

## Endothelial activation is associated with albuminuria in multibacillary leprosy

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### ABSTRACT

Leprosy may present kidney and endothelial abnormalities, being a risk factor for complications. However, the involvement of renal and vascular endothelia has been poorly investigated. We aimed to investigate if the levels of systemic endothelial biomarkers are associated with kidney abnormalities and the clinical forms of leprosy. This is a cross-sectional study with leprosy patients enrolled in January 2017 to December 2018, before the initiation of the multidrug therapy. Leprosy-associated clinical and epidemiological data were collected. Two groups were investigated: Paucibacillary (PB) and Multibacillary (MB) infections, for the comparisons. Serum and urine samples were obtained for laboratory analysis. In serum samples, were evaluated the endothelial biomarkers VCAM-1 and ICAM-1. In total, 101 leprosy patients were included, the mean age was  $48 \pm 15$  years and 71 (70%) were male. The multibacillary form occurred in 81 cases (80%), among which 22 had the Virchowian form. Serum creatinine was more elevated in the MB group than in PB patients. In addition, VCAM-1 was elevated in the MB group and was correlated with the bacteriological index ( $\rho = 0.372$ ,  $p < 0.01$ ), the duration of disease symptoms ( $\rho = 0.234$ ,  $p = 0.04$ ), and the number of skin lesions ( $\rho = 0.468$ ,  $p < 0.001$ ). Moreover, in MB patients who presented albuminuria  $> 15$  mg/g of creatinine, VCAM-1 showed a significant correlation with increased albuminuria and improved the correlation with the number of skin lesions ( $\rho = 0.563$ ,  $p = 0.010$ ). In conclusion, higher systemic VCAM-1 levels were associated with the multibacillary clinical form of leprosy and with increased albuminuria. Prospective studies are necessary to establish a cause-effect and evaluate the preventive role of these biomarkers to improve the clinical care.

**KEYWORDS:** Leprosy. Endothelium. Kidney disease. Albuminuria. Paucibacillary leprosy. Multibacillary leprosy.

### INTRODUCTION

Leprosy is a millennial disease and also an important neglected disease. Despite a major progress in its care and treatment, the global incidence of leprosy remains high and is frequently associated with clinical complications in the long term<sup>1</sup>. After the implementation of multidrug therapy, clinical manifestations of leprosy have been controlled, including secondary alterations such as renal abnormalities<sup>2</sup>.

However, patients with multibacillary leprosy are at high risk of developing kidney disease according to kidney injury scores<sup>3</sup>. Subclinical renal parameters such

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as albuminuria and urinary MCP-1 levels were previously correlated with clinical aspects of the multibacillary form of leprosy, including a higher bacteriological index in skin smears<sup>3</sup>.

It is not fully understood if in the long term, even after the specific treatment, leprosy patients would have a higher incidence of chronic diseases like chronic kidney disease (CKD) and hypertension. Nevertheless, investigations on subclinical alterations and incipient renal damage during leprosy patients care have not been evaluated<sup>4</sup>. In addition, it is not clear if leprosy patients that suffer from other previous chronic diseases, like diabetes and hypertension, have a higher risk for renal diseases and other complications<sup>5</sup>.

Endothelial biomarkers have been associated with renal dysfunctions, representing an important tool for the early diagnose of renal diseases in various clinical contexts<sup>6,7</sup>. VCAM-1 and ICAM-1 are important molecules in the mediation of immunological responses against *Mycobacterium leprae*<sup>8</sup>. Multiple studies evaluated the systemic levels of these endothelial molecules in various clinical forms of leprosy<sup>8</sup>. However, the relationship between these levels and important renal parameters has not yet been investigated in leprosy patients.

Thus, the present study aims to seek the association of endothelial biomarkers with kidney injury parameters and provide important data regarding vascular and renal changes in leprosy patients.

## MATERIALS AND METHODS

### Ethical aspects

The study protocol was reviewed and approved by the Ethics Committee of the Federal University of Ceara (CAAE N° 63841417.3.0000.5054), and all the participants gave their written informed consent prior to the study enrolment.

