MOLECULAR CHARACTERIZATION OF *E.HISTOLYTICA* STRAINS AND THE IMPACT OF HOST GENETICS ON AMOEBIC INFECTION IN LIMPOPO AND GAUTENG PROVINCE, SOUTH AFRICA.

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ABSTRACT

BACKGROUND: *Entamoeba histolytica* is a protozoan parasite known to be pathogenic to human. It is the causative agent of amebiasis, which ranges from asymptomatic to symptomatic diseases. What determines the outcome of this infection is not well understood and the factors that make some infected people sick while others do not get the disease are not clearly defined. Studies have suggested that there is a genetic component that contributes to susceptibility and possibly outcomes of infection and that parasite genotypes and host responses upon exposure to a pathogen can determine a wide spectrum of illness from subclinical or mild to severe diseases. Interleukin (IL)-10, an anti-inflammatory cytokine with pleiotropic properties is involved in the progression of the disease, but the relationship between the genetic variants of IL-10 has not been extensively studied in people with amoebiasis. Therefore the present study aimed at determining the molecular characteristics of *E. histolytica* in relation to disease presentation and to determine the role that host genetics play in the susceptibility to amoebic infection. **METHOD**: This study was approved by the research and ethical committee of the University of Venda, and authorization was obtained from the Department of Health and Welfare in Polokwane. Diarrheal and non-diarrheal stool samples were collected from patients of all ages at Giyani and Pretoria. All the stool (111) samples were examined under a light microscope for the presence of *Entamoeba* cysts and trophozoites. PCR method was used to amplify the serine rich *E. histolytica* protein (SREHP) and chitinase genes using published primers.

A total of 647 Peripheral blood was collected from patients infected with HIV, who were attending different hospitals and clinics throughout the Limpopo province. Genomic DNA was extracted from buffy coat using Gen ELUTE Blood Genomic DNA kit from Sigma-Adrich. The levels of interleukin-10 in serum were measured using enzyme-linked immunosorbent assay

(ELISA) from MABTECH. The serum samples were also used to detect antibodies against *E. histolytica* using *E. histolytica* serology ELISA kit from Techlab. The Techlab *E. histolytica* II kit was used to detect the antigen (GalNAc lectin) against *E. histolytica*. Single nucleotide polymorphisms (SNPs) at positions -1082A/G and -592C/A in the IL-10 gene promoter were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) by using MnlI and RsaI restrictions enzymes respectively. **RESULTS PART 1:** From the 111 stool samples collected, 51 were positive by either PCR or microscopy and 14 samples were positive by both methods. The serine- rich *E. histolytica* protein was successfully amplified in 26 samples positive by microscopy or PCR. Out of the 26 samples (19) SREHP profiles were obtained. SREHP #2 was obtained in 5 different isolates, 4 from Pretoria and 1 from Giyani (2 symptomatic and 3 asymptomatic), based on these findings this profile is related with diarrhea, since it was also found in patients with watery stools. This profile was found to be more prevalent in Pretoria than Giyani. The chitinase gene amplified 59 samples positive by microscopy and PCR, and out of the 59 samples 22 chitinase profiles were obtained. Two interesting chitinase profiles (profile #4 and 18) were obtained in this study. Profile #4 was found in 6 different isolates, 5 from Giyani and 1 from Pretoria (3 symptomatic and 3 asymptomatic), this profile was found to corelate with diarrhea since it was found in isolates with watery stools and loose stools. However, profile # 18 was not found to corelate with diarrhea since it was only found in isolates with formed stools, in all the isolates from Giyani. All the chitinase and serine rich *E. histolytica* profiles were more prevalent in Giyani (75.1%) compared to Pretoria (24.3%). **RESULTS. PART 2:** Out of the 647 serum samples used in this study, 422 (65.2%) were positive for antibodies against *E. histolytica* by ELISA. The *E. histolytica* antigens were detected in only 3 (0.5%) samples. The seroprevalence was higher in females (66.1%) than in males

(63.5%), with high prevalence in rural areas (66. 5%) than in urban areas (63.9%). The young (0 to 25) and old aged group (46 to 80) were more infected than people aged 26 to 45, with a prevalence rate of 68.9%, 67.6% and 64% respectively. **RESULTS PART 3:** The patients who were positive for antibodies against *E. histolytica* were found to produce high levels of IL-10 (68.1%) than the sero- negative group (34.2%) by ELISA, and the difference was significant *(P=0.016).* There was no significant difference in IL-10 expression in relation to gender and age *(P>0.005).* However, the results of this study showed that more females had high IL-10 (30.3%) than males (25.9%). The patients aged 46-80 were found to produce more IL-10 compared with the other groups (0-25 and 26-45 age groups) with percentages of 35.3%, 27.9% and 27.3% respectively. **RESULTS PART 4:** In this study we evaluated the association between the polymorphisms of the IL-10 promoter at position -592 A/C and -1082 A/C and the *E. histolytica* seroprevalence. For the IL-10 -1082A/G polymorphism, there was significant difference between the genotypes and the *E. histolytica* infection, however, the homozygous AA (68.1%) and heterozygous AG (67.5%) genotypes were associated with susceptibility to amoebiasis and the homozygous GG genotype was associated with reduced risk of amoebiasis (P=0.945). There was no significant difference between the -592 A/C polymorphism with *E. histolytica* infection. However, this study showed that AC genotype was associated with reduced risk of amoebiasis. The homozogous CC and AA genotypes were common among sero-positive patients (71.3% and 64.1 %) in comparison with the sero-negative subjects (28.7% and 37.6%) (P=0.259) respectively. There was no significant difference between IL-10 levels and the IL-10 gene promoter polymorphisms in the study. **CONCLUSIONS PART 1**: The results obtained in this study have further confirmed the genetic heterogeneity of *E. histolytica* for the SREHP and chitinase genes which might have a role in the presentation of amoebiasis (symptomatic and

asymptomatic infections) depending on the genetic profile of the infecting strain. **PART 2:** The high seroprevalence of antibodies against *E. histolytica* suggest that the majority of the people in this study have been exposed to *E. histolytica*. Moreover, this prevalence also shows that amoebiasis is endemic in these areas. The low prevalence of antigens against *E. histolytica* in this study, suggests that amoebic liver abscess might not be common in the region. **PART 3**: IL-10 concentrations might influence the progression of the disease. This study revealed that elevated level of IL-10 is associated with reduced risk of amoebiasis, the higher the level of IL-10 in the serum the lower the infection rate. **PART 4**: This study reports for the first time that IL-10 promoter polymorphisms participate in the progression of amoebiasis. The IL-10 -1082 A/G polymorphism was more associated with amoebiasis susceptibility as compared to -592 A/C polymorphism

Keywords: *Entamoeba histolytica*, SREHP, chitinase and IL-10, genetic Polymorphisms.