VETERINARSKI ARHIV 72 (2), 101-108, 2002

Urine albumin level in mice infected with *Trypanosoma brucei*

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AGU, W. E., A. N. EGBUJI: Urine albumin level in mice infected with *Trypanosoma brucei*. Vet. arhiv 72, 101-108, 2002.

ABSTRACT

Urine albumin levels were evaluated in mice infected with *Trypanosoma brucei* using urine strips (Medi-Test Combi 9, Macherey-Nagel, Neumann-Neander-Strabe 6-8, D-52355 Duren). The urine albumin level in some of the infected mice began to increase from the 6th day post-infection, and by the 12th day all infected mice showed a high level of albumin in urine which progressively increased with time. There was a high level of significance ($F=161.9025$, $P<0.001$) with mean urine albumin levels being significantly higher in trypanosome-infected mice (1.9302) than non-infected control mice (1.4771) As infection progressed there was also a significant increase ($F=152721$, $P<0.001$) with mean urine albumin levels increasing with time. It was concluded that urine albumin could be used to indicate trypanosomosis.

Key words: mice, *Trypanosoma brucei*, albumin, urine strips, trypanosomosis, proteinuria

Introduction

The report of the 25th meeting and Golden Jubilee of ISCTRC, Mombassa, Kenya, 27th September - 1st October 1999, stated, among other things, that the infection rate in human trypanosomosis is back to where it was in the 1930s, with over half of the local population being affected.

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ISSN 0372-5480
Printed in Croatia

One recommendation made at the meeting was that "urgent and particular attention be given to surveillance, to intervention in epidemic areas, and drug availability and resistance" (ANONYMOUS, 1999). Surveillance is therefore very necessary to facilitate early diagnosis and treatment of the disease. In the area of epidemiology and surveillance some improvements in African trypanosomosis diagnosis have obviously been achieved with recent techniques such as ELISA and PCR (BUSCHAIR, 2001). For example, REBESKI et al. (1999; 2000) have made remarkable efforts to standardize an ELISA for *T. vivax* and *T. congolense* in bovine and to have it validated and introduced in many African laboratories. Moreover, to increase sensitivity and specificity, PCR is sometimes combined with hybridization (CLAUSEN et al., 1999). However, despite such improvements these techniques are poorly standardized, and the current cost and technical requirements of molecular diagnostics are prohibitive for their general application in developing countries where the disease is endemic. Apart from that, evidence is available that the tests suffer unexplained false negatives (KABIRI et al., 1999; SIMO et al., 1999) and false positives which remain positive long after a successful cure (PEREIRA DE ALIMEIDA, 1999). Therefore, with the present diagnostic methods available, which emphasize parasitological, serological and molecular diagnostic techniques, surveillance would still be limited, expensive and time consuming, thereby leaving most cases of trypanosomosis undiagnosed and untreated.

The present study was designed using test strips, a rapid screening test, to evaluate the albumin levels in urine of mice infected with *Trypanosoma brucei* and thus to use albumin levels to indicate trypanosomosis. It is envisaged that the work would be used as a model to evaluate trypanosomosis in man through albumin levels in urine and so serve as a rapid and relatively inexpensive method of screening large populations of people for trypanosomosis.

Materials and methods

Outbred mice aged between 5 and 8 weeks obtained from the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, were used for the experiment. They were kept in appropriate rat/mice cages and fed on pelleted mice feed, with water *ad libitum*. The *T. brucei* used for the work

was isolated from a dog at the University of Nigeria Veterinary Teaching Hospital in 1998, and had since been maintained by continuous mice passages. The blood of mice showing heavy parasitaemia was used as material for initiating infection in the experimental animals. Each of the 20 infected mice received 1 x 10^6 trypanosomes intraperitoneally contained in 0.2 ml of diluted infected blood, while each of the 10 uninfected control mice received 0.2 ml sterile normal saline given intraperitoneally. Microscopic examinations of the blood from tail vein were performed daily on wet blood films made from each mouse. Urine samples were collected from each animal every three days. Mice were isolated during the period of urine collection, one to a clean plastic case, and allowed to urinate naturally. The urine sample was pipetted into a clean tube and immediately tested for albumin. The Test strip (Medi-Test Combi 9, Macherey-Neagel, Neumann-Neander-Strabe 6-8, D-52355 Duren) was dipped into the fresh urine for approximately one second and immediately drawn across the rim of the tube to remove excess urine. After 30-60 seconds the test strip was compared with the colour scale and the colour change was noted and converted to the corresponding albumin concentration (mg/dl).

Statistical analysis. Changes in mean urine albumin levels of both infected and non-infected control mice were analysed by a 2-way ANOVA. Unequal subclass numbers (method of weighted squares of means) with main effects as treatment (2 levels corresponding to trypanosome-infected and non-infected groups) and time (8 levels corresponding to 0 to 21 days). Data on albumin readings (mg/dl) were not normally distributed and were therefore subjected to logarithmic transformation prior to analysis.

Results

Results of the infection showed that the pre-patent period of the *T. brucei* in mice was 4 to 5 days. By the 5th day of inoculation all infected mice showed trypanosomes in their peripheral blood. The results of urine albumin levels in the infected mice showed a high level of significance ($F=161,9025$, $P<0.001$) with mean urine albumin levels being significantly higher in trypanosome-infected mice (1.9302) than in non-infected control

mice (1.4771). Also, as infection progressed there was a significant increase (F=15.2721, P<0.001), with mean urine albumin levels increasing with time (Table 1). Similarly, there was a significantly positive interaction between trypanosome infection and time (F=14.4989, P<0.001) which facilitated the detection of between treatment and within treatment differences in mean urine albumin levels at any point in time.

