ASSESSMENT OF THE PREVALENCE OF *Leishmania* AMASTIGOTES IN BUFFY COAT FILMS IN PATIENTS IN A RENAL UNIT IN A CUTANEOUS LEISHMANIASIS ENDEMIC AREA IN SRI LANKA.

W.M.C.W. Menike[1*], R.T. Dasanayake[2], R. Wickremasinghe[1], J.C. Dassanayake[1], S. Kahatapitiya[1], I. S. Wijesiriwardene[4], I. De Alwis[3], P.H.K.I.S. Ranasinghe[1]

[1] Department of Parasitology,University of Sri Jayewardenepura
[2] Nephrology Unit, Teaching Hospital, Anuradhapura
[3] Blood Bank, Provincial General Hospital, Ratnapuara
[4]Department of Pathology,University of Sri Jayewardenepura

INTRODUCTION

Sri Lanka is one of the newly emerged leishmaniasis disease foci in the world (Karunaweera et al. 2003, Alvar et al. 2012). The disease presents in three major clinical forms; cutaneous leishmaniasis (CL) which is confined to the skin, mutilating mucocutaneous leishmaniasis (MCL)/mucosal leishmaniasis (ML) which involves the mucocutaneous junctions of the oro-nasal cavity and potentially fatal visceral leishmaniasis (VL) (WHO 2010).

Sri Lanka mainly report CL with few cases of VL & ML (Epidemiology Unit Ministry of Health Sri Lanka (EUMHS) 2012, Ranasinghe et al. 2012).

Currently more than 500 CL patients have been reported annually from Anuradhapura, Polonnaruwa, Matara, Thangalle and Hambanthota districts (EUMHS 2012).

It was revealed in a recent study that both VL and CL causing strains in Sri Lanka are similar in their iso-enzyme migration patterns and belong to the same zymodeme *L. donovani MON-37* (Karunaweera et al. 2003, Ranasinghe et al. 2012). VL may be asymptomatic or subclinical and self- resolving, but usually runs a sub-acute or chronic course (Mpaka et al. 2009). It is well reported that visceral leishmaniasis could remain asymptomatic up to 20% of an endemic community (Franca et al. 2013). Out of those only 5% will progress to VL depending on the patient's immunity and nutrition (Franca et al. 2013). Leishmaniasis is transmitted to humans mainly by the bite of an infected sandfly. However, it has been reported to have been transmitted via blood transfusions and organ transplantations (Dettwiler et al. 2010). About 100 cases of VL after kidney transplantation in the world are reported in the literature (Bauchekoua et al. 2014). It was also well documented that reactivation of asymptomatic or cured VL could occur when a person becomes immunosuppressed (WHO 2010, Dettwiler et al. 2010).

In Sri Lanka chronic kidney disease (CKD) is on the rise due to known and unknown aetiologies. Chronic kidney disease of unknown aetiology (CKDu) is reported from some parts of the country which include the CL endemic districts. Although CKD patients require repeated blood transfusions at several stages of the course of the illness and are prone to transfusion transmission of VL, there is no screening for *Leishmania* amastigotes carried out among blood donors in Sri Lanka.

Detection of *Leishmania* amastigotes in peripheral blood buffy coat smears is an alternative and minimally invasive procedure for the parasitological diagnosis of VL. Its sensitivity ranged from 50 to 99% (Monica 1998). As a definitive diagnostic tool, buffy coat smear has the potential to be carried out for point-of-care VL case management (Salam et al. 2012).

The objective of the study was to assess the presence of *Leishmania* amastigotes in buffy coat films among CKD and renal transplant patients in a renal unit in a cutaneous leishmaniasis endemic area in Sri Lanka.

[*] *Corresponding author: Email –* ***cmenike@gmail.com***

METHODOLOGY

Data related to clinical features and history of Leishmaniasis and blood samples from 170 individuals were collected from June to October, 2015. The population comprised individuals with CKD who had undergone repeated blood transfusions, immunosuppressive treatments and KT attending clinics or being treated at the Renal Unit (RU), Teaching Hospital, Anuradhapura (THA). Diagnosis and selection of patients meeting the inclusion criteria were done by the Consultant Nephrologist at THA according to the criteria described in Kidney Disease Improving Global Outcomes (KDIGO) 2012, the clinical practice guideline for the evaluation and management of CKD.

