SESSION 8: ONCOGENIC VIRUSES

Organizer : *Dr. Peter Wanzala and Dr. Odada Sumba*

Chair: *Prof. Rosemary Rochford*

ORAL PRESENTATIONS

1. **Early age of EBV infection in infants from Western Kenya: clues to the etiology of Burkitt lymphoma**

Erwan Piriou[1,2], Amolo S. Asito[2,3], Peter O. Sumba[2], Nancy Fiore[1], Jaap M. Middeldorp[4], Ann M. Moormann[5], Robert Ploutz-Snyder[1,6], and Rosemary Rochford[1]

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Background: Infection with Epstein-Barr virus (EBV) early in life and repeated malaria exposures have been proposed as risk factors for endemic Burkitt lymphoma (eBL).

Objective: In this study, we followed infants from two areas of rural Kenya with divergent malaria exposure and differential risk for eBL to address the question of the effects of age of primary EBV infection on EBV persistence.

Methods: Infants were enrolled from two rural sites in Kenya: Kisumu District where malaria transmission is holoendemic and risk for eBL is high and Nandi District where malaria transmission is limited and the risk for eBL is low. Blood samples were taken through 2 years of age to measure EBV viral load, EBV antibodies, and malaria parasitemia. EBV viral load and malaria parasitemia were measured by Q-PCR and antibodies to EBV were measured by a Luminex multiplexed bead array assay.

Results: We observed a significantly earlier age of primary EBV infection in children from Kisumu compared to Nandi [mean age of 7.28 mo (+/- 0.33 SEM) in Kisumu vs. 8.39 mo (+/- 0.26 SEM) in Nandi] with 35.3% of children in Kisumu infected before 6 months of age. To analyze how different predictors affected EBV viral load over time, we did multi-level mixed modeling which revealed that residence in Kisumu, and earlier age at first EBV infection were significant predictors for having a higher EBV viral load throughout the period of observation.

Conclusion: These data demonstrate that infants from a region in Kenya with high malaria exposure were infected with EBV earlier in life and that earlier age of infection leads to poor control of the virus after primary infection. The data presented is consistent with the hypothesis that infection by EBV very early in infancy may set the stage for eBL development.

2. **Burkitt lymphoma incidence correlate with differences in malnutrition, malaria and Epstein-Barr virus**

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Background: Endemic Burkitt lymphoma (eBL) has been associated with Epstein-Barr virus (EBV) and holoendemic Plasmodium falciparum malaria. However, recent eBL incidence maps showing more refined spatial clustering within malaria holoendemic areas, suggest that other risk factors may be involved in the etiology of eBL. Therefore, we hypothesized that the selenoprotein glutathione peroxidase (GPx), a surrogate of nutritional status, is an important biomarker for eBL risk. Methods: This study measured plasma GPx, anthropometric markers of malnutrition, EBV viral loads and malaria parasitemia in children aged 1-9 years (n=258) from two Locations in Nyanza Province, Kenya with higher than expected and lower than expected incidence of eBL. The study participants were malaria asymptomatic children from the community.

Results: Children from eBL high incidence areas had significantly lower GPx levels, high EBV viral load and more evidence of chronic malnutrition than children from eBL low incidence areas (all $p < 0.001$). Additionally, GPx levels were significantly lower in children with the highest EBV viral load and for those with P. falciparum infections ($p=0.035$ and $p=0.004$, respectively). Conclusions: Taken together, these results suggest that selenium deficiency may be an additional risk factor that needs to be considered in the etiology of endemic Burkitt lymphoma.

3. **Epstein Barr virus latent - lytic proteomic dynamics in HIV-1 infection in Nairobi and its implications on diagnosis and management of HIV associated lymphomas**

D'lima M[1], *Waihenya R*[2], *Johnson NW*[3], *Wanzala P*[4]

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Background: EBV is maintained in a latent state within the infected host cell. During any immunosuppressed state this latency is untenable and viral replication, host cell lysis and lymphoproloiferative disease occur, unchecked. HIV infection is associated with a wide spectrum of EBV associated lymphomas which manifest in advanced stages of HIV clinical disease. These lymphomas, autoimmune disease and other reactive disorders are typically associated with high serological titers against Early antigen and Viral capsid antigens with low Epstein Barr nuclear antigen titers.