### Study design

This is a cross-sectional study with leprosy patients. Patients were evaluated in Dona Libania Dermatology Reference Center, in downtown Fortaleza, Ceara State, Brazil, from January 2017 to December 2018.

Leprosy patients of both genders, with ages between 18 and 60 years old, without kidney diseases, presenting with paucibacillary or multibacillary forms of leprosy (according to the operational classification of the World Health Organization - WHO) were invited to participate in this study before the beginning of the multidrug therapy. Leprosy patients with reactional episodes were excluded.

### Diagnosis and clinical aspects of leprosy

The leprosy diagnosis was based on clinical characteristics with the following cardinal signs<sup>9</sup>: a) Hypopigmented or erythematous macules or plaques with sensitivity loss; b) Involvement of peripheral nerves, with or without thickening, associated with sensitive or motor alterations; c) identification of alcohol-acid resistant bacilli (BAAR) in skin smears or in biopsies.

Depending on the number of lesions, WHO has classified leprosy into paucibacillary and multibacillary disease. Multibacillary disease is considered when the affected individual has > 5 skin lesions or > 1 nerve involvement or a positive skin smear in any site. The paucibacillary form is diagnosed if there are fewer skin lesions or no nerve involvement, or if there is only one nerve involvement with negative skin smears in all sites. This classification is useful from a therapeutic perspective, as paucibacillary cases are treated for 6 months, while multibacillary cases for 12 months using the same treatment regimens (rifampicin, dapsone and clofazimine)<sup>10</sup>. Moreover, the time of disease was evaluated according to questionnaires containing the patients' subjective answers, according to their perception of the first symptom.

### Collection and processing of the biological samples

Venous blood and urine samples were collected. The urine samples were collected in a sterile collector cup. Urine samples were centrifuged during 15 min 1,000 g for the removal of urinary sediments and debris. The urinary supernatant was aliquoted and immediately frozen at -80 °C until analysis.

Venous blood samples were collected in appropriate tubes for serum isolation. After 15 min, the blood was centrifuged, aliquoted and frozen at -80 °C until analysis.

### Laboratorial analysis

The following parameters were evaluated in urine samples: creatinine (Cobas C111, Roche®), proteinuria (Labtest®) and glycosuria (Cobas C111, Roche®) through enzymatic colorimetric methods; and albuminuria by an immunoturbidimetry assay (Cobas C111, Roche®). Albuminuria levels over 30 mg/g-Cr may characterize a renal alteration<sup>11</sup>. All the urinary clinical markers evaluated had their values adjusted by the urinary creatinine values, eliminating the bias of the differences in urinary concentrations among patients' samples<sup>12</sup>.

Endothelial biomarkers were quantified in the serum samples through an immunoenzyme assay (ELISA). Specific

test kits were acquired for VCAM-1 (ab47355, Abcam) and ICAM-1 (ab47349, Abcam) and the manufacturer instructions were followed.

### Statistical analysis

Categorical data were expressed as absolute counts, and frequencies in percentages. Frequencies were compared using the Qui-square test. Continuous data were initially tested for normal distribution using the Kolmogorov-Smirnov test and histograms evaluation. Normal or almost normal data were reported as mean  $\pm$  standard deviation. Non-normal data were expressed as medians and interquartile ranges. The Student t test or the Mann-Whitney test were used for comparisons between Paucibacillary and Multibacillary groups, according to the distribution of the data in each group. Correlations between the endothelial biomarkers and renal parameters were evaluated through the Spearman correlation (rho de Spearman). The statistical analysis used the SPSS program for Macintosh, version 23.0 (IBM, Armonk, NY, USA). A  $p < 0.05$  was considered statistical significant for all the analyses.

## RESULTS

### Clinical aspects and evaluated groups

In total, 101 leprosy patients were included before the beginning of the specific treatment. There was one or there

were more signs for the leprosy classification of the patients in this study. It was observed that 64 patients had more than five skin lesions, 56 patients had positive bacilloscopy in skin smears containing lymph, and 16 patients needed confirmation by biopsy. From the total of 101 patients, 20 had the paucibacillary form (PB group) and 81 had the multibacillary form (MB group) of leprosy. Into the MB group, 22 patients (27%) had the Virchowian clinical form. The MB group had significantly more lesions in comparison with the PB group (median [IQR]: 10 [6 – 14] vs 1 [1 - 1] skin lesions,  $p < 0.001$ ).

