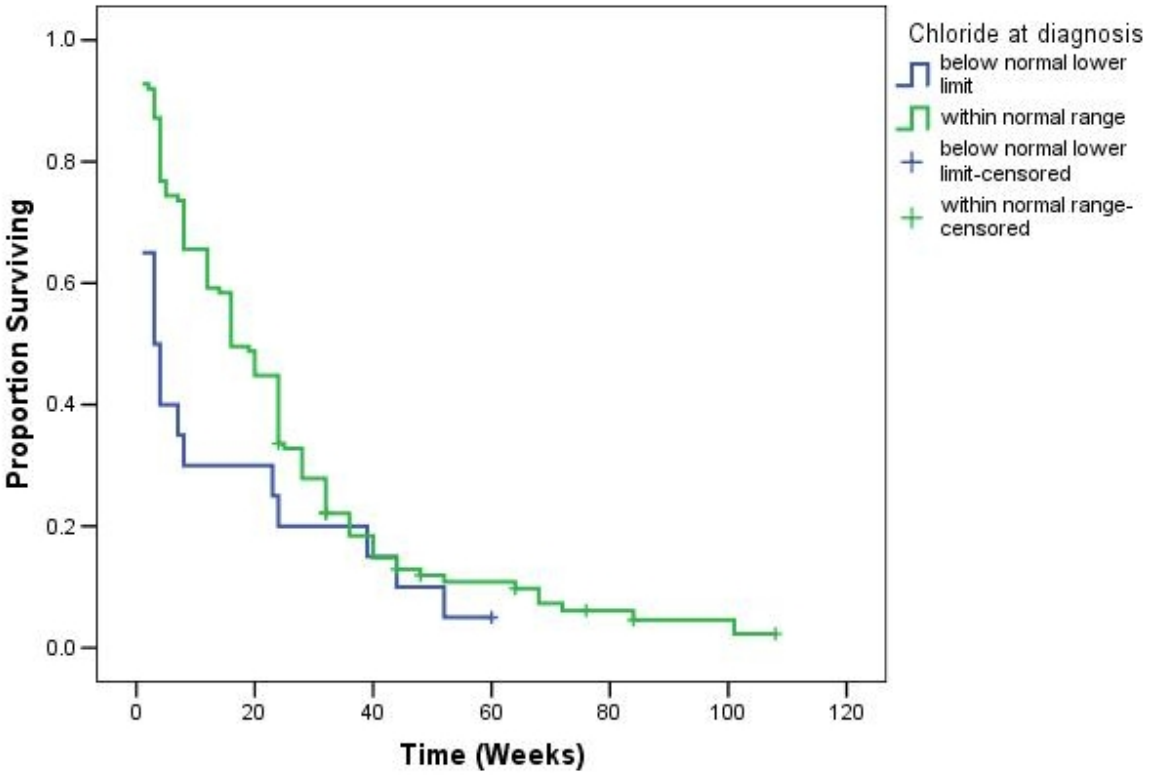


Figure 12: Overall survival (OS) of BL patients with respect to serum chloride level at diagnosis

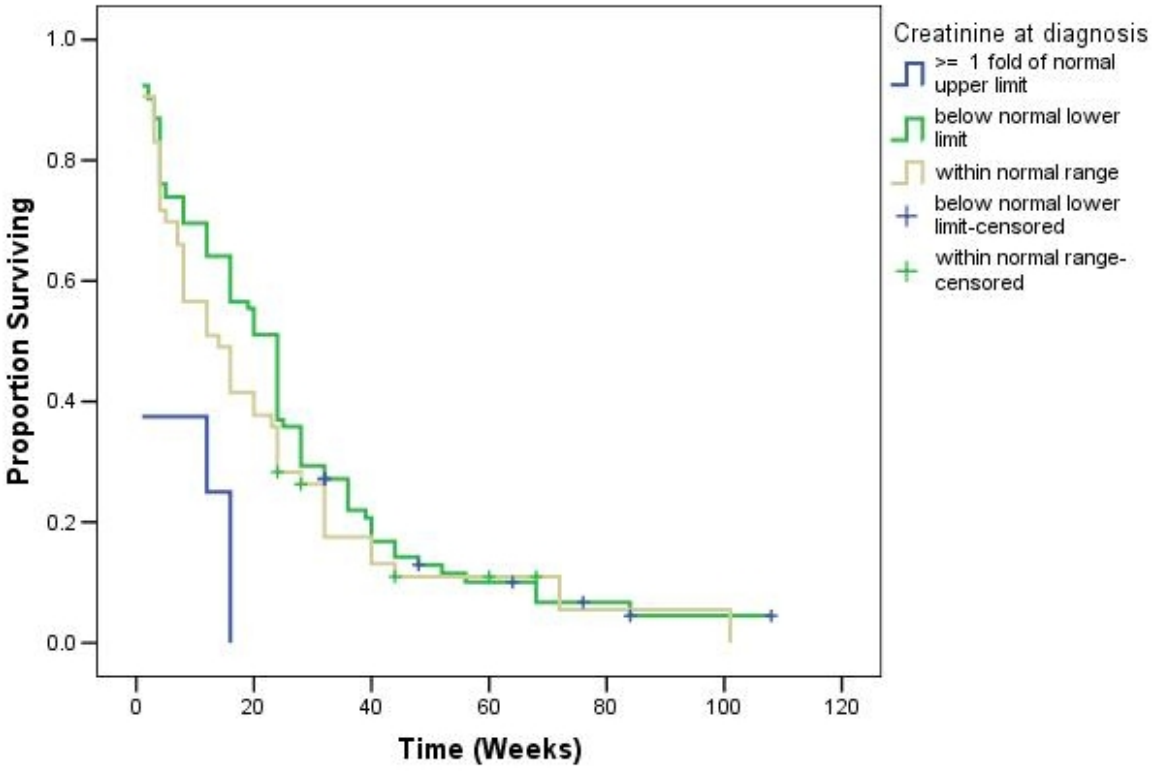


Figure 13: Overall survival of BL patients according to serum creatinine level at diagnosis

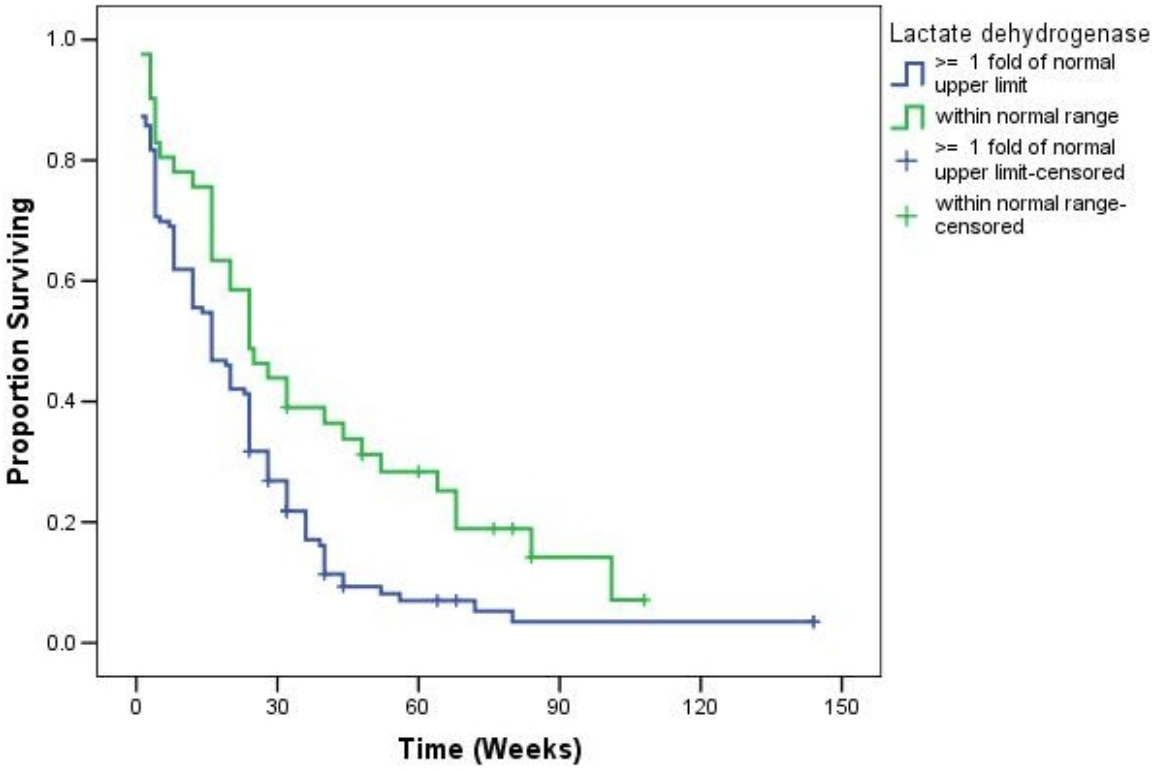


Figure 14: Overall survival (OS) of BL patients according to serum LDH level at diagnosis

