Title (Upper case)

EPSTEIN-BARR VIRUS(EBV) CO-INFECTION AS A RISK FACTOR FOR DEVELOPMENT OF SEVERE MALARIA IN CHILDREN

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Introduction *(include hypothesis)*

Malaria is the leading cause of morbidity in Uganda with 90–95 % of the population at risk and it contributing to 13 % of under-five mortality (D. Roberts, 2016). Children in sub-Saharan Africa experience their primary Epstein-Barr virus (EBV) infection within the first years of life, with majority of children becoming seropositive by 12 months of age (CG Matar, 2015). Given that the B cell compartment is the primary niche for EBV persistence, it is plausible that the humoral immune response to malaria may be altered during EBV co-infection (CG Matar, 2015).Total IgM and IgG was seen to be reduced in mice co-infected with mouse models of EBV and malaria as compared to those singly affected with the latter (CG Matar, 2015). Infection with EBV was seen to reduce antibody responses to meningococcal polysaccharide and measles vaccines in Gambian children while CMV didn't have much effect (B Holder et al, 2010).We hypothesize that children with both malaria and EBV will have decreased production of antibody against Plasmodium falciparum and will thus develop a more severe form of malaria.

Methods (*include source of funding and ethical approval if required)*

A total of 384 children (< 10years of age) who are confirmed to have malaria will be recruited from Mulago Hospital Paediatric Clinic and Assessment Centre. Two drops of blood will be spread as thick smears, dried on a labelled slide, and stained with Leishman's stain for 2-3 minutes. The slides will be evaluated under a microscope; Malaria positivity will be quantified as parasitemia levels 1+ to 4+.

Anaemia will be determined by measuring Haemoglobin levels in a Hemoglobinometer.Blood will be collected by capillary puncture into a hemoglobinometer chamber to determine the haemoglobin levels, as instructed by the manufacturer.

Approximately 3-7 mls of blood will be drawn and processed to collect plasma for ELISA. Saliva will be collected by chewing on a sponge for 1 minute; the sponge will be placed in Phosphate Buffer Saline (PBS) and specimens stored at 22°C.

Automated DNA extraction from saliva and/plasma will be performed using a QIACube.DNA will be quantified and purified by NanoDrop Spectrophotometry. Screening for EBV will be performed using Polymerase Chain Reaction, with Epstein Barr Nuclear Antigen 1(EBNA 1) as the forward and reverse primers. Amplification of target sequences will be performed in a Thermocycler.These amplification products will later be taken through automated electrophoresis using QIAExcel.

The indirect ELISA method will be done using human MSP-1 ELISA kits to determine the presence, and the quantity of opsonizing IgG antibodies against Merozoite Surface Protein (MSP). Antibody responses will be compared between 127 children with malaria and EBV and 257 children with malaria only.

Interpretation of Results

The number of children found to have malaria and EBV will tell us the prevalence of EBV co-infection. Anaemia will be confirmed when children have haemoglobin levels <11g/dL. Parasitemia levels and anaemia will be compared between the cases and controls to determine whether children with EBV have a more severe form of malaria. Determination of the presence and quantity of antibody will be done by getting the optical density of the colour product formed using a spectrophotometer. A correlation will also be made between the presence/absence of antibody and the children that have severe forms of malaria in order to determine the extent to which EBV co-infection poses a threat. This information will be compiled in Excel in the form of numbers and percentages. Odds ratios will be used to determine odds of occurrence and bar graphs will be made to show the difference between the various parameters above between the two groups.

Conclusions

A decrease in the presence and quantity of antibody is expected to be seen in children with both malaria and EBV, as well as an increased severity of malaria. If this is so, and the number of children with EBV co-infection outweighs those that don't have EBV then the research findings can be used to advocate for the use of Novirin as an antiviral agent or the development of an EBV vaccine.

References *(include acknowledgement here if appropriate)*

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-Holder B, Miles DJC, Kaye S, Crozier S, Mohammed NI, et al. (2010) Epstein-Barr Virus but Not Cytomegalovirus Is Associated with Reduced Vaccine Antibody Responses in Gambian Infants. PLoS ONE 5(11): e14013. doi:10.1371/journal.pone.0014013.
-Matar CG, Anthony NR, O'Flaherty BM, Jacobs NT, Priyamvada L, Engwerda CR, et al. (2015) Gammaherpesvirus Co-infection with Malaria Suppresses Anti-parasitic Humoral Immunity. PLoS Pathog 11(5): e1004858. doi:10.1371/journal.ppat.1004858.