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Short Communication

Markers of Vascular Damage with Potential to Predict Amputation in Patients with Critical Limb Ischemia

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Abstract

Critical limb ischemia (CLI) is the advanced presentation of peripheral arterial disease (PAD), characterized by persistent pain at rest and/or necrosis for more than two weeks. Endothelial dysfunction (ED) is considered a constant process during vascular damage. Aim: To explore the predictive ability of some markers of vascular damage in relation of lower limb amputation. Methods: We assessed the predictive ability of some biochemical (MDA, malondialdehyde and VEGF–A, Vascular Endothelial Growth Factor-alpha) determined from the femoral arterial circulation) and functional (FMD, flow mediated dilation) markers in relation with lower limb amputation during a follow-up of 30-days post-angioplasty in a population with CLI. Results: Significantly abnormal FMD pre-angioplasty values were associated with 67% of cases submitted to major amputation during the follow up period (p=0.03). MDA and VEGF-A reflected changes related with the endovascular procedure.

ABBREVIATIONS

PAD: Peripheral Artery Disease; ED: Endothelial Dysfunction; FMD: Flow-Mediated Dilation; VEGF-A: Vascular Endothelial Growth Factor - A; MDA: Malondialdehyde

INTRODUCTION

Peripheral Artery Disease (PAD) is a progressive disease that occurs as a result of plaque formation in the arterial vascular system, which progressively decreases the blood flow to the limbs, such as the arms, legs as well as the vital organs [1,2]. It is a frequent worldwide disease. In Mexico, the prevalence of PAD is approximately 10% and mainly affects older male adults [3].

Critical Limb Ischemia (CLI) is the advanced presentation of PAD, characterized by persistent pain at rest and/or necrosis for longer periods than two weeks. This condition is more common in a population with a systolic pressure lower than 50mmHg measured at ankle level, or the presence of ischemic ulcers; and it is frequently associated with loss of a limb and/or death [4,5]. Some common findings in these patients are the presence of Endothelial Dysfunction (ED), considered one the first manifestations of vascular damage [6], which represents the loss of the endothelial ability to maintain an adaptive physiological response in the vascular system according to the peripheral tissue´s blood demand. Likewise, early atherosclerotic changes can be observed in individuals with vascular risk factors such as systemic arterial hypertension, diabetes mellitusor dyslipidemia [7,8]. Therefore, biomarkers of vascular damage may be related with a decrease of lower limb blood flow or with amputation risk in patients with CLI. Lower limb angioplasty represents a minimally invasive endovascular procedure that improves arterial blood flow and thus the quality of life. However, the prognosis of vascular complications, such as the progression of the disease, restenosis or the requirement of further major lower limb amputation, is variable.

Currently, there is no trust worthy biomarkers to predict major vascular complications not even in patients with CLI. Likewise, biomarkers related to vascular damage, such as malondialdehyde (MDA) and VEGF-A, have been insufficiently explored in patients with PAD and lower limb amputation. Therefore, this study was aimed to explore the predictive ability of biomarkers of vascular damage in relation to lower limb amputation within the first 30 days of post-angioplasty in population with CLI.

MATERIALS AND METHODS

Design and type of study

Observational, quasi-experimental, prospective design with correlational analysis.

Study population

Twenty patients with CLI, candidates for lower limbs vascular angioplasty, with different degrees of affected areas and ED. These patients were consecutively recruited from the Angiology and Vascular Surgery Department, Centro Médico Nacional "20 de Noviembre", ISSSTE, from May 2016 to Jan 2017. The protocol was approved by the local Committee for Research, Biosafety and Ethics; and all patients signed the informed consent.

Sampling

Eight milliliters of blood were obtained from the femoral arterial blood circulation, at the atherosclerotic lesion site, during the angioplasty procedure. In order to evaluate immediate effects of the angioplasty, pre-angioplasty blood sample (4ml), and subsequent sample (4ml) of blood obtained 30 minutes after the angioplasty, were collected and analyzed.