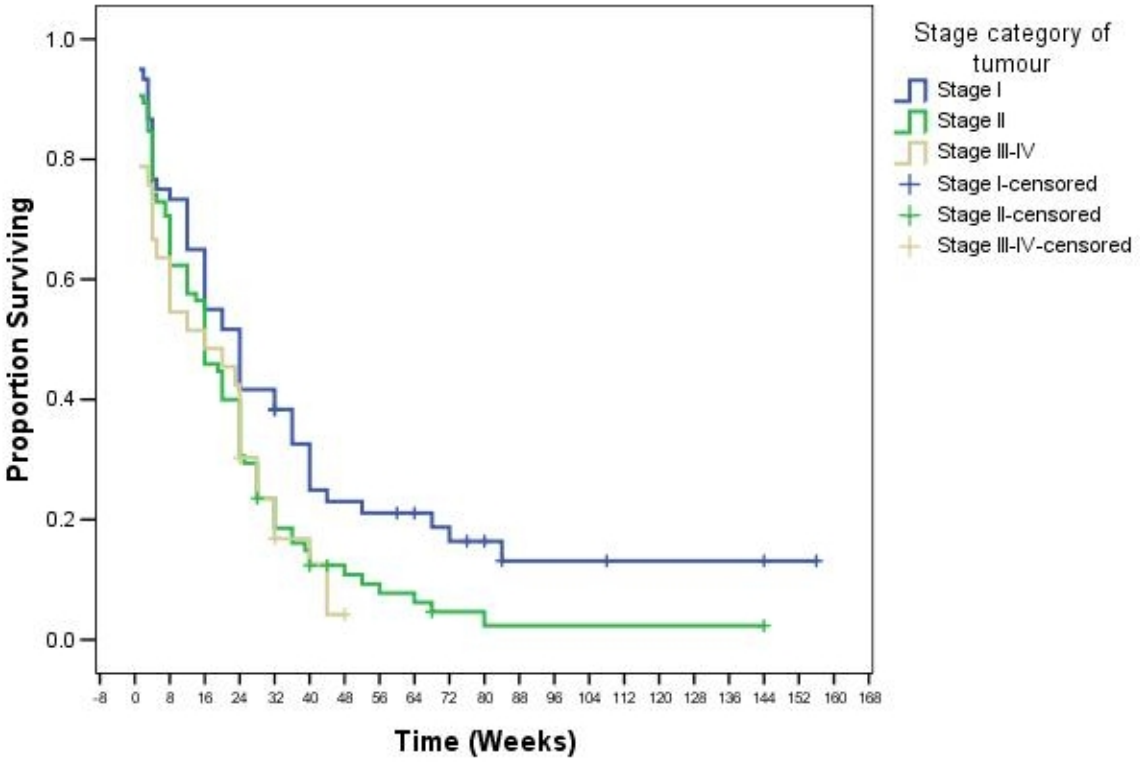


Figure 15: Overall survival (OS) of BL patients with respect to Ann Arbor tumour stage classification at diagnosis

These four independent prognostic factors were employed as covariates in Cox proportional hazard modeling using a forward stepwise algorithm (Forward: LR method). In this process, a variable is retained at a particular step during the model-building process only when it significantly ($p< 0.05$) contributes to the model and it is removed when the change it brings into the model is not significant ($p> 0.10$) to generate a hazard function. The hazard function measures the potential for an event to occur at time *t*, given that the event has not yet occurred. Larger values of the hazard function indicate greater potential for the event to occur. The value of the observed hazard [Exp (B)] is the product of the baseline hazard and the covariate effect. Thus, the ratio of the hazards for any two cases at any time period is the ratio of their

covariate effects (Proportional hazard assumption). Using category "within normal range" as the reference category (Appendix B) for stepwise comparison (without interactions) in the model, Crt, LDH and Stage were observed to contribute significantly to patient OS (Tables 5a and 5b). However, when interactions were permitted, Cl also showed some level of significance for OS (Tables 6a and 6b). The overall survival hazard function is shown graphically in Figure 16.

Table 5a: Omnibus Tests of Model Coefficients

Step	Overall (score)			Change From Previous Step			Change From Previous Block		
	Chi-square	df	Sig.	Chi-square	df	Sig.	Chi-square	df	Sig.
1(a)	15.167	2	0.001	10.403	2	.006	10.403	2	.006
2(b)	25.708	3	<0.001	11.561	1	.001	21.964	3	< 0.001
3(c)	31.248	5	<0.001	6.525	2	.038	28.489	5	< 0.001

a Variable(s) Entered at Step Number 1: Crcat
b Variable(s) Entered at Step Number 2: LDHcat
c Variable(s) Entered at Step Number 3: stage

Table 5b: Variables in the Equation (Model)

		df	Sig.	Exp[B]	95.0% CI for Exp[B]	
					Lower	Upper
Step 1	Crcat	2	.001			
	Crcat(1)	1	.159	.751	.504	1.118
	Crcat(2)	1	**.005**	**3.039**	1.388	6.653
Step 2	LDHcat	1	**.001**	**2.183**	1.352	3.526
	Crcat	2	.002			
	Crcat(1)	1	.445	.854	.571	1.279
	Crcat(2)	1	**.003**	**3.343**	1.517	7.367
Step 3	stage	2	.041			
	stage(1)	1	**.015**	**1.744**	1.113	2.734
	stage(2)	1	.492	1.234	.677	2.247
	LDHcat	1	**.004**	**2.038**	1.250	3.324
	Crcat	2	.001			
	Crcat(1)	1	.440	.850	.562	1.285
	Crcat(2)	1	**.002**	**3.593**	1.619	7.975

Exp[B]= Proportional hazard estimator/ ratio

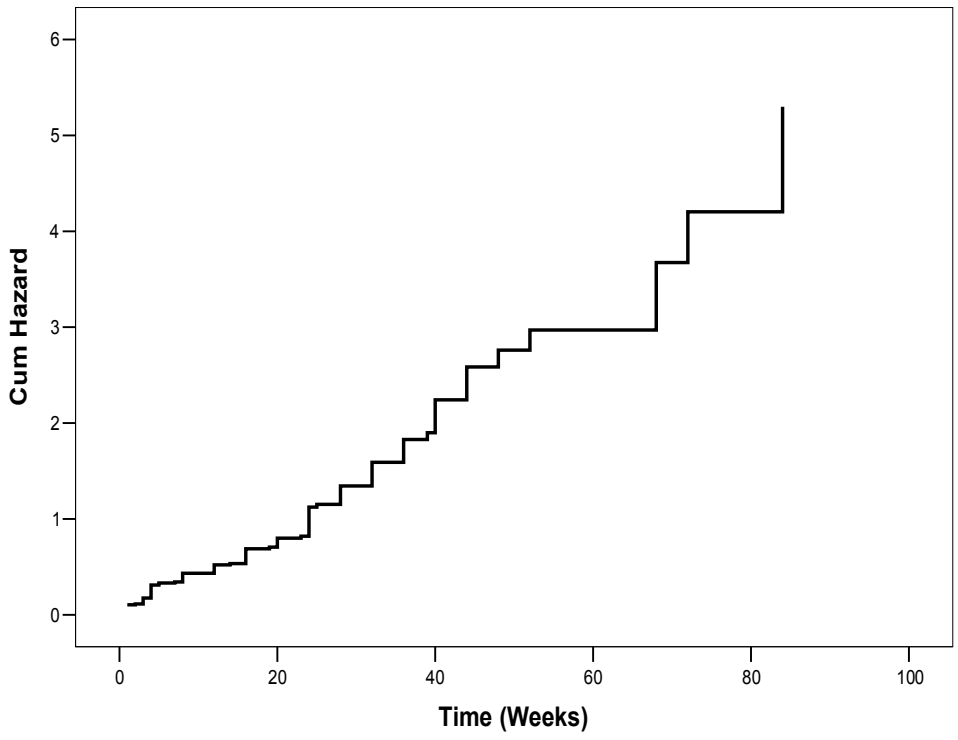


Figure 16: Hazard function for OS at means of covariates

Table 6a: Omnibus Tests of Model Coefficients

Step	Overall (score)			Change From Previous Step			Change From Previous Block		
	Chi-square	df	Sig.	Chi-square	df	Sig.	Chi-square	df	Sig.
1(a)	25.112	4	<0.001	11.044	4	.026	11.044	4	.026