The mean age of the leprosy patients was  $48 \pm 15$  years and 71 (70%) were male. Only six patients (6%) had diabetes and seven (7%) presented cardiac arrhythmias. The frequency of diabetes was similar in the PB and the MB group (5% vs 6.2% in the MB group,  $p = 0.672$ ) (Table 1).

Albuminuria levels over 30 mg/g-Cr were observed in eight patients (8%) (Table 1). Moreover, the patients were stratified according to the third quartile (15 mg/g-Cr), to uncover a potential more susceptible group and to eliminate very low values of albuminuria, a possible bias in the correlation analysis. A group of 20 patients with albuminuria higher than 15 mg/g-Cr was obtained.

### Laboratorial aspects

Laboratory data were compared between the paucibacillary and the multibacillary forms. It was observed that only the serum creatinine presented significance

**Table 1** - Clinical aspects evaluated in the studied leprosy patients according to the operational classification.

	Leprosy patients (n=101)	Paucibacillary (n=20)	Multibacillary (n=81)	p*
Gender (Male)	71 (70)	15 (75)	64 (81)	0.946
Age (years)	48 $\pm$ 15	47.9 $\pm$ 13.37	48.22 $\pm$ 15.55	0.934
IMC (kg/m <sup>2</sup> )	20.1 $\pm$ 11.3	22.98 $\pm$ 12.29	19.77 $\pm$ 10.71	0.249
Diabetes	6 (6)	1 (5)	5 (6.2)	0.672
Arrhythmias	7 (7)	0 (0)	7 (9)	0.514
Albuminuria > 30 mg/g-Cr	8 (8)	0 (0)	8 (10)	0.124
Albuminuria > third quartile <sup>#</sup>	20 (21)	3 (15)	17 (21)	0.689
<b>Clinical aspects of the disease</b>				
Paucibacillary Form	20 (20)	-	-	-
Multibacillary Form	81 (80)	-	-	-
Virchowian form	22 (27)	-	-	-
Number of skin lesions	6 (1 – 14)	1 (1 - 1)	10 (6 – 14)	<0.001
Bacillary charge	2.48 $\pm$ 0.5	-	2.48 $\pm$ 0.5	-
Duration of disease (years)	1.6 $\pm$ 0.6	1.62 $\pm$ 0.72	1.59 $\pm$ 0.58	0.828

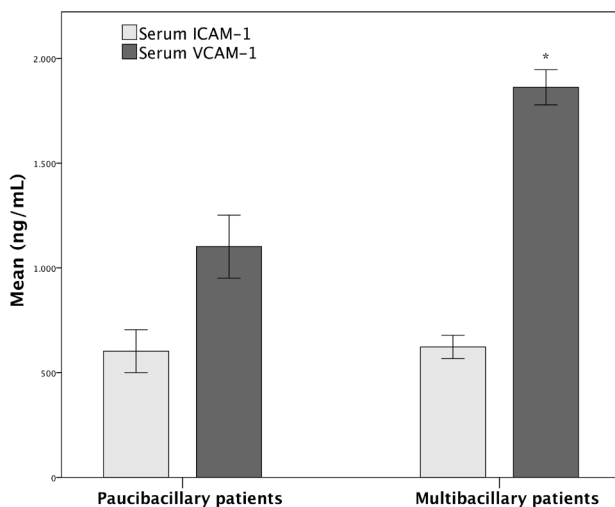
Quantitative data expressed as mean  $\pm$  standard deviation for normal data, and as median and interquartile range for non-normal data. Categorical data were expressed as absolute counts and percentages between parenthesis; # Quartile 1 = 3.9 / Quartile 2 = 7.1 and Quartile 3 = 15.0 mg/g-Cr; \*Groups were compared using the Student t test for quantitative data. For categorical data, the chi-square test or the Fisher exact test were used.

