CEREBROSPINAL FLUID RESPONSES AS A MEANS OF PREDICTING LATE STAGE HUMAN AFRICAN TRYPANOSOMIASIS IN THE MONKEY MODEL

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Abstract

Human African Trypanosomiasis (HAT), also known as sleeping sickness (Kennedy P.G. E. 2004) is caused by a protozoa parasite *Trypanosoma brucei gambience* or *Trypanosome brucei rhodesiense.* The two forms of the disease are transmitted by tsetse flies in the genus *Glossina* (order Diptera).Both are fatal if left untreated (Chappuis F. *et al,* 2005) The disease takes two forms, the early and the late form. The late form normally has a CNS involvement and there is activation of astrocytes. Activation of these cells normally signifies entry of the parasite into brain tissue. The main aim of the study is to determine the time at which this happens. This will be done by performing immunohistochemistry on vervet brain samples euthanized at various time points of the infection. 15 brain samples will be used 4 serving as negative controls (none infected) and 11 infected.

Introduction: Human African Trypanosomiasis is a neglected tropical disease whose infection rate is currently estimated at over 300,000 cases annually with about 60 million people at risk of infection worldwide of whom, less than 4 million are under medical surveillance (WHO 1998 & 2006; Sternberg *et al.,* 2005). Its prevalence is estimated at 400,000, incidence at 60,000, and mortality at 40,000 per annum. Field diagnosis of HAT still remains a challenge and especially detection of parasite entry into the brain leading to meningoencephalitis. Astrocytosis is the earliest indicator that the parasite has entered the brain which marks the beginning of late stage disease.

Objectives: The study was therefore aimed at determining the diagnostic test that could best predict

time of onset of astrocytosis. This was important so as to know the transition of early to late stage disease since therapy of the two stages is different. Another aim of the study was to asses astrocytosis as an indicator of meningoencephalitis and the correlation between astrocytosis and parasitaemia.

Methodology:The study was a continuation of another project which had worked on fourteen vervet monkeys. One of the monkeys was used as a control while thirteen were infected with approximately 104 *T.b. rhodesiense* (isolate: KETRI 2537). The monkeys were then treated with Diaminazine Aceturate at day 28 so as to let the animals survive long for the late stage disease to set in. The animals were then euthanized fortnightly from day 42 and brain samples harvested at the level of the third ventricle and preserved in 10% formalin and then embedded in wax. At day 140 a treatment for late stage trypanosomiasis was administered with Melasoprol. The animals surviving up to this point were kept until day 462 when they were euthanized together with the control animal. During the study, data on clinical signs, immunology – (IL 10, Serum IgG, IgM, CSF IgG, and IgM) and haematology were collected fortnightly. The fourteen vervet monkey brain samples were then used in this experiment. An immunohistochemical analysis was performed on the brain samples so as to stain for astrocytes. The slides were then examined and pathology graded from grade 0-4 using a predetermined scale. Astrocyte distribution in the brain and their sizes were determined.

Results: From Immunohistochemistry results the earliest time that astrocytosis was detected was at day 42 post infection which showed few small sized astrocytes at the area near the choriod plexus. The distribution into the brain parenchyma and cell size increased with days post infection to peak at day 98 post infection when grade 4 pathology characterized by stumpy astrocytes – (large cell bodies with short processes) was evident. An animal that died at day 178 showed that a healing process had set in and this was after treatment with Melasoprol at day 140 post infection and had pathology similar to grade 2. By the time the last animal was euthanized at day 462 post infection the astrocytes had significantly reduced in size and the brain parenchyma was almost healed. There was no detectable parasitaemia throughout the study except for two animals on days 42 and 70 hence this was

insignificant.

Correlation analysis showed CSF levels of IgG, IgM and CSF white cell counts to be strongly associated with astrocytosis (p-values of 0.003, 0.0036, and 0.0034 and correlation coefficients of 0.887, 0.883 and 0.886 respectively). Serum parasite specific IgG and IgM as well as IL 10 had no association with astrocytosis (p-values of 0.3643, 0.1743 and 0.8225 and correlation coefficients of 0.407, 0.532 and -0.095 respectively). Clinical signs which included body temperature, respiration rate, pulse rate and lymph node enlargement also had no association with astrocytosis (p-values of 0.0679, 0.1254, 0.8976, and 0.1924 respectively).

Conclusion The study found that the earliest time astrocytes activation occurs during *T.b. rhodesiense* HAT infection is at day 42 post infection and CSF levels of IgG and IgM as well as white cell counts in the fluid could be used to predict astrocytosis. However serum parasite specific IgM, IgG, PCV as well as clinical signs cannot be used to predict astrocytosis. In addition there was no evidence to relate parasitaemia to astrocytosis.