Objective: This study sought to study EBV protein serology in HIV infected outpatients and to determine whether EBV reactivation and neoplastic transformation are associated with a particular distribution of these proteins during early and advanced clinical HIV infection.
Methodology: This was a cross sectional study conducted on 101 adult outpatients, clinically classified at various stages of HIV infection. Subjects underwent venepuncture for solid phase EBV ELISA using Euroline ® test kits (Medizinische Labordiagnostika AG, Lubeck, Germany). The presence of antibodies to a panel of 5 proteins was demonstrated on the ELISA strip.
Results: When a cluster analysis of the various EBV proteins were done in early and advanced HIV clinical disease, the following clusters emerged in decreasing order of strength of mean Approximately Unbiased (AU) P value evidence (p19 and p22;mean AU P value 96.5%) then (gp125,p22 and p19;mean AU P value 95.5%) and finally (EBNA-1 and EAD; mean AU P value 69%).EBNA-1 was the runt in early HIV and EAD was the runt in late HIV. When an AU P value: standard error plot for the cluster pairs was done EBNA -1 and EAD had the highest Standard error (SE) without any overlap in the SE (0.025) with other protein clusters.

Conclusions: EBNA-1 and EAD was the weakest but most statistically significantly different cluster in both early and late clinical HIV disease.EBNA-1 and EAD are good candidates for research and development into a diagnostic and monitoring tool for HIV associated lymphomas.

4. **A Cross Sectional Survey On The Seroprevalence Of Hhv8 In The Haart Naïve Hiv Patients At Kenyatta National Hospital Comprehensive Care Clinic**

Einstein M. Tsuma, Omu Anzala, Erastus O. Amayo, Gladwell K. Kiarie, Marybeth C. Maritim and Peter Wanzala

Abstract

Background: The HIV/AIDS pandemic is one of the leading health challenges in the world and Kenya today. Kaposi's sarcoma is the leading neoplasia in HIV/AIDS and is an AIDS defining illness. Human Herpesvirus 8 is the causative organism of this neoplasia. The interaction of HHV8 with HIV is critical in the development of the neoplasia and other HHV8 associated illnesses. HAART and the reversal of immunosuppression has been shown to be the best option of treating and preventing diseases associated with HHV8.The seroprevalence of HHV8 in the HIV patients is not very well studied in Kenya.

Objectives: The study aimed to determine the seroprevalence of HHV8 and its associated factors (demographic and behavioral) in the HAART naïve HIV patients attending the Kenyatta National Hospital Comprehensive Care Clinic (CCC).

Study design: Cross sectional descriptive survey

Methods: 378 consecutive HAART naïve HIV patients were recruited from the Kenyatta National Hospital Comprehensive Care Clinic between Dec. 2010 and April 2011. Demographic and behavioral data was collected using a pretested structured questionnaire. HHV8 serology and CD4 counts from collected blood was determined by use of the ABI HHV8 immunoglobulin G antibody Elisa method and CyFlow respectively.

Data management: Accrued data was entered and analyzed using in SPSS version 17.

Results: The seroprevalence of HHV8 was 54.4%. Seropositivity was associated with male gender and a low CD4 of < 350 cells/mm^3 on the multivariate analysis. OR 1.90, 95% CI [1.21-2.99] and OR 1.88, 95% CI [1.18-2.99] respectively.

Seropositivity was not related to other demographic or behavioral factors.

Conclusion: The seroprevalence of HHV8 is high in this population. Male gender and a low CD4 counts is associated with HHV8 seropositivity.

5. **Prevalence of Human Papillomavirus infection by age and cervical cytology in Thika, Kenya**

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Abstract

Background: Human papillomavirus (HPV) infections cause cervical cancer and premalignant dysplasia. Data on HPV and cervical cancer in Kenya are scarce. Type-specific HPV prevalence data provides a basis for assessing the impact of HPV vaccination programs on cervical cytology and how HPV based screening will influence cervical cancer prevention.

Objective: We aimed at describing HPV infections in a population in Thika, Kenya.