**Table 2** - Laboratory parameters of leprosy patients, according to the clinical form of the disease.

	Leprosy patients (n=101)	Paucibacillary (n=20)	Multibacillary (n=81)	p*
Leucocytes	6,756 ± 1,402	7.073 ± 1.528	6.677 ± 1.381	0.455
Platelets (10 <sup>3</sup> /mm <sup>3</sup> )	246.9 ± 85.1	218.8 ± 65.2	253.9 ± 88.8	0.273
Urea (mg/dL)	25.66 ± 8.52	24.86 ± 6.68	26.1 ± 9.0	0.582
Creatinine (mg/dL)	0.75 ± 0.24	0.65 ± 0.23	0.78 ± 0.24	<b>0.030*</b>
Glucose (mg/dL)	109 ± 51.9	111 ± 58.1	108 ± 50.3	0.826
AST (U/L)	26.7 ± 8.9	27.1 ± 11.2	26.6 ± 8.6	0.885
ALT (U/L)	27.5 ± 16.1	31.5 ± 19.1	26.7 ± 15.5	0.420
Albumin (g/dL)	4.0 ± 1.0	4.03 ± 0.8	3.9 ± 0.8	0.362
Proteinuria (mg/g-Cr)	99.8 (65 - 151)	88.2 (65 - 122)	103 (65 - 155)	0.513
Albuminuria (mg/g-Cr)	7.1 (3.9 - 15)	6.3 (3.8 - 14.4)	6.8 (3.9 - 17.1)	0.579
ICAM-1 (ng/mL)	611.29 ± 471.26	602 ± 446	623 ± 485	0.871
VCAM-1 (ng/mL)	1,708.81 ± 765.87	1.101 ± 659	1.826 ± 730	<b>&lt;0.001</b>

AST = aspartate transaminase; ALT = alanine transaminase; data expressed as median, an interquartile amplitude between parenthesis for nonparametric data, and as average ± standard deviation for data with normal distribution according to Kolmogorov-Smirnov; \*groups were compared using the Student t test for normal data and the Mann-Whitney test for non-normal data.

and was higher in the multibacillary patients (Table 2). Regarding the endothelial biomarkers, only VCAM-1 was elevated in the multibacillary group (Figure 1).



**Figure 1** - Level of VCAM-1 and ICAM-1 in leprosy patients accordingly with the clinical form used to choose the specific treatment. \*p<0,05 between groups in VCAM-1 levels.

#### Association between clinical aspects of leprosy with VCAM-1 and albuminuria

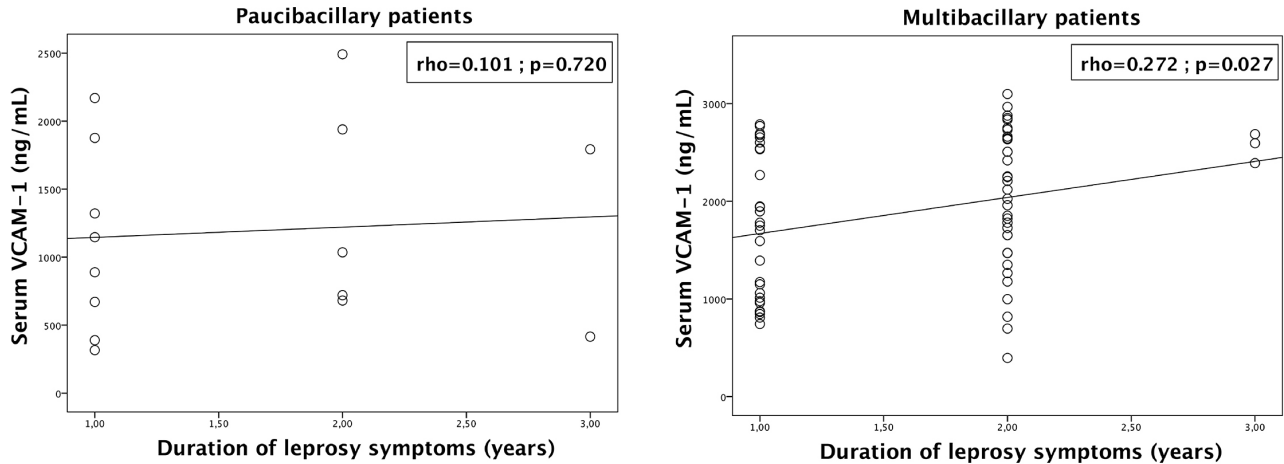
In the correlation analysis using VCAM-1 and ICAM-1, only VCAM-1 presented significant correlations. A modest correlation was observed between VCAM-1 and the bacteriological index in the skin smears ( $\rho = 0.372$ ,  $p < 0.01$ ) and the number of skin lesions ( $\rho = 0.468$ ,  $p < 0.001$ ). In addition, VCAM-1 had a weak association