a: Variable(s) Entered at Step Number 1: Clcat*Crcat*LDHcat*stage

Table 6b: Variables in the Equation

		df	Sig.	Exp[B]	95.0% CI for Exp[B]	
					Lower	Upper
Step 1	Clcat*Crcat*LDHcat*stage	4	.002			
	Clcat*Crcat(1)*LDHcat*stage(1)	1	.113	1.954	.854	4.469
	Clcat*Crcat(2)*LDHcat*stage(1)	1	**.001**	**11.980**	2.648	54.187
	Clcat*Crcat(1)*LDHcat*stage(2)	1	.418	1.514	.555	4.128
	Clcat*Crcat(2)*LDHcat*stage(2)	1	**.018**	**11.980**	1.544	92.967

Exp[B]= Proportional hazard estimator/ ratio

4.1.2. Prospective Study

4.1.2.1 Trend of Biochemical Indices during Cyclical Chemotherapy

A total of 76 patients were recruited for the prospective study but some were lost though death or discontinuation of medical review at the end of 84 days at 42 days intervals (Table 7). Forty-two days interval was chosen for sampling and analysis of patients' blood in order to estimate the effect of two treatment cycles on patients at each sampling point. From table 7, the changes in the levels of serum LDH, Na and Ca during chemotherapy were

significantly different using the Kruskal-Wallis test. Students-Newman-Keuls (SNK) multiple range test showed independent differences within these biochemical indices at diagnosis (Dx), 42nd day, 84th day and with control healthy age-sex-matched children, figures 17, 18 and 19 for LDH, Na and Ca respectively.

Table 7: Median (range) score for serum LDH, Crt, Cl, Na, K, Phos, UA and Ca in control and patients with BL over time

Test	Control (C) *n =20*	Diagnosis (Dx) *n = 76*	42nd Day *n = 45*	84th Day *n = 28*	*p*-value
LDH	189.5 (131- 262)	405.5 (176- 3417)	281 (141- 729)	258.5 (76- 996)	**< 0.001***
Crt	53 (44.2- 79.6))	53 (27.7- 341)	52.9 (17.7- 353.6)	47.7 (24.8- 99)	0.301
Cl	100.33 (99.1- 101.34)	99 (73- 128)	100 69- 110)	101.18 (59- 127)	0.651
Na	142.56 (142.44- 142.76)	137 (104- 150)	137 (95- 147)	140 (116- 446)	**< 0.001***
K	4.14 (4.03- 4.31)	4.4 (2.9- 7.9)	4.3 (1.9- 6.4)	4.2 (2.2- 6.4)	0.132
Phos	1.5 (1.3- 1.8)	1.5 (0.5- 5.23)	1.4 (0.6- 3.97)	1.4 (0.6- 2.1)	0.374
UA	220.15 (142.8- 291.6)	255.9 (6- 1707.7)	255.85 (5.9- 940)	261.8 (113.1- 678.3)	0.096
Ca	2.5 (2.4- 2.7)	2.42 (0.1- 10)	2.4 (1.85- 9.8)	2.4 (1.55- 2.83)	**0.013***

LDH: Lactate dehydrogenase, Crt: Creatinine, Cl: Chloride, Na: Sodium, K: Potassium, Phos: Phosphorus, UA: Uric Acid, Ca: Calcium, *n*: number of patients

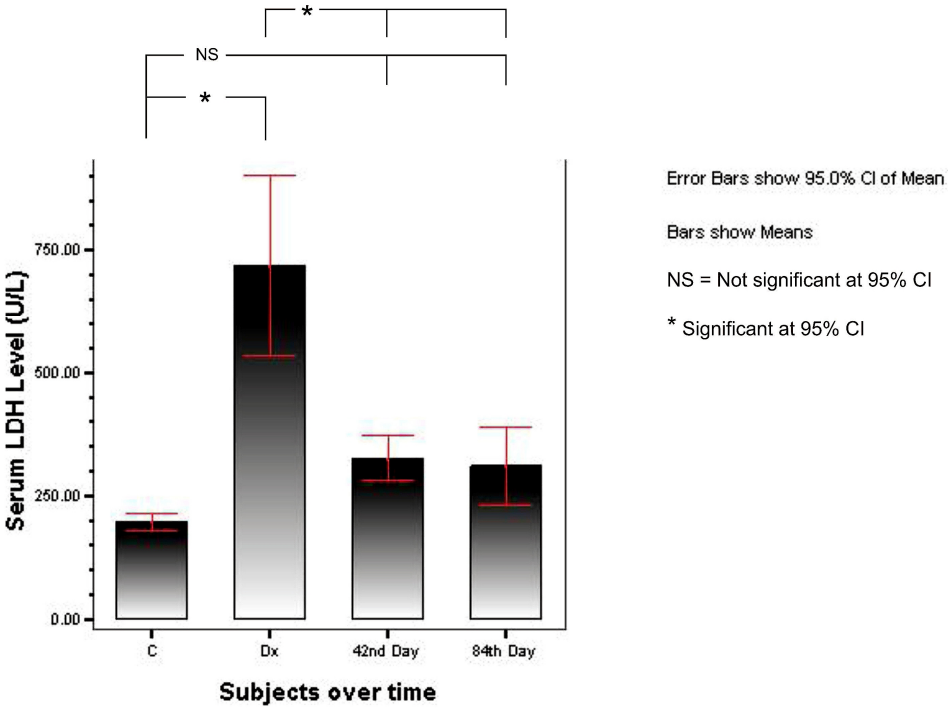


Figure 17: Schematic representation of the mean (SEM) score for serum level of LDH in controls (C) and in patients with BL at diagnosis (Dx) through 84-day period

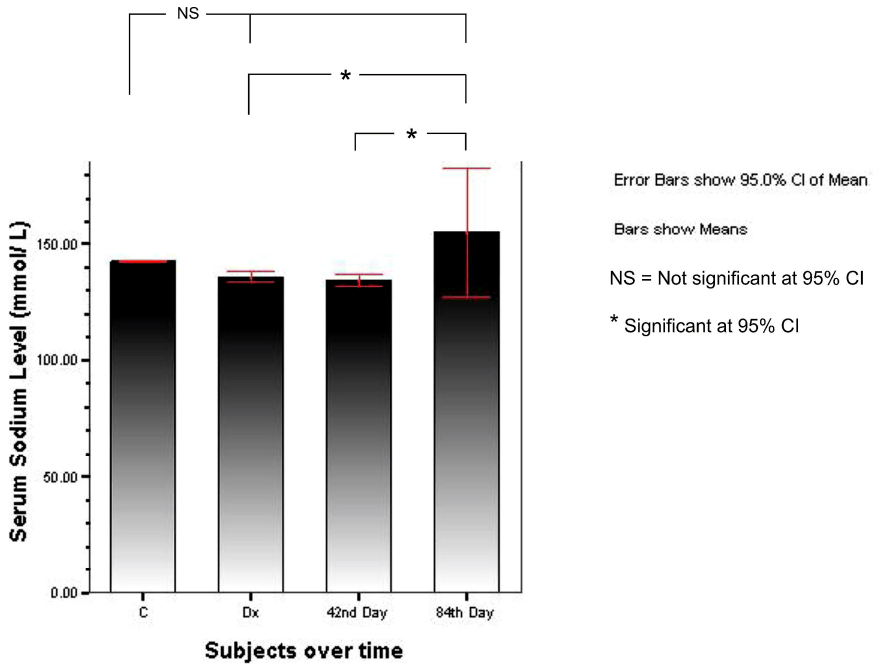


Figure 18: Schematic representation of the mean (SEM) score for serum level of sodium in controls (C) and in patients with BL at diagnosis (Dx) through 84-day period