Taking of the routine history and examination of patients were carried out by a medical officer in the RU, THA. Questionnaires were administered to collect additional information, such as socio economic factors that are related to acquiring leishmaniasis. Routine investigation reports and diagnostic cards of patients that are recorded in the RU were also used to collect information relevant to this study.

Venipuncture was done under sterile condition by a trained nursing officer. Two milliliters of venous blood was collected from each patient to an EDTA bottle. Collected blood was centrifuged in low speed (2000rpm/ for 10 mins with two- three break offs in four to five minutes intervals. (HIP. 2015) to separate buffy coat and thin layer (film) was drawn on a microscope slide with 10 µl of buffy coat.

The smears were fixed with absolute methanol and stained with Giemsa stain and examined under the microscope under oil emersion (100X) for the presence of *Leishmania* amastigotes.

RESULTS AND DISCUSSION

Results

Table 1. Categorization of patients and buffy coat examination.

Criteria	Male	Female	Amastigotes
1. BT only	98 (58%)	15 (9 %)	Not present
2. IM only	05 (03%)	05 (03%)	Not present
3. KT only	00 (00%)	00 (00%)	Not present
4. BT & IM	00 (00%)	03 (02%)	Not present
5. BT & KT	00 (00%)	00 (00%)	Not present
6. IM & KT	26 (15%)	03 (02%)	Not present
7. BT, IM & KT	14 (08%)	01 (0.6%)	Not present
Clinical Features			
1. Yes	42 (25%)	07 (04%)	Not present
2. No	101 (59%)	20 (12%)	Not present
Skin Lesions			
1. Yes	01 (0.6%)	00 (00%)	Not present
2. No	00 (00%)	00 (00%)	Not present

BT: Blood transfusions; IM: Immunosuppressive Treatments; KT: Kidney transplant

131 (77%) patients had a history of blood transfusions and 44 (26%) had undergone kidney transplant within the last two years. 57 (34%) patients were on immunosuppressive treatments within last two months and continues to be on treatment.

Out of 170 patients studied, none of the patient had clinical features suggestive of VL

(Fever, anaemia, lymphadenopathy, hepatosplenomegaly etc…) However, there were 04% with fever, 03 % with lymphadenopathy and about 03% of hepatosplenomegaly. Those clinical features could be due to chronic infections that are usually found in that group of patients. There were 18% of patients present with pallor, but it could not be regarded as clinical feature that is directly related to leishmaniasis. Only one patient (0.6%) had a lesion that could be suspected as Leishmaniasis, but diagnosed as negative by Slit Skin Smear and biopsy examination.

From the 170 individuals tested, all patients were negative for *Leishmania* amastigotes in buffy coat films.

DISCUSSION

In the last 20 years, the increasing frequency of organ transplantations and the improvement of associated immunosuppressive treatments have led to the recognition of several cases of VL complicating organ transplantation (Bauchekoua et al. 2014).
Since the advent of HIV infection and increased use of immunosuppression for transplantation and chemotherapy, populations that suffer from the disease have increased (WHO 2010).
There was a demonstration of a reactivity of 37% to VL in haemodialysed polytransfused patients from Brazil during the burden of VL (Luz et al. 1997). The presence of antibodies against *Leishmania* and its DNA in blood donors has been found (Luz et al. 1997). This indicates that there are asymptomatic reservoirs. Thus, blood transfusion might be a risk factor for VL.

As all the patients in this study were negative for *Leishmania* amastigotes there was no association to be shownb between CKD/KT and VL according this study.

This study reveals that VL may not still be an acute problem among CKD/KT patients community in a Cutaneous Leishmaniasis (CL) endemic areas in Sri Lanka, none of the patients had past history of VL and none of the patients had proven past history of CL.

Although demonstration of even a single amastigote upon microscopic examination of tissue smears or multiple promastigotes in cultures is considered sufficient for positive diagnosis of the disease, the sensitivity of the tissue examination, except in the case of splenic aspirate, is low (Sundar & Rai 2002).

Therefore, antibody detection should be performed in this group of patients to see whether asymptomatic VL exists among could be an aetiological factor for CKD in a CL endemic area in Sri Lanka.

CONCLUSIONS/RECOMMENDATIONS

Visceral Leishmaniasis may not still be an acute problem among CKD/KT patients in a Cutaneous Leishmaniasis (CL) endemic area in Sri Lanka. However, serological tests are recommended to arrive at further conclusions regarding correlation between CKD/KT patient's population and VL in a cutaneous leishmaniasis endemic area in Sri Lanka.