with the duration of disease symptoms in leprosy patients ( $\rho = 0.234$ ,  $p = 0.04$ ). Importantly, when the patients were stratified according to the operational classification, in the PB group, no significant correlation between VCAM-1 and duration of disease symptoms was observed ( $\rho = 0.101$ ,  $p = 0.72$ ). On the other hand, in the MB group, there was a significant correlation among them ( $\rho = 0.272$ ,  $p = 0.03$ ) (Figure 2).

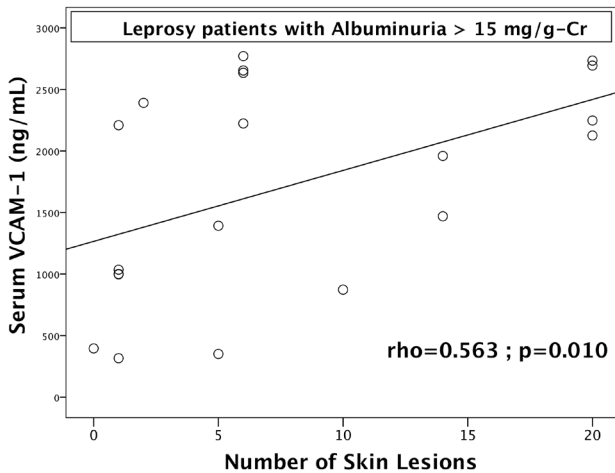
When the correlation between VCAM-1 and albuminuria was evaluated in the whole group as well as in the multibacillary patients, no significant correlations were found. However, due to the large amount of tests showing low albuminuria, we decided to evaluate a new group with samples presenting higher albuminuria levels, according to the third quartile ( $> 15$  mg/g-Cr). In this group VCAM-1 presented a significant correlation with the albuminuria levels ( $\rho = 0.341$ ,  $p < 0.05$ ). Furthermore, an improvement in the correlation between VCAM-1 and the number of skin lesions ( $\rho = 0.563$ ,  $p = 0.010$ ) was also observed (Figure 3).

#### DISCUSSION

The main goal of the present study was to investigate the levels of endothelial biomarkers and their association with albuminuria in multibacillary leprosy. Leprosy can affect renal function through different mechanisms, mostly immunological, favoring the development of diverse clinical complications<sup>2</sup>. Recently, biomarkers associated with endothelial activation, such as VCAM-1, ICAM-1 and syndecan-1 have been associated with renal diseases in multiples clinical contexts such as other infectious diseases



**Figure 2** - Scatter plot showing the Spearman correlation between systemic VCAM-1 and duration of leprosy symptoms in paucibacillary and multibacillary patients.



**Figure 3** - Scatter plot showing the Spearman correlation between systemic VCAM-1 and number of skin lesions in leprosy group that presented albuminuria > 15mg/g-Creatinine.

like leptospirosis and AIDS<sup>13-16</sup>. In the present study, for the first time, the endothelial biomarker VCAM-1 was associated with clinical aspects of leprosy, and also with albuminuria levels, a renal parameter that often increase in multibacillary leprosy patients<sup>17</sup>.

The endothelial activation in leprosy has not totally been elucidated. The expression of VCAM-1 in the endothelial cells occurs in a constitutive manner, but may be changed by inflammatory stimuli according to different cytokines profiles<sup>18</sup>. The surrounding products of the activated endothelium-like VCAM-1 may be detectable earlier, becoming a clinically useful parameter, acting as a predictive biomarker in systemic infectious diseases. The present study shows that levels of VCAM-1 were increased in multibacillary leprosy patients (Virchowians or with the “dimorphic” form) than in the paucibacillary group.