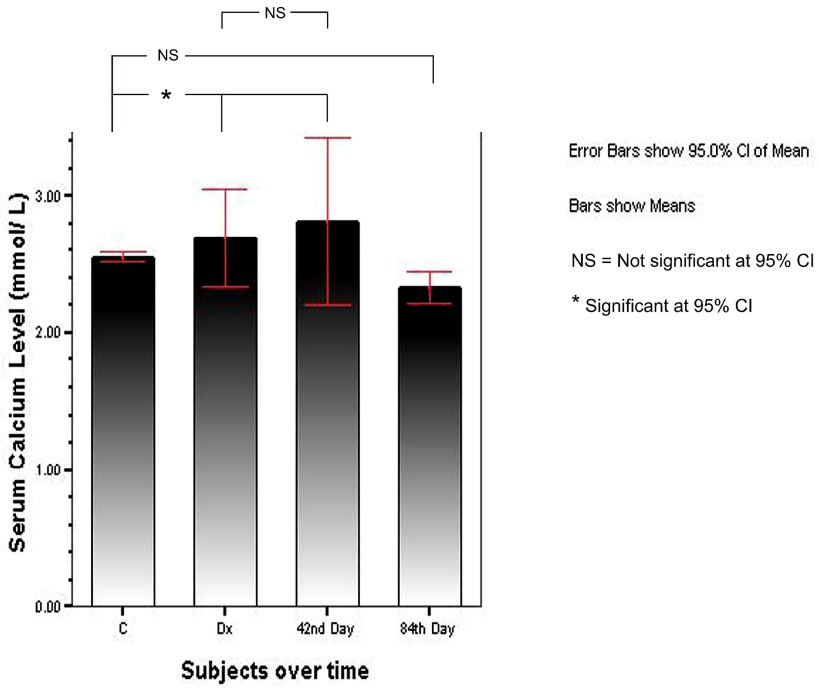


Figure 19: Schematic representation of the mean (SEM) score for serum level of calcium in controls (C) and in patients with BL at diagnosis (Dx) through 84-day period

To further investigate the prognostic significance of these biochemical indices, the final 28 patients were used as test subjects and observed for another three month. Two main groups, treatment event and remission, were defined. Treatment event group (72.7%) represented patients who experienced either relapse or death within the test period while 27.3% of the patients were still alive and disease-free (remission group) at the end of the test period. Creatinine, chloride and phosphorus showed no significant ($p>$ 0.05) association with these outcomes (graphs not shown). Potassium, uric acid, lactate dehydrogenase, calcium and sodium demonstrated significant ($p<$ 0.05) associations with the two defined outcome groups at their mean serum levels, illustrated by Figures 20, 21, 22, 23 and 24 respectively.

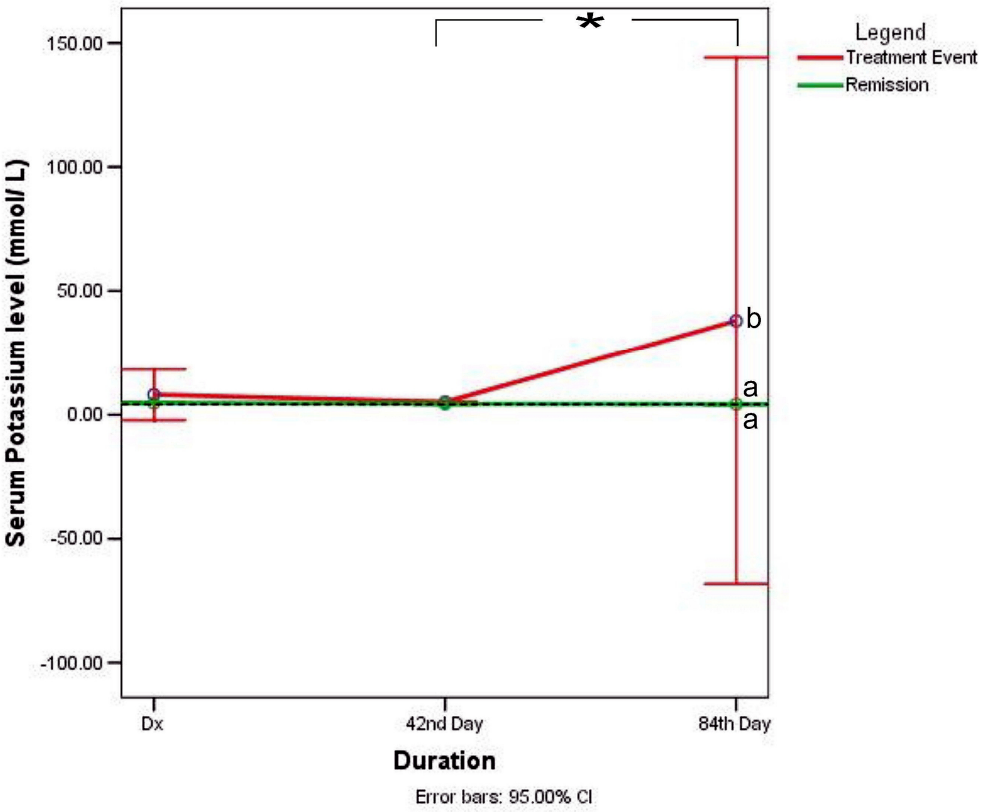


Figure 20: Effect of cyclical chemotherapy on serum potassium in children during BL treatment. Values are means SEM. Dotted horizontal line is the mean serum potassium of 20 healthy control children. The single asterisk indicates a significantly different serum potassium level between time points [$p < 0.05$ by repeated measures of analysis of variance (ANOVA)] The mean SEM values at each time point with different alphabets are significantly different ($p< 0.05$ by Scheffe's multiple comparison test.).

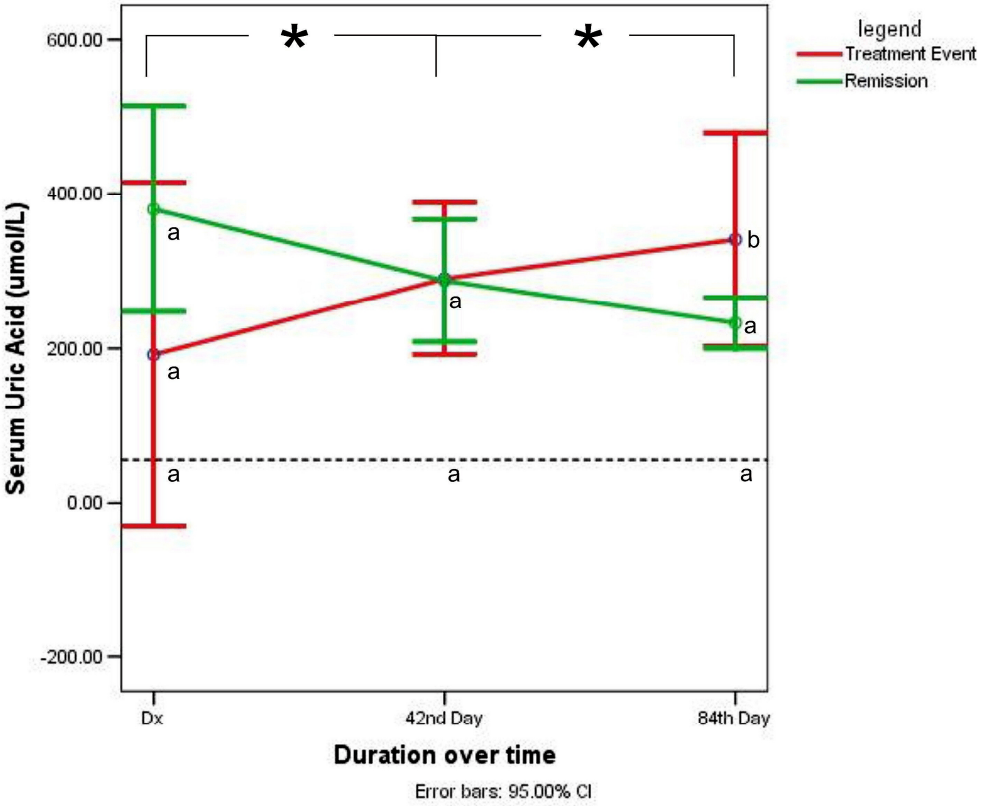


Figure 21: Effect of cyclical chemotherapy on serum uric acid in children during BL treatment. Values are means SEM. Dotted horizontal line is the mean serum uric acid of 20 healthy control children. The single asterisk indicates a significantly different serum uric acid level between time points [$p < 0.05$ by repeated measures of analysis of variance (ANOVA)] The mean SEM values at each time point with different alphabets are significantly different ($p < 0.05$ by Scheffe's multiple comparison test.).