Methodology: We obtained cervical cells specimen from 498 women in a population in Thika district. The study was conducted between January to May 2010. Pap smears were performed, HR HPV DNA were detected by Digene Hybrid capture 2® (hc2) test and HPV genotyping was performed with Multiplex Luminex HPV genotyping kit (Multimetrix, Progen, Germany).

Results: We report HPV type specific prevalence and distribution data for 498 women (age range 18-74 years; mean age 36 years) recruited into the study in relation to age and cervical cytology. Samples from 106 women (21.3%) tested positive for HPV. Multiple HPV types were detected in 40 (37.7% of HC2-positive samples) and the rest had infection with single HPV type. The most common HR HPV type at all ages was HPV16, 52, 56, 66, and 18. There was a marked decline in the prevalence of HR-HPV with age.

Conclusion: The pattern of HR HPV distribution in this population was slightly different from existing literature, which has important consequences for HPV vaccination and prevention programs.

SESSION 8: POSTER PRESENTATIONS

1. **Effect Of DMPA ON HSV-2 IgG Serology In Asymptomatic HAART-NAÏVE HIV Positive Women**.

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Background: Worldwide about 16 million women are infected with HIV, most heterosexually and 80% are in Sub-Sahara Africa. Over one quarter of the HIV infections have been directly attributed to HSV-2, in areas with high HSV-2 prevalence and up to 6% of HIV infections have been attributed to DMPA use in some studies. In Kisumu-Kenya where HIV-1 prevalence is estimated at over 14%, about 17% of women on modern contraception are on DMPA. These rates may be higher among the HIV positive women. Data on HSV 2 prevalence in DMPA use among HIV positive women is
Main Objective: To investigate the effect of DMPA on HSV-2 IgG index values in asymptomatic HAART-naïve HIV positive women using DMPA.

Methodology: The study was conducted at the Research Training and Care Program/Family Aids Care and Education Services (RTCP/FACES) comprehensive care clinic in Lumumba Health Centre in Kisumu, Kenya. This was a case-control study with an arm consisting of women on progesterone only contraceptives; DMPA and a control arm consisting of women not on any hormone-containing contraceptives. Samples were collected for determination of vaginal flora and HSV-2 titres. Analysis was conducted at the RTCP/FACES laboratories in Kisumu and the Immunology laboratory, Department of Human Pathology, University of Nairobi. ANOVA was used to compare index values between the two groups.

Results: A total of 112 asymptomatic confirmed HIV infected were recruited to participate in the study, of which 51 were DMPA users while 61 were not on any hormonal contraceptive. The mean HSV-2 IgG titres were 3.327±0.114 (mean ± sem) and 2.471±0.143 (mean ± sem) for the cases and controls respectively. There was a significant difference between the case and the control titres with p-value <0.05. This significance of difference is lost (n control =20, n-cases = 12, p = 0.0298) with CD4 below 500 with means of (3.364±0.383) and (2.904±0.232) for cases and controls respectively. The significant difference in titres is maintained at higher CD4 counts even with much smaller samples (CD4 > 1000, n control=2, n cases=10, $p<0.05$).

Conclusion: From these findings it is clear that DMPA use is associated with higher HSV-2 IgG titres in HIV-1 infection. It is possible that DMPA may cause higher HSV-2 viral load or increase the frequency of reactivations thus increasing frequency of exposure. The loss of significance with lower CD4 counts may be a possible reflection of altered mucosal and systemic immune responses at low CD4 counts.

2. **Comparison of EBV and KSHV Seroprevalence in a Cohort of Children in Western Kenya**

Ibrahim I. Daud[1, 2, 3], Amolo S. Asito[1,2], Erwan Piriou[1,2], Peter O. Sumba[2], Wendell Miley[4], Denise Whitby[4], and Rosemary Rochford[1]
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Background: Kaposi's sarcoma herpesvirus (KSHV) is associated with a number of human malignancies including Kaposi's sarcoma and primary effusion lymphoma. KSHV is endemic in many areas of sub-Saharan Africa including Kenya. Interestingly, there is different geographical distribution of KSHV suggesting differences in etiologic factors that promote transmission of the virus. KSHV is similar to another member of the gammaherpesvirus family, Epstein-Barr virus (EBV) in that it is transmitted through saliva in endemic regions.
Objective: To compare EBV and KSHV seroprevalence in a cohort of children from western Kenya.