According to our literature review, this is the first study that evaluates systemic levels of VCAM-1 and correlate

them with clinical parameters of leprosy. A recent study showed the expression of endothelial biomarkers in skin lesions through the histochemical analysis of biopsies<sup>18</sup>. In that study, augmented levels of ICAM-1 and more importantly of VCAM-1 were significantly present in patients with the tuberculoid or the paucibacillary form, which, in part, contradicting our findings. In the tuberculoid form, the patients present an effective immune response, with the augmentation of endothelial adhesion cells that optimize the arrival of leukocytes to the infection site<sup>19</sup>. However, despite the VCAM-1 role in the surface of active endothelial cells, it is not surprising that the soluble forms of VCAM-1 can be released from endothelial cells into the bloodstream<sup>20</sup>. Hence, we suggest that, considering that multibacillary patients present a higher dissemination of the infection and more inflammatory stimuli throughout the whole body<sup>21</sup>, the endothelial activation may be augmented and, consequently, more VCAM-1 molecules will be potentially released. In fact, in the present study, VCAM-1 levels in leprosy patients showed a significant correlation with the number of skin lesions, with the bacteriological index in skin smears and with the duration of the disease. All these parameters are associated with the widespread inflammatory activation<sup>2</sup>.

Moreover, in the present study, leprosy patients with albuminuria > 15 mg/g-Cr (mostly multibacillary) had increased VCAM-1 levels correlated with both, albuminuria levels and number of skin lesions. Endothelial dysfunctions have been associated with renal dysfunction parameters in infectious diseases and with renal diseases in other clinical contexts<sup>7,8,22</sup>. Patients with leptospirosis have major lesions in the vascular endothelium due to the invasion of *Leptospira* and as a consequence, endothelial biomarkers levels were associated with acute kidney injury (AKI)<sup>14</sup>. In patients with CKD, endothelial dysfunctions were associated



with increased proteinuria and serum creatinine, and decreased glomerular filtration rates<sup>19</sup>. Albuminuria and histological findings in leprosy patients represent a CKD profile<sup>2,3</sup>. In diabetic patients, it has already been shown that systemic VCAM-1 levels were increased in patients with albuminuria > 30 mg/day in comparison with the normal albuminuria level group<sup>23-25</sup>. In a previous study of our team, the albuminuria was associated with increased urinary MCP-1 levels, a biomarker involved in inflammation of the renal tissue and glomerular dysfunction<sup>25</sup>, and also with increased bacillary charges<sup>3</sup>. Hence, we suggest a potential use of VCAM-1 as an early biomarker for leprosy complications, including kidney disfunctions.

The present study has limitations. Some patients had diabetes, which may have contributed to changes in endothelial biomarkers in the leprosy patients. However, the frequencies of diabetes were similar among PB and MB groups, without statistical significance. In addition, the present results with a higher baseline level of VCAM-1 does not necessarily mean 'activation' from a baseline state, because another biomarkers of activation would be needed to support this hypothesis. In addition, the correlation of albuminuria with higher VCAM-1 levels does not prove a cause-effect relationship regarding nephropathies. Thus, further prospective studies are necessary to establish the risk for the onset of kidney diseases and their outcomes.

## CONCLUSION

In conclusion, leprosy patients with multibacillary forms had higher systemic VCAM-1 levels which were associated with poorer clinical aspects of leprosy and with increased albuminuria, an important marker of kidney diseases progression. Further prospective studies are necessary to establish a cause-effect relationship and evaluate the predictive role of these biomarkers. Moreover, VCAM-1 and albuminuria screening may provide a preventive tool of kidney diseases in leprosy patients, aiming to improve the clinical care.

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## AUTHORS' CONTRIBUTIONS

The following authors contributed to samples processing, laboratory analysis, ELISA experiments and support for

creating the: LDT, GFB, TPR and IEPA. MAAP provided support for the patients' care and collection of clinical data of hospitalized patients. GCM performed the statistical analysis, tables and figures, created the aims, the research proposal and wrote the manuscript that was revised and altered by GBSJ, EDFD and AMCM.

## CONFLICT OF INTERESTS

All authors declare no conflict of interests.

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