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BRIEF COMMUNICATION

EVALUATION OF ORAL MUCOSAL TRANSUDATE FOR IMMUNODIAGNOSIS OF CHAGAS´ DISEASE

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The infectious diseases are diagnosed by the detection of specific immunoglobulins in serum, and the drawn blood with needlestick injuries makes this procedure somewhat unattractive. Oral mucosal transudate (OMT), a saliva component, is a serous fluid rich in IgG that comes from transudation at the gingival crevice and across oral mucosal surfaces. This biological fluid has been used as an alternative for detection of antibodies against a variety of viral agents including hepatitis A, B and C virus, HIV and *S. mansoni* antigens [2,3,10]. This alternative method have shown results as accurate as those obtained with serum samples.

In the present communication we report the evaluation of oral mucosal transudate for the diagnosis of *Trypanosoma cruzi* infection using an immunoenzymatic assay.

Twenty-one individuals proved (clinically, electrocardiographically, serologically and epidemiologically) to have chronic Chagas´ disease: 10 with cardiac form, 2 with digestive form, 6 with mixed form and 3 with asymptomatic form were studied. Seven individuals from endemic area, with negative serology, constituted the control group. Blood samples from these groups were taken by venopuncture and the sera obtained were stored at –20 ºC. All individuals were tested for Chagas´ disease using two serological assay according to LUQUETTI *et al*.[5].

For the colection of the OMT from both groups, the collect device OraSure®, Epitope Inc., Beaverton, OR, USA, was used. The paper pad was placed between the lower gum and cheek for 4 min. After collection, the pad was placed in a tube containing 1 ml of a bacteriostatic solution and stored at 4 ºC by four days. The sample was recovered from the pad by centrifugation and stored at –20 ºC until use.

The Abbott Chagas antibody enzyme immunoassay (EIA) kit was used to detect anti-*T. cruzi* IgG. We used the 1:2 dilution for assaying OMT samples that were tested in duplicate. The cut-off was calculated on the basis of the optical density (OD) values from negative controls (oral samples from endemic area) plus two standard deviations (SD).

The results of individual anti-*T. cruzi* specific IgG in OMT samples are shown in the Fig. 1. Twenty from twenty one chagasic patients presented the OD above the cut-off value (0.140) and were considered positive for Chagas´ disease. None of the OMT samples from the seronegative individuals were positive. The sensitivity and specificity were 95% and 100%, respectively. The confidence interval to sensitivity of 95% was 86% to 100%.

Recently, a ELISA test, using whole saliva, was developed by PINHO *et al*. (1999) to detect specific IgG in a population of *T. cruzi* infected individuals. The sensitivity and specificity in this test were 90.4% and 95%, respectively. Whole saliva is a mixture of secretions from the parotid, submandibular, and minor salivary glands, and serum transudates from the capillaries beneath the bucal mucosae and from the gingival crevice as well as mucin, bacteria, leukocytes, and sloughed epithelial cells. The primary limitation of whole saliva as a viable testing fluid is that it contains relatively low titers of IgG, which is concentrated in two components of whole saliva: gingival crevicular fluid and mucosal transudate[6,7,8]. Second, microorganisms in whole saliva generate proteases that degrade immunoglobulin over a relatively short time period, making it difficult to store saliva samples for transport to the laboratory[4]. In addition, mucins and various particulates in saliva give it high viscosity, causing problems with accurate pipetting[2]. Contrarily, OMT collected by the procedure described in the present communication, contains IgG levels 4-fold greater than those found in ordinary whole saliva. On the other hand, the sample quality is further improved by the use of a non toxic stabilizing preservative that prevents bacterial growth and degradation of IgG by bacterial proteases[1,7]. These facts can explain the values of sensitivity (90.4%) and specificity (95%) found by PINHO *et al*.[9] by using the whole saliva, when compared with our results (sensitivity = 95% and specificity = 100%) by using OMT, and justify the use of the later oral fluid to detect specific IgG anti-*T. cruzi*. In conclusion, the preliminary results demonstrate that the OMT could be used as alternative biologic fluid for diagnosis of Chagas´ disease. In addition, the availability of a simple, noninvasive, reliable alternative to blood should