Method: Infants were enrolled from two rural sites in Kenya: Kisumu District where malaria transmission is holoendemic and Nandi District where malaria transmission is limited. Plasma from venous blood taken at 3 years of age was analyzed for KSHV seroprevalence using ELISA to detect antibodies to ORF73 and K8.1. Presence of antibodies to either ORF73 or K8.1 were used to indicate whether children were seropositive. EBV seroprevalence was determined by detection of antibodies to VCA and EBNA1 from 1 month through 3 years of age.

Results: By 3 years of age, KSHV seroprevalence was only detected in 8 of 57 children from Kisumu District (14%) and in 3 of 75 children in Nandi (4%). In contrast, all children in the study were EBV seropositive by 16 months of age.
Conclusion: These results show that although both EBV and KSHV are transmitted through saliva in endemic regions, EBV infection is transmitted much earlier in life than KSHV.

3. **Increased frequencies of surface membrane immunoglobulin light chain-negative B cells in peripheral circulation of endemic Burkitt's lymphoma patients**

Amolo S. Asito [1, 2], Erwan Piriou [2,3,#], Jeff A. Bailey [4], Walter G. Z. O. Jura [1], Collins Ouma [1, 2], Ann M. Moormann[4], Rosemary Rochford [3]*

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Background: Endemic Burkitt's lymphoma (eBL) is a B cell neoplasm that is thought to arise from arrested maturation of germinal center B cells. However, information is currently lacking on the peripheral B cell compartment and whether changes in B cell subsets mirror elevated Epstein-Barr virus (EBV) loads in eBL patients. Methods. We used flow cytometry to analyze peripheral B cell subset distribution and real-time quantitative polymerase chain reaction (Q-PCR) to analyze EBV viral loads in 32 children presenting with eBL and 25 age-matched controls from western Kenya. Results. No cells with a BL tumor phenotype (CD19+CD10+CD77+CD38+) were found in the peripheral circulation of either eBL patients or controls. The frequencies of naive (CD19+IgD+CD27-) and classical memory (CD19+CD10-IgD-CD27+) B cells were comparable in the two groups, while there were significantly higher frequencies of surface membrane immunoglobulin light chain-negative B cells (CD19+CD10-κ-λ-) and CD19+CD27-CD5-CD10- B cells in eBL patients as relative to controls (all, $p<0.0001$). Importantly, EBV viral load was more elevated in eBL patients as compared to controls ($p<0.0001$), and was positively correlated with the frequency of CD19+CD27-CD5-CD10- B cells ($r=0.4952$, $p=0.0191$). Conclusions. Our results demonstrate increased peripheral circulation of B cells that lack surface immunoglobulin light chains suggesting that there is profound perturbation of B cell homeostasis in eBL patients with concomitant elevation of EBV viral loads not associated with peripheral circulation of tumor cells.

4. Exploring obstacles in management of childhood cancers in Western Kenya

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Introduction: Since its discovery in East Africa, BL has posed a challenge to healthcare workers in the region. It is under this background that Epidemiology of Burkitt lymphoma in East African Children and Minors (EMBLEM) program, a case-control study is conducting multidisciplinary cancer community surveillance (MCCs) module in the three countries of East Africa.

The major obstacle in Kenya is lack of budgetary allocation and apical approach in addressing cancer related illnesses. The MCCs implementation in the hospitals and the control communities, require considerable effort by administrators, clinicians, researchers and community advisory members to pass cancer awareness education module, sustain participant interest and patient benefit as being realized in Western Kenya, Uganda and Tanzania.

Objectives

1. To test the hypothesis that genetic resistance to malaria is associated with a lower risk of BL and use genome-wide association methods to discover genetic variation that may be associated with decreased or increased risk of B lymphoma

2. To explore scientific based evidence of spatial distribution of Burkitt lymphoma from the region

3. To evaluate the power of community involvement in disease surveillance and treatment outcome

Methodology: Case control study to involve 1500 BL cases and 3000 age, sex and residence matched controls from general population. The investigation will focus on modern cancer diagnosis and treatment to identify the etiologic agents in childhood cancer causal matrix. The multimodal carcinogenic investigations are based on two categories: causes that are intrinsic to the patient (heredity and hormones) and extrinsic cases (chemicals, dietary factors, radiation, viruses, microbes and other environmental toxicants).