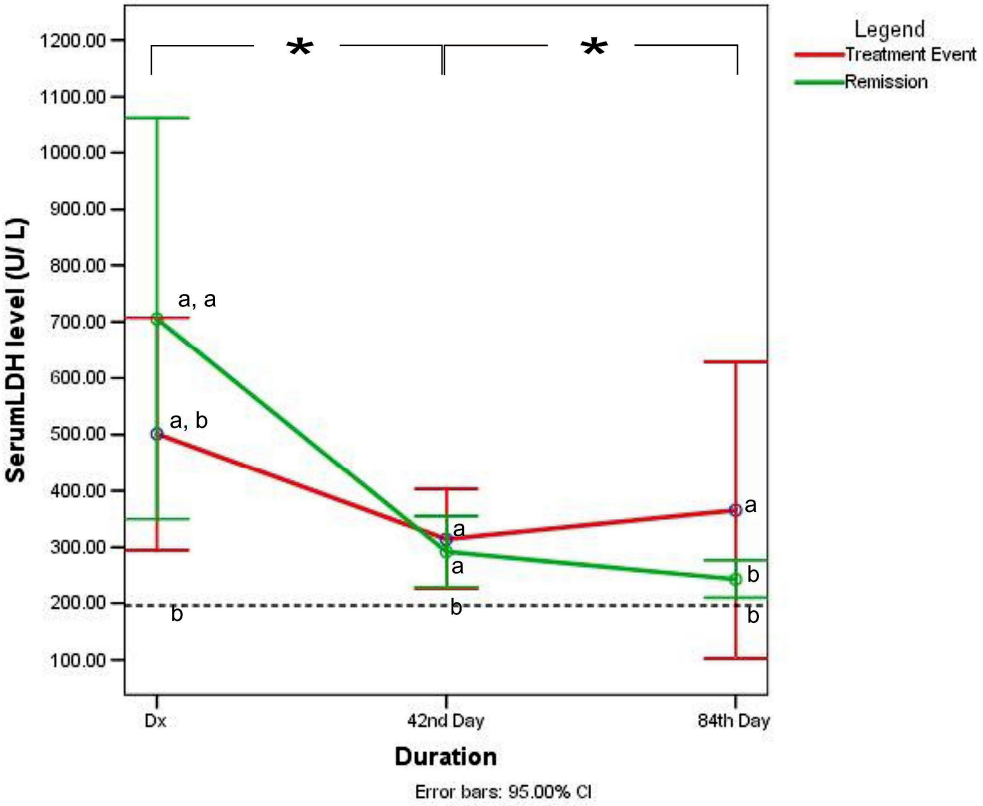


Figure 22: Effect of cyclical chemotherapy on serum lactate dehydrogenase (LDH) in children during BL treatment. Values are means SEM. Dotted horizontal line is the mean serum LDH of 20 healthy control children. The single asterisk indicates a significantly different serum LDH level between time points [$p < 0.05$ by repeated measures of analysis of variance (ANOVA)] The mean SEM values at each time point with different alphabets are significantly different ($p< 0.05$ by Scheffe's multiple comparison test.).

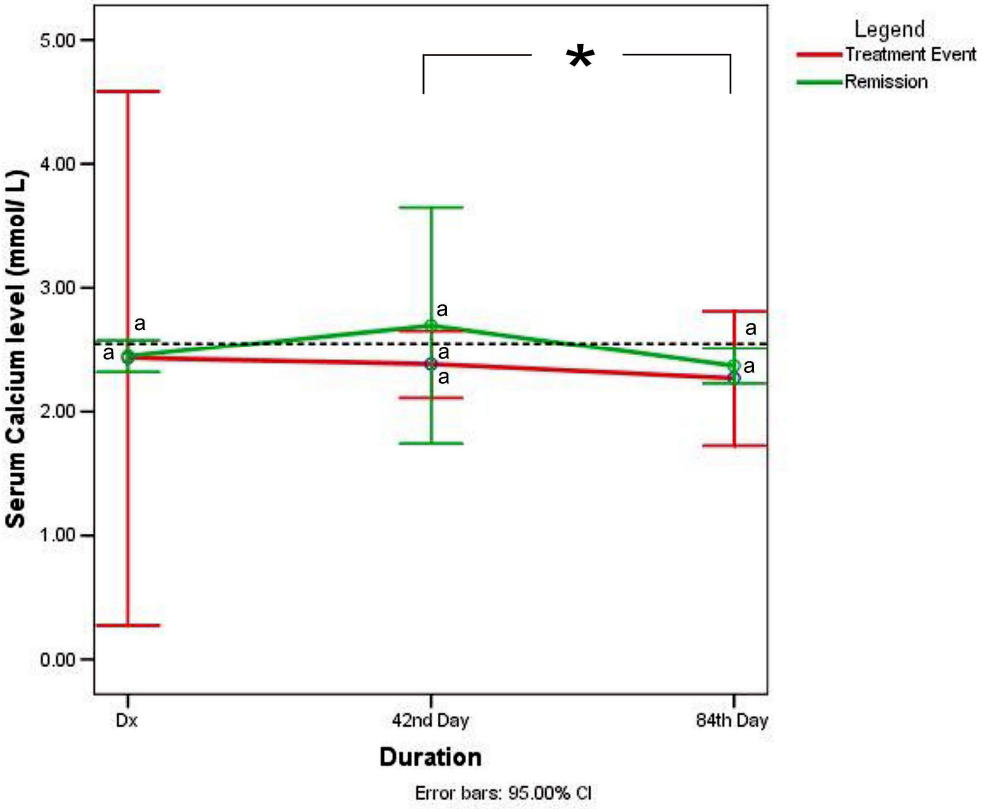


Figure 23: Effect of cyclical chemotherapy on serum calcium in children during BL treatment. Values are means SEM. Dotted horizontal line is the mean serum calcium of 20 healthy control children. The single asterisk indicates a significantly different serum calcium level between time points [$p < 0.05$ by repeated measures of analysis of variance (ANOVA)] The mean SEM values at each time point with different alphabets are significantly different ($p < 0.05$ by Scheffe's multiple comparison test).