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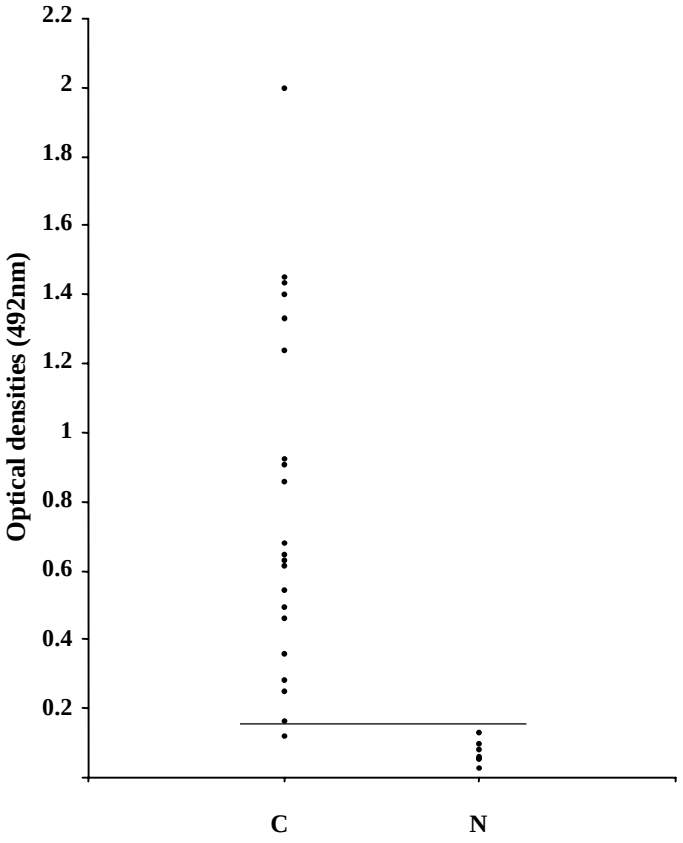

Fig. 1 - IgG response against *T. cruzi* antigen in oral mucosal transudate of chagasic patients (C) and normal individuals (N) measured by ELISA. The line indicates cut-off values.

have wide application in epidemiological studies. We are currently increasing the number of individuals in order to validate the present results including comparative analysis among OMT and serum samples.

RESUMO

Avaliação do transudato da mucosa oral no imunodiagnóstico da Doença de Chagas

Anticorpos anti-*Trypanosoma cruzi* (isotipo IgG) foram detectados no transudato da mucosa oral (TMO) através de um ensaio imunoenzimático. Foram estudados 21 indivíduos com doença de Chagas crônica comprovada através de diagnóstico clínico, eletrocardiográfico, epidemiológico e sorológico: 10 com forma cardíaca, 2 com forma digestiva, 6 com forma mista e 3 com forma assintomática. Sete indivíduos de área endêmica, com sorologia negativa, constituiram o grupo controle. O soro destes grupos foi armazenado a –20 ºC. A coleta de TMO de ambos os grupos foi realizada com o dispositivo OraSure® seguindo orientação do fabricante (OraSure®, Epitope Inc., Beaverton, OR, USA). As amostras de TMO foram diluídas (1:2) e testadas em duplicata através de um ensaio imunoenzimático da Abbott Laboratories para detectar anticorpos IgG contra doença de Chagas. Vinte dos vinte e um pacientes chagásicos apresentaram densidade óptica acima do limiar de reatividade e foram considerados positivos para doença de Chagas. Nenhuma das amostras provenientes de indivíduos soronegativos foi positiva. A sensibilidade e especificidade foram de 95% e 100%, respectivamente. Estes resultados indicam que TMO poderá ser utilizado como um fluido biológico alternativo para o diagnóstico da doença de Chagas. Nós estamos aumentando o número de indivíduos para validar estes resultados incluindo a análise comparativa entre amostras de TMO e soro.

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