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ETIOLOGICAL RISK FACTORS AND CLINICAL CHARACTERISTICS OF CHILDHOOD NON-HODGKIN LYMPHOMA IN UGANDA

ACADEMIC THESIS

The public defence for the degree of Doctor of Philosophy at Karolinska Institutet and Makerere University will be held at Davies Lecture Theatre, Makerere University, College of Health Sciences, Kampala, Uganda.

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by

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ABSTRACT

Introduction: Incidence of non-Hodgkin lymphoma (NHL) has increased greatly over time, especially in children. Improved diagnostic methods alone cannot explain this increase, especially the increase observed in sub-Saharan Africa, where diagnostic capabilities are low.

Objectives and aims: The objectives of this study were to better understand known risk factors for NHL, such as Epstein-Barr virus (EBV), and their impact on disease characteristics. The specific aims were: I. to understand the background role of EBV, II. to elucidate the basis for and strength of the diagnosis of childhood NHL in Uganda, III. to highlight trends in characteristics of childhood NHL, and IV. to examine the impact of human immunodeficiency virus (HIV) infection.

Subject and Method: Aims I and II were studied in **Papers I and II** using samples and data from a case-control study carried out at the Mulago National Referral Hospital between 2004 and 2008. This study enrolled children with suspected tumours or masses referred to the Departments of Paediatrics, Paediatric surgery, Orthopaedics and to the Uganda Cancer Institute. In **Paper I**, EBV viral load was measured in saliva, whole blood, and white blood cells by real-time PCR, serological values for IgG-VCA, EBNA1, and EAd-IgG were measured and compared in NHL and chronic inflammatory conditions (CIC). Comparisons were also done by NHL subtypes (Burkitt lymphoma, BL and other NHL). Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated. In **Paper II** children were diagnosed with suspected NHL based on initial clinical examination; tissue samples were then taken and examined in Uganda and sent thereafter to a pathology laboratory in The Netherlands for re-examination and additional tests. Agreement between diagnoses assigned in Uganda and The Netherlands were compared using kappa statistics.

For aims III and IV a review of routine clinical records of paediatric BL patients seen at the Uganda Cancer Institute was done and reported in **Papers III and IV**. Information on demographic characteristics (age and sex), clinical features (symptoms, signs, disease site, and stage), treatment response and vital status information were obtained. In **Paper III** the frequency distribution of the clinical characteristics, treatment, and outcome of childhood BL over 20 years were summarised by means and standard deviations (SD), or proportions; differences were tested by the 2 test, t-test, z-test or analysis of variance (ANOVA) procedures. In **Paper IV** descriptive statistics of frequencies, means and SD were done using Student's t-test and Chi-square test statistic and ORs, CIs and P-values were obtained. Survival analysis was performed using the Kaplan-Meier method.

Results: In **Paper I** the most common clinical presentations were fever, night sweats and weight loss. EBV viral load in blood was elevated in BL vs other NHL (OR 6.67, 95% CI 1.32-33.69; P-value=0.04) and a significant difference in EAd-IgG was observed in NHL vs CIC (OR 0.19, 95% CI 0.07-0.51; P-value=0.001). In **Paper II**, the agreement between clinical and pathological diagnoses of NHL in Uganda was 91% (95% CI 84-95; kappa 0.84; P-value=0.001). The agreement between clinical diagnoses in Uganda and pathological diagnoses in The Netherlands was 49% (95% CI 40-59; kappa 0.04; P-value=0.612). The agreement between all pathological diagnoses assigned in Uganda and The Netherlands was 36% (95% CI 28-46; kappa 0.11; P-value=0.046). In **Paper III**, facial tumour (n=945, 77.65%) and abdominal disease (n=842, 69.19%) were the most common presentations. Significant presentation with advanced-stage disease (hepatic mass, malignant pleocytosis) was noted (P-value <0.01). Mortality was higher in older children, children with advanced-stage BL, and HIV-positive children. In **Paper IV** HIV-positive children presented significantly more often with disease in the lymph nodes (67%), liver (51%), and chest (10%). Response to chemotherapy was similar in HIV-positive and HIV-negative children although survival was poorer in HIV-positive children (median survival of 11.79 months, 95% CI 8.65-14.92; P-value<0.000).

Conclusion: This study provides additional understanding of the role of EBV in childhood NHL, shown by the significant association between virological and serological markers and common general features, suggesting a common factor. We noted a weak basis for diagnosis of childhood NHL in Uganda with a high probability of error. The presenting features of childhood NHL have not changed with time, although more children present late, especially those with HIV. Improvements in the cancer care system in Uganda should include better diagnostic and treatment services for children as a basis for better understanding of disease and high-quality research.

Key Words: Epstein-Barr virus; non-Hodgkin lymphoma; Burkitt lymphoma; cancer; HIV; characteristics; Africa; children; paediatrics; Uganda.