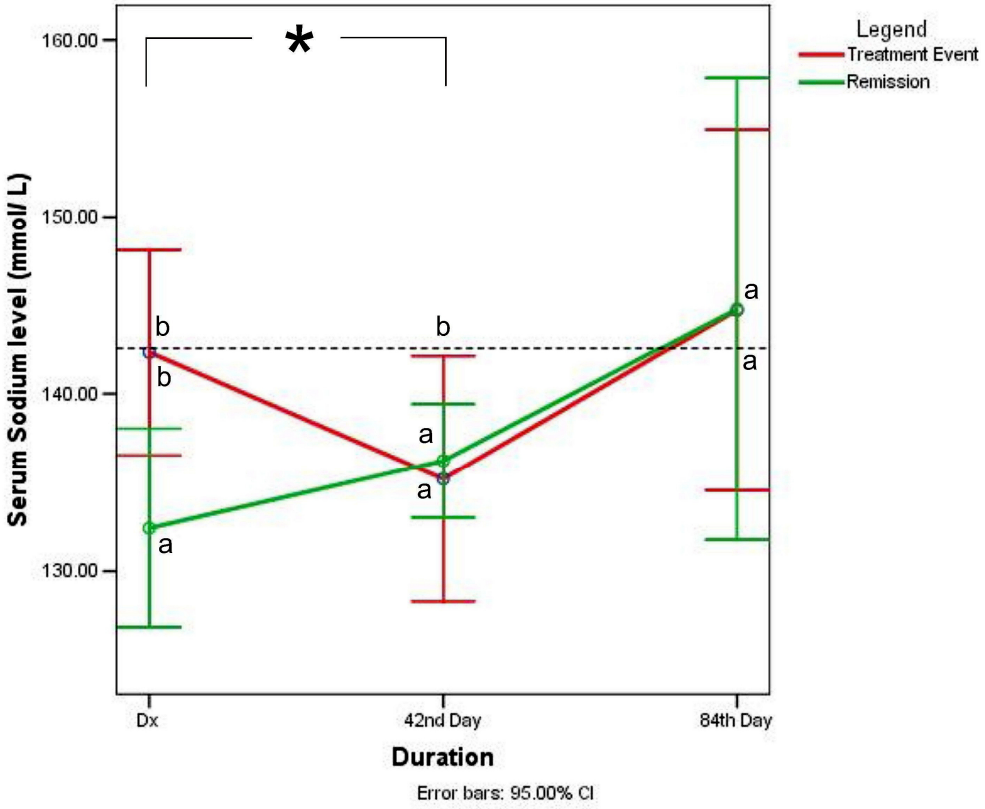


Figure 24: Effect of cyclical chemotherapy on serum sodium in children during BL treatment. Values are means SEM. Dotted horizontal line is the mean serum sodium of 20 healthy control children. The single asterisk indicates a significantly different serum sodium level between time points [$p < 0.05$ by repeated measures of analysis of variance (ANOVA)] The mean SEM values at each time point with different alphabets are significantly different ($p < 0.05$ by Scheffe's multiple comparison test.).

4.2 DISCUSSION

4.2.1. Retrospective study

4.2.1.1. Epidemiological study

Burkitt's lymphoma has long been tagged 'endemic' to Africa because it uniquely stands out as the predominant childhood malignancy (Magrath, 1990, Orem *et al.*, 2007). Scanty information can be found on this important juvenile neoplasm in both local and international literature on Ghana, which is among the worst hit countries in the world (Nkrumah and Olweny, 1985). According to Orem and colleagues (2007), Kyadondo county, Uganda recorded the highest BL incidence, followed by Malawi inferring from cancer registries available in Africa. The lack of a coordinated cancer registry in Ghana makes it impossible to estimate the overall incidence of BL in the country. Nkrumah and Olweny (1985) reported an annual BL incidence of approximately forty cases for an eleven-year study conducted in Accra, Ghana. This current study reported not less than eighty cases annually at KATH in Kumasi, Ghana. This figure might even be an underestimation because of limited treatment centres where people could easily report. The overall preponderance ratio among the sexes was M:F: 1.42:1 and this is comparable to ratios reported elsewhere in Africa such as 1.5:1 for Uganda (Orem *et al.*, 2007), 1.8:1 for Nigeria (Kagu *et al.*, 2004) and 1.7:1 for a 1985 Ghanaian report (Nkrumah and Olweny, 1985). In contrast, Algeria, Brazil and Europe (Pedrosa *et al.*, 2007; Aboulola *et al.*, 1985; Philip, 1985) exhibited higher male-to-female ratios of 2.26:1, 2.4:1 and 3.7:1 respectively for the few cases that were observed. The reason why most western countries

exhibit even higher male dominance is unclear but this has been well documented by Sandlund *et al.*, 1996.

Even though an overall mean age of approximately 6.9 (mode: 7) was observed, which is comparable to literature across the globe (Sandlund, Downing and Crist, 1996, Orem *et al.*, 2007; Donati *et al.*, 2004, Nkrumah and Olweny, 1985, Wackenhut and Barnwell, 1979), there was an intriguing observation as to the relationship between specific age range (within 95% CI) and primary site of tumour presentation. All facial involvements were seen to dominate among younger children (4-7 years), abdominal cases dominated within the age range of 6-11 years and cases involving combined facial and abdominal took an intermediate range of 5-9 years, illustrated by Figures 8 and 9. The overall age range for this study was 1-16 years and in reference to Parkins *et al.*, 2007, who observed an age range of 2-14 for BL patients in their study at Korle-Bu in Accra, one can speculate that BL is rare among children less than a year old in Ghana. Though, NHLs are said to have no clear age-related incidence (Forestier and Schmiegelow, 2005), this study identified Ghanaian children of age 4-8 years to be significantly at risk for BL.

After about age 10, facial presentations decrease in incidence, even among males, but abdominal cases remain fairly high, especially among females (Figures 7 and 8). The general trend is that facial cases manifest at early age with male dominance while females dominates abdominal presentations,

which tend to appear at a latter age, with respect to general BL age range. Even though this observation is subtle, it raises the question of whether sex and age influence whatever factor(s) that dictate the preferred site(s) of tumour presentation. It is worth noting that malaria incidence and mortality among Ghanaian children, in general, have been reported to assume an age-sex pattern whereby males exhibit higher susceptibility until puberty age when there is a reverse in incidence among the sexes (Adams *et al*, 2004). These observations therefore call for further studies into the age-sex patterns of both BL and malaria to define a putative crossroad of these diseases.

Approximately 3% of the total patients with BL had CNS involvement which suggests that the nervous system is not a common site of primary involvement compared to either facial or abdominal clinical presentations among Ghanaian children (Figure 3, Table 3). The observation that facial and combined facial and abdominal presentations exhibit direct opposite incidence (Figure 6) needs validation from other BL registries. Once verified, clinical-presentation prediction models could be developed to anticipate the predominant form in a giving year.

Ever since the empirical proof that certain climatic conditions, especially moisture and warmth, characterize endemic regions of BL (Haddow, 1963) the search continues to identify direct etiologic factor(s) for this cancer. Ghana is divided into three agro-ecological zones, namely, savannah, forest and coastal. All the three regions within the forest zone demonstrated

significant incidence of BL ($p< 0.05$) compared to regions within the other zones (figure 10, table 8 in appendix). As originally noted by Burkitt (1962), the tumour is rarely seen in areas with yearly mean temperature below 15.5^{o}C, annual rainfall less than 50cm and high altitude. These factors, as investigated by Burkitt, also showed a strong association with malaria endemicity. The minimum annual mean rainfall and temperature for the three regions within the forest zone are 125cm and 26^{o}C respectively with humidity of 70- 80% (Asante and Asenso-Okyere, 2003). Additionally, Ashanti region demonstrated the highest malaria incidence (22%) followed by Brong- Ahafo region (16%) with Western region contributing 6% to the over 3.5 million malaria cases recorded in 2003 nationwide, of which 40% were children under five years old (Roll Back Malaria Monitoring and Evaluation; country profile- Ghana, 2005). These conditions clearly satisfy the characteristics prevalent at BL endemic regions as defined by Burkitt and hence the significant BL incidences observed in this zone. The other zones exhibit one or two of the tributary conditions but high fluctuations exist within compared to the relatively stable conditions of the forest zone ((Asante and Asenso-Okyere, 2003).

An alternative reason for the high frequency of BL cases recorded at KATH may be due to proximity of patients within the Ashanti, Brong-Ahafo and Western regions to the treatment centre. Notwithstanding, there were very few BL cases from the Upper East, Upper West and Northern regions which are also close to the treatment centre. This observation may be due to their geographic location in the savannah zone of Ghana.