REFERENCES

Bouchekoua, M.,Trabelsi,S.,Ben,A.T.,Khaled,,S.2014. Visceral leishmaniasis afterkidney transplantation: report of a new case and a review of the literature. 28,32-5.

Dettwiler,S., McKee,T., Hadaya,K., Chappuis,F., van Delden,C., Mol,l S. 2010. Visceral leishmaniasis ina kidney transplant recipient: parasitic interstitial nephritis, a cause of renal dysfunction. Am J Transplant10, 1486-9.

Epidemiology Unit, Ministry of Health Sri Lanka (EUMHNSL) 2012.Newly introduced notifiable diseases.Weekly Epidemiological Reports,39: 3. Available at: http://www.epid.gov.lk/wer.htm. [Accessed December 28, 2014].

Franca,A.de O., Castro,V.L., Junior,M.S., Pontes,E.R., Dorval,M.E. 2013. Anti leishmania antibodies in blood donors from the Midwest region of Brazil. Transfuse Apher sci49,627 –30.

HIP (Human Immunology Portal) available at http://www.humanimmunologyportal.com/protocols/preparing-a-buffy-coat-from-whole-blood [Accessed June, 15, 2015].

Karunaweera,N.D., Pratlong,F., Siriwardana,H.V., Ihalamulla,R.L., Dedet,J.P.2003. Sri Lankan cutaneous leishmaniasis is caused by *Leishmania donovani*zymodeme MON-37. *Trans Roy Soc Trop Med Hyg97*,380-81.

KDIGO 2012. Clinical practice Guideline for the Evaluation and Management of chronic kidney Disease. *Kidney international suppliments3*,5 -14.

Luz,K.G., Da Silva,V.O., Gomes,E.M., Machado,F.C., Araujo M.A., Fonseca H.E., et al. 1997.Prevalence of anti-Leishmania donovani antibody among Brazilian blood donors and multiply transfused hemodialysispatients. *Am J Trop Med Hyg 57*, 168—71.

Maia,Z.1., Lírio,M., Mistro,S., Mendes,C.M., Mehta,S.R., Badaro,R.2012. Comparative study of rK39 Leishmania antigen for serodiagnosis of visceral leishmaniasis: systematic review with meta-analysis. *PLoS Negl Trop Dis*. *6*,e1484.

Monica, C. 1998. *District laboratory practice in tropical countries, part I*, p 275–280 Cambridge University Press, U.K.

Mpaka,M.A., Daniil,Z., Kyriakou,D.S., Zakynthinos,E.2009. Septic shock due to visceral leishmaniasis, probably transmitted from blood transfusion. *J Infect Dev Ctries*. 3, 479-83.

Ranasinghe, S., Wickremasinghe, R., Munasinghe, A., Hulangamuw,a S., Sundaramoorthy, S. *et al.* 2013. A cross sectional study to assess risk factors for leishmaniasis in an endemic region of Sri Lanka. *Am J Trop Med Hyg* 89, 742-9.

Ranasinghe, S., Zhang, W., Wickremasinghe, R., Abeygunasekera, A., Chandrasekharan, V.*et al.* 2012. *Leishmania donovani* zymodeme MON-37 isolated from an autochthonous visceral leishmaniasis patient in Sri Lanka. *Pathog Glob Health* 106, 421-4.

Salam, A.M., Khan, M.G.M., Bhaskar, K.R.H., Afrad, M.H., Huda, M.M., Mondal, D. 2012 Peripheral Blood Buffy Coat Smear: a Promising Tool for Diagnosis of Visceral Leishmaniasis J Clin Microbiol 50, 837-840.

Sundar, S., and Rai, M. 2002. Laboratory diagnosis of Visceral Leishmaniasis. Clin Diagn, Lab Immunol, 9, 951-8.

ACKNOWLEDGMENTS

The authors would like to acknowledge Faculty of Medical Sciences, University of Sri Jayewardenepura for funding (Grant No. ASP/01/RE/MED/2015/45), Dr. Iresha Darmasena, Consultant Haematologist, Teaching Hospital Anuradhapura for providing laboratory facilities for sample preparation and Ms. Sarala Kooragamage, Nursing officer, Renal Unit, Teaching Hospital, Anuradhapura for assisting sample collection.