Table 1. Albumin level (mg/dl) in infected and control mice mean ± SE

Days	0	3	6	9	12	15	18	21
Infected group	30.0 ± 0	30.0 ± 0	64.0 ± 23.65	94.5 ± 31.75	176.84 ± 39.65	176.84 ± 51.06	340.0 ± 65.32	328.57 ± 80.81
Non-infected	30.0 ± 0	30.0 ± 0	30.0 ± 0	30.0 ± 0	30.0 ± 0	30.0 ± 0	30.0:1:0	30.0 ± 0

Note: The test is for rapid screening for protein in urine with range: negative, 30, 100, 500 mg/dl. Thus, any trace of protein not up to 100 mg/dl is recorded as 30 mg/dl. This means that each of the controls has a trace of protein, which can only be recorded as 30 mg/dl.

Discussion

The results of this study indicate that within 5 days of inoculation with *T. brucei* all infected mice showed trypanosomes in their peripheral blood. The urine albumin level in some of the infected mice began to increase from the 6th day post-infection, and by the 12th day all infected mice were showing high levels of albumin in urine which progressively increased with time (Table 1). Urine albumin was significantly higher in infected mice P<0.001) than in non-infected mice. Normal mice showed traces of concentration (30 mg/dl) of albumin in their urine (Table 1).

The observed increase in urine level in infected animals could probably be attributed to degenerative changes in the kidneys, resulting in increased permeability of the glomerular filter. LOSOS and IKEDE (1972) reported extensive inflammatory, degenerative and necrotic changes in connective tissues of rodents infected with *T. brucei*, especially in the cells of the liver, heart, skeletal muscles and kidney. VAN DEN INGH et al. (1976a) observed kidney lesions in *T. vivax* infected goats. These lesions included

hypercellular glomeruli and dilatation of proximal tubules. STEPHEN (1986) noted hypertrophy and hyperplasia of cells of the epithelium of Bowman's capsule and the endothelium of capillary network of the glomeruli, in *T.vivax* infection of ruminants. Severe tubular necrosis and round cell infiltration has also been reported in animal trypanosomosis (FIENNES, 1970). In other cases, accumulation of proteinaceous exudates in the Bowman' s capsule and in the tubules of cattle infected with *T. vivax* has been noted (ISOUN and ESURUOSO, 1972; MWONGELA et al., 1981) was observed. A number of workers have noted a remarkable decrease in the levels of serum albumin as infection progressed in cattle and sheep infected with *T. vivax* (ANOSA and ISOUN, 1976; TABEL et al., 1979; VAN DEN INGH, 1976). Proteinuria had also been reported in ovine *T. vivax* infection (ANTIA, 1981). It is probable that the increase in urine albumin noted in the present study could be due to a reduction in serum albumin resulting from lesions in the kidneys, giving rise to increased permeability of the glomerular filter.

Whatever the pathogenesis of increased albumin in the urine of mice infected with *T. brucei* may be, the important factor is that the increase in urine albumin can be used to indicate trypanosomosis. From the present study, it is obvious that within two weeks of infection all infected animals showed a high level of albumin in urine, and this was easily and relatively inexpensively detected using urine strips, and could therefore also be used to indicate trypanosomosis. It is pertinent to note that traditional diagnostic observations include the distinctive smell of the urine and taste of milk in *T. evansi* infections of camels (SCHILLHORN VAN VEEN, 1997). Obviously, other diseases and conditions could also lead to proteinuria, but the use of urine strips to indicate trypansosomosis through albumin levels could be useful in screening large populations of people in an endemic area, especially now, when there are reports of an increase in the intensity of the disease, with over 50% disease prevalence (ANONYMOUS, 1998; EDEGHERE et al., 1998; ANCELLE, 1996). In this case, only those people showing albumin in their urine would be subjected to other parasitological diagnostic tests. It is suggested that such use of urine strips to indicate trypanosomosis through urine albumin levels be attempted in endemic areas of human trypanosomosis to evaluate its potential for diagnosis.

Acknowledgements
We are grateful to Mr. A. B. Chime of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, for his technical assistance, and to Professor M.M. Ikeme for providing the statistical analysis.

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Received: 25 January 2001
Accepted: 25 April 2002

AGU, W. E., A. N. EGBUJI: Razina albumina u miševa invadiranih protozoonom *Trypanosoma brucei*. Vet. arhiv 72, 101-108, 2002.

SAŽETAK

Razina albumina u miševa invadiranih protozoonom *Trypanosoma brucei* određena je aproksimativnom metodom s urin trakom (Medi-Test Combi 9, Macherey-Nagel, Neumann-Neander-Strabe 6-8, D-52355 Duren). Razina albumina u nekih invadiranih miševa porasla je šestog dana nakon invazije. Dvanaestog dana nakon invazije primijećen je porast u svih miševa. U invadiranih miševa utvrđena je statistički značajna razlika u odnosu na kontrolu. Autori smatraju da se koncentracija albumina može pouzdano koristiti u dijagnostici tripanosomoze.

Ključne riječi: miš, *Trypanosoma brucei*, albumin, tripanosomoza, proteinurija