4.2.1.2. Biochemical Prognostic Modelling based on Pre-treatment Clinical Indices

The attempt to define prognostic factors for cancer has received much attention in the last three decades (Cabanilla *et al.*, 1978, Scheneider *et al.*, 1980, Fisher *et al.*, 1981, Coiffier *et al.*, 1991, Hoskin *et al.*, 1991, Shipp, 1993). The International NHL Prognostic Factor Project (IPI) for cancer was initiated and executed by 16 institutions in Europe, Canada and United States of America to address this issue. The IPI project considered a number of aggressive NHLs and proposed candidate prognostic factors (Shipp, 1993). Some of these factors have been validated against specific forms of cancers and has yielded mixed results (Leonard *et al.*, 1991, Lopez-Guillermo *et al.*, 1994, Hermans *et al.*, 1995, Federico *et al.*, 2000). Unfortunately, little has been done to define prognostic indices specific for paediatric cancers and of much importance, BL. Pedrosa and colleagues (2007), attempted to define prognostic factors for cancer patients, mostly children suffering from BL, using various clinical, epidemiological and socioeconomic factors. Unfortunately, none of these factors exhibited any significant ($p > 0.05$) association with overall survival (OS) as reported for adult cancers and they attributed their observation to small sample size and socioeconomic support given to families of patients. This study examined a total of 180 BL patients (2000- 2007) who lived in their normal homes with no special socioeconomic support given them from the study, other than the free diagnosis and treatment they received.

Total serum LDH is a known prognostic index (PI) for adult cancers and other systemic infection (Scheneider *et al.*, 1980). Results from this work also confirmed total serum LDH to be an independent prognostic factor for children suffering from eBL which is in contrast to what Pedrosa and colleagues reported. The level of total serum LDH has been reported to be an index for the rate of cellular proliferation and invasiveness potential in cancers (Pan *et al*., 1991) hence it is reasonable that patients who had their serum LDH level within normal range at diagnosis (Fig. 14) were significantly favoured ($p = 0.003$) towards survival compared to their cohorts who had serum LDH level at or above one fold upper limit of the hospital's normal clinical range (80- 286 U/ L). The hazard function which estimates the probability of an event (relapse or death) occurring, also predicted that patients with abnormally higher LDH level had twice the potential to experience an event at any time point compared to their cohorts with normal LDH level (Table 5b:Exp [B] = 2.038, $p= 0.004$). Total serum LDH is therefore an important prognostic index to be considered during paediatric BL diagnosis.

Higher than normal serum levels of phosphate, creatinine, chloride and potassium are associated with renal dysfunction which might be due to advanced lymphoma or haemolysis, renal impairment, metabolic acidosis and/ or alkalosis respectively (Gaw *et al.*, 1999). Among the candidates in this category, serum chloride which generally does not give much clinical

information on its own, proved to be an independent prognostic index. Patients who had normal serum chloride were of a survival advantage compared to those with lower than normal serum chloride level (Fig. 12, p= 0.039). In the survival hazard modelling, serum chloride did not prove significant (p> 0.05) when interaction with other factors was restricted. However, when interaction with higher than normal LDH level, creatinine level and stage of disease, which were all independent prognostic indices, was permitted in the modelling process, serum chloride contributed its effect to the model by predicting a hazard probability (Table 6b: Exp [B] of 11.980, p= 0.001 for stage II and p= 0.018 for stage III-IV) compared to basal or normal levels of all these parameters. Chloride ion is the major extracellular anion and with sodium ion, they give insight about the hydration status of an individual. Therefore in an event of body fluid loss, through either excessive vomiting and/ or diarrhoea which are known adverse effect of chemotherapy, the patient loses chloride ions and may suffer metabolic alkalosis (Gaw *et al.*, 1999). It is paramount to consider serum Cl in association with other indices as a prognostic index.

Serum creatinine proved to be an independent prognostic factor in the univariate analysis (Fig. 13, p< 0.001). Poorer overall survival (OS) was observed with patients who had their pre-treatment serum creatinine level above the normal upper limit (62- 115 μmol/L). Patients who had higher than normal creatinine level were about 3.6 times (Exp [B]) at risk of either treatment failure or relapse or death (p= 0.002) compared to those with either

normal or lower than normal creatinine level at diagnosis. The biochemical measurement of serum creatinine, in association with other parameters, gives information about the renal excretion efficiency of an individual. Patients with any renal impairment have higher serum level due to inefficient renal excretion. Such patients might demonstrate similar inefficient excretion of the cytotoxic and cellular contents after tumour lyses. These drugs and their metabolites as well as product of tumour lyses might overwhelm the inefficient renal excretion system and finally become toxic or induce lytic shock in such individuals. Patients whose pre-treatment serum creatinine level, in association with other renal function indices, is normal give the general information about the good renal excretion system and the ability to efficiently excrete the cytotoxics and tumour products, hence a mark of treatment tolerability. It is therefore consistent that patients who had higher serum creatinine (an indication of renal impairment, with all things being equal) showed poorer OS.

Age, though was not an independent prognostic factor, is worth mentioning. This is because it has been identified as an important prognostic index in several studies with large numbers of older patients (Dixon *et al*, 1986, Hoskin *et al*, 1991, Shipp, 1993). In these studies, OS was poorer among older (<60 years verses > 60 years) patients but as reported by Pedrosa and colleagues (2007) in conformity with this data (Table 4), age is of no prognostic importance (p= 0.568) in paediatric cancer or BL to be specific. To reduce treatment-related toxicity, it is prudent to treat older patients with

lower doses of cytotoxics (Dixon *et al*, 1986) so as not to overwhelm their aged organs involved in drug metabolism. Children, either above or below eight years who were considered in this study or the five-year stratification approach for children below 16 years used by Pedrosa and colleagues, 2007, showed no significant difference in overall survival with respect to age. Therefore, young patients generally tend to tolerate intensive chemotherapy better than older (>60 years) patients as reflected by the non-significance of age as an independent prognostic factor in children (Table 4).

Tumour staging has over the years been used as an important non-invasive means to estimate the extent of tumour spread within patients and has been demonstrated to be of prognostic importance (Velasquez *et al.*, 1991, Shipp, 1993) for high grade non-Hodgkin's lymphomas. The stage of patients proved to be of prognostic significance (p= 0.018) as reported in literature. However, when stage I was used as reference against stages II and III-IV in the hazards modelling, stage II significantly (Fig. 15, p= 0.015) contributed to the model with a hazard of 1.744. This means that any patient who reported with a stage II tumour had about 1.744 (hazard ratio) probability of experiencing either treatment failure or relapse or death (treatment event) compared to any stage I case. The potential to experience any "treatment event" was also 1.234 times higher for stage III-IV patients compared to stage I patients but lacked significance (p= 0.492). This observation may be due to the smaller number of patients within this group since both stages (II and III-IV) in association with the other independent prognostic factors (Cl, Crt and LDH) had a similar

hazard estimator/ ratio (Exp [B]=11.980) when interactions were permitted in the modelling process (Table 6a and 6b).

4.2.2 Prospective Study

4.2.2.1 Effect and Implication of Cyclical Chemotherapy on some Biochemical Indices and Treatment Outcome

Burkitt's lymphoma patients at KATH receive an intensive cyclophosphamide-based chemotherapy at 21- 28 days interval for six month (induction period) which is followed with a one-and-half years of less intensive maintenance period. Survival, three months post-induction has been described as a good index of treatment response and hence general survival (Forestier and Schmiegelow, 2005). Hence in addition to the general patients' monitoring, at approximately two months intervals during cyclical chemotherapy, patients' re-evaluation (for volunteers) was done. Patients that had successfully completed their intensive chemotherapy period were examined after three months for any event of relapse or death (treatment outcome) or disease/event-free remission (remission). Patients who had experienced any treatment outcome or in remission were noted and the trends of their biochemical indices were analysed along side those of healthy cohort.

Serum LDH level was significantly different ($p< 0.05$) between healthy subjects (control) and patients at diagnosis but the levels normalized (no significant difference) after 42 and 84 days of cyclical chemotherapy (Fig. 17). Level of serum LDH for patients at diagnosis was also significantly

different compared to levels at 42nd and 84th day after receiving chemotherapy. These differences can be attributed to the effect of the cytotoxics used for chemotherapy that actively kill highly proliferating malignant cells, compared to normal cells. Thus, as more and more malignant cells are eliminated, the highly abnormal level of serum LDH which was produced chiefly by the malignant cells approaches normality. Figure 22 illustrates this point in that patients, who were in disease-free remission after 84 days, had similar serum level of LDH compared to normal healthy children. As such, the progressive monitoring of serum LDH as part of patient's clinical review protocol and monitoring plan is essential.