Expected outcome: Community involvement has a rich factor to understand underlying disease ecological variations and socio-demographic factors influencing health seeking behavior. The hospital spotting will act as community catchment indicators as previously outlined in Alma Ata declaration 1978.

Provisional indicators

The study's community interaction module and enrollment approach has shown improved BL treatment outcome in Lacor, Gulu, Uganda and Shirati Tanzania. This based on BL community spotting and early diagnosis. The health outcomes based on this module can be extrapolated anywhere once the analysis of the preliminary data is completed.

5. Seroprevalence of Cytomegalovirus Infection in a Cohort of children in Western Kenya

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Background: Cytomegalovirus is a member of the herpesviridae group of viruses that occurs worldwide without seasonal variations. Cytomegalovirus infection is a serious cause of congenital disease in western countries where it causes sensoneural hearing loss and neurodevelopmental disorders. The prevalence of CMV infection in American adults is approximately 54% while approximately 85% of Gambian children acquire CMV infection by their first birthday. However, little is known about the prevalence of CMV in Kenyan children.

Objective: Our aim was to determine the CMV seroprevalence in a cohort of children from western Kenya.
Method: Infants were enrolled from two rural sites in Kenya: Kisumu District where malaria transmission is holoendemic and Nandi District where malaria transmission is limited. Blood samples from infants born to HIV-seronegative mothers were taken from 1 month through 2 years of age to measure CMV viral load in peripheral blood and CMV antibodies. CMV-specific IgG was assessed using a luminex bead based array assay and viral loads were measured in DNA extracted from whole blood using quantitative PCR.
Results: Preliminary results shows that CMV seroprevalence increases with age with a seroprevalence of 60% and 22% at three months of in malaria holoendemic and sporadic area respectively, by the age of six months, 75% and 54%, by 12 months, 89% and 84% and by 24 months, the seroprevalence was 97% and 92% respectively. Studies are ongoing to analyze CMV IgM to confirm primary infection and not detection of maternal antibodies at 3 and 6 months of age.
Conclusion: These preliminary results show higher seroprevalence of CMV in infants living in a malaria endemic area within the first year of life compared to infants living in a region where malaria is not as prevalent. Further studies are needed to understand why seroprevalence of CMV in a malaria endemic region is so high early in infancy.

6. **Epidemiology Of Burkitt Lymphoma In East African Children And Minors (Emblem) – Answering Old Questions With New Technology**

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Background: Burkitt lymphoma (BL) is an aggressive monoclonal B-cell malignancy that is endemic in equatorial Africa. Epstein – Barr Virus (EBV) and malaria are epidemiology linked to endemic BL, but both diseases affect substantially more people than those who develop BL, suggesting that poorly understood co-factors contribute.
To answer this question, we are conducting the EMBLEM study to investigate the role of malaria and EVB in BL in using genome – wide scan methods.

Study Objectives: To investigate whether or not genetic resistance to malaria lowers risk of Burkitt Lymphoma. To use genome-wide scan methods to discover genetic variation that may be associated with risk of Burkitt Lymphoma.

Study Design: EMBLEM is a case-control study of 1500 BL cases and 3000 age-, sex- and residence – frequency matched controls to be conducted in four rural regions in three countries of East Africa (Uganda, Tanzania and Kenya) where malaria transmission is holo-endemic all year round.

Methodology: Cases will be enrolled at hospitals i.e. Webuye and Homa-Bay District Hospital in Kenya .Control will be enrolled from children attending health facilities where cases originate. Clinical, demographic and risk factor information will be obtained by structured questionnaire and blood and saliva samples for genotyping will be obtained from cases and controls to measure host and EBV genetic variance and malaria parasite, antigens and antibodies and measure EBV copies, quantity and types.
Conclusion: EMBLEM will be the largest study of BL in Africa with adequate power to answer its primary objectives and also provide new resources to address questions about tumor biology, viral and host co-factors, and tumor responses in different settings in addition, EMBLEM provides a unique model for intra-African scientific collaborations for training in research and mentoring. Discoveries from EMBLEM may lead to improvement in diagnostic methods, treatment and prevention of BL.