Serum creatinine did not prove to be a significant factor (Table 7) for monitoring cyclical chemotherapy. This is because once a patient is assessed to have a good renal excretion system before the commencement of cyclical chemotherapy, serum creatinine level is not expected to alter much hence the insignificant changes observed over time (p= 0.301) and lack of association with treatment outcome.

The level of serum potassium also did not change significantly during cyclical chemotherapy (p= 0.132) but mean levels at the 84^{th} day for patients who achieved remission and those who experienced treatment event were insightful. Serum potassium does not vary much with water loss (dehydration) but does vary with disease or certain type of drugs/ treatment that has the potential to induce acidosis and/ or tumour lysis syndrom (Gaw *et*

al., 1999). Also, the kidney serves as the primary excretory organ for potassium hence any impairment of renal filtration/ function as well as the incidence of tumour lysis syndrome would result in higher than normal serum potassium. Patients who were in remission, after the test period, were observed to have similar serum potassium level (mean) as their healthy cohort (Fig. 20) but those who experienced adverse treatment outcome had significantly ($p< 0.05$) higher mean serum potassium (Fig. 20). This observation could be due to the long term effect of cytotoxics on renal function and/ or in the event of acidosis or tumour lysis syndrome in these patients where potassium ions are released from cells.

Serum chloride did not show to be an important monitoring index ($p= 0.651$) but serum sodium was significantly ($p< 0.001$) affected by cyclical chemotherapy (Table 7 and Fig. 18). Sodium ion, the major extracellular cation, has a significant relationship with water (free body water) balance in a person. The serum level is affected by dietary intake, renal excretion and body fluid loss (Gaw *et al.*, 1999). The group of patients who experienced hyponeutrimea at the initial stage of chemotherapy (before day 42), due to excessive fluid loss through vomiting, were less likely to be in remission three months post-induction (Fig. 24). Notwithstanding, the effect of the observed change was not significantly different between patients in disease-free remission and those who experienced "treatment event" after 42 days of monitoring, though both categories demonstrated significantly different ($p< 0.05$) serum sodium levels compared to healthy controls (Fig. 24).

Unlike the empirical facts about the positive link between serum uric acid (SUA) and hypertension in children and adults (Alper *et al.*, 2005 and Sundström *et al.*, 2005), mixed conclusions can be drawn for cancers (Ames *et al.*, 1981, Kolonel *et al.*, 1994, Peterson and Trell, 1983 and Hiatt and Fireman, 1988). Serum uric acid was not an independent prognostic index (p= 0.548) but its relationship with treatment outcome after cyclical chemotherapy was insightful. Patients who experienced adverse treatment outcome had their SUA level significantly higher ($p< 0.05$) than healthy control children and disease-free remission patients at 84th day during chemotherapy (Fig. 21). Higher SUA is associated with increased DNA turnover which can be induced by rapid proliferating malignant cells and the spill of purine nucleic acids from cells (Ultmann, 1962, Kolonel *et al.*, 1994). Hence in the event of tumour relapse and/ or treatment failure, categorized as treatment event, SUA would increase to correspond with re-growth, drug resistance of tumour and/ or tumour lysis syndrome. In this light, it can be proposed that high SUA level does not play any protective role against cancer as suggested by others (Peterson and Trell, 1983 and Hiatt and Fireman, 1988, Kolonel *et al.*, 1994) or support chemotherapy for enhanced survival.

Serum calcium level can be altered by cancer in the event of metastasis to the bone or the production of parathyroid-hormone like substances by the tumour (Gaw *et al.*, 1999). Though there was a significant difference ($p<0.05$) between serum calcium level at 42^{nd} and 84^{th} day of cyclical chemotherapy (Fig. 23), the observed difference between healthy control and the defined

treatment outcomes (Fig. 23) were not significant (p>0.05) at all time points. Calcium level, as reflected in the serum, is affected by several factors including serum magnesium, vitamin D and parathyroid hormone (Ryzen *et al*., 1987, Estep *et al.*, 1969) hence the observed change (p= 0.013, Table 7) during chemotherapy might be multifactorial (Lee *et al*., 1978) which can not be thoroughly explained within the scope of this current data.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Burkitt's lymphoma of all clinical presentations, especially facial and abdominal (80.7% cumulatively), is fairly common among Ghanaian children below age seventeen. At ages 6 years, 10 years and 13 years, the abdominal cases were observed to peak while facial cases declined. Most cases of BL were from the forest zone with few cases reporting from the savannah zone of Ghana. Notwithstanding, none of the clinical presentations can be explicitly associated with any particular region within the boundaries of Ghana.

In addition to the serum level of lactate dehydrogenase (LDH) and tumour stage which were used as prognostic markers at diagnosis, serum chloride and creatinine levels were identified as important prognostic makers specific for African/ endemic Burkitt's lymphoma (eBL).

Serum LDH, uric acid, potassium, calcium and sodium were identified as important biochemical indices that must be routinely assessed during cyclophosphamide-based cyclical chemotherapy in the treatment of eBL as a means to identify responders and non-responders of the chemotherapy regimen being administered. This will make it possible for any expeditious alternative or intervention treatment before patient experience any treatment event.

In summary, this small real-country based study has demonstrated that geographical assessment within endemic countries is necessary to establish demographic maps of incidence, management and monitoring protocols for BL cases. This study thus confirms reported findings of ecological importance in BL pathogenesis and proposes some new prognostic and monitoring indices.

5.2 RECOMMENDATIONS

Based on the insight gained through this work, the under listed recommendations are suggested:

It is imperative to conduct similar investigations at other treatment centres in Ghana and other countries of high BL incidence.

The newly-identified prognostic indices should be carried out at diagnosis to complement the existing ones for better prognostic insight.

Finally, the biochemical indices that were shown to be associated with treatment outcome, based on cyclical chemotherapy, should be verified in a larger prospective cohort study and once established should be made part of patient's routine laboratory investigative panel.

Further biochemical investigations should be carried out to explain the trend of serum calcium during cyclical chemotherapy.

APPENDIX

Appendix A: Regional incidence of BL cases in Ghana (KATH, 2000- 2007)

Region	Facial only (%)	Abdominal only (%)	Facial and Abdominal (%)	Facial and CNS (%)	Abdominal and CNS (%)	CNS only (%)
Ashanti	149 (60.1)	120 (71)	53 (64.6)	3 (37.5)	5 (62.5)	0 (0)
Western	44 (17.7)	20 (11.8)	16 (19.5)	2 (25)	3 (37.5)	1 (100)
Volta	3 (1.2)	3 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)
Eastern	6 (2.4)	1 (0.6)	2 (2.4)	1 (12.5)	0 (0)	0 (0)
Brong-Ahafo	36 (14.5)	19 (11.2)	10 (12.2)	2 (25)	0 (0)	0 (0)
Central	5 (2)	5 (3)	1 (1.2)	0 (0)	0 (0)	0 (0)
Upper East	2 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Upper West	1 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Northern	2 (0.8)	1 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)
Total	**248 (100)**	**169 (100)**	**82 (100)**	**8 (100)**	**8 (100)**	**1 (100)**

Appendix B: Categorical Variable Coding (b,c,d,e)

		Frequency	(1)	(2)
stage(a)	1=Stage I	42	0	0
	2=Stage II	63	1	0
	3=Stage III-IV	22	0	1
LDHcat(a)	1=within normal range	31	0	
	3=>= 1 fold of normal upper limit	96	1	
Clcat(a)	1=within normal range	110	0	
	2=below normal lower limit	17	1	
Crcat(a)	1=within normal range	41	0	0
	2=below normal lower limit	78	1	0
	3=>= 1 fold of normal upper limit	8	0	1

a Indicator Parameter Coding
b Category variable: stage (Stage category of tumour)
c Category variable: LDHcat (Lactate dehydrogenase)
d Category variable: Clcat (Chloride at diagnosis)
e Category variable: Crcat (Creatinine at diagnosis)

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