Endothelial dysfunction

FMD was measured at the brachial artery above the antecubital fossa, according to the recommendations of the International Brachial Artery Reactivity Task Force, with a Hitachi ultrasound equipment, Aloka Prosound Alfa-7 (2009) and a vascular linear transducer of 5-10 Mhz.

ELISA assay

Plasma MDA concentration was determined by ELISA kits, (MIT KIT, CEA597Ge Cloud-Clone Corp.) as well as VEGF–A (Human VEGF-A Platinum ELISA BMS277 / 2 / BMS277 / 2TEN, affymetrixe Bioscience). The procedure was performed following the supplier's recommendations.

Follow up

Patients were followed up for 30 days after performing lower limb angioplasty. Follow up was carried out by telephone calls twice a week and appointments for medical consultation every 15 days. We registered the number and percentage of patients who required major lower limb amputation at level where blood flow is still preserved and good functional prognosis is expected".

Table 1: Study Population (n = 20).

Clinical-Demographic Characteristics	
Age (years old)	68 (64.7 - 76.5)
Male sex	10 (50)
Smoking	10 (50)
Comorbidities	
Diabetes Mellitus	20 (100)
Systemic Arterial Hypertension	14 (70)
Ischemic Heart Disease	4 (20)
Dyslipidemia	11 (55)
Cerebral vascular disease	2 (10)
Chronic Kidney Disease	3 (15)
Clinical Laboratory	
Fasting glycemia (mg/dL)	180.5 (144.5-216.5)
HbA1C (%)	8.3(7.8-9.1)
Creatinine (mg/dL)	0.98(0.63-1.55)
BUN (mg/dL)	19.8(14.2-37.3)
Triglycerides (mg/dL)	175.0(125.0-213.7)
Total cholesterol (mg/dL)	120.0(102.0-130.0)
Results were expressed as median (p25-p75) or n (%).	

Table 2: Evaluation of Vascular Damage (n = 20).

Clinical Indicators of Vascular Damage	
FMD (%)	-7.8 (-9.3 a 7.1)
Index ankle-arm	
Right	0.5 (0.40 – 0.73)
Left	0.6 (0.56 – 0.70)
Rutherford Classification	
I to III	0 (0)
IV	2 (10)
V	2 (10)
VI	16 (80)
History of Revascularization	7 (35)
Biomarkers related to Vascular Damage	
MDA (nM/L) [baseline]	0.86 (0.83 – 0.87)
MDA (nM/L) [30 min post-angioplasty]	0.93 (0.88 – 0.97)
VEGF-A (pg/mL)[baseline]	110.0 (106.5 – 116.6)
VEGF-A (pg/mL) [30 min post-angioplasty]	140.0 (124.2 – 148.7)
Results were expressed as median (p25-p75) or n (%). Abbreviations: FMD, Flow Mediated Dilation; VEGF-A, Vascular Endothelial Growth Factor - A.	

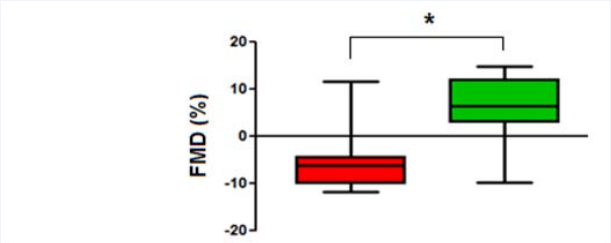
RESULTS AND DISCUSSION

Clinical-demographic characteristics of our study population are shown in Table 1. It was constituted of twenty patients, 10 (50%) male, mean age 68 years old, who met the selection criteria. Our population shared similarities with previous studies, in terms of high prevalence of diabetes mellitus, hypertension and dyslipidemia, as the main entities associated with vascular damage [8].

The degree of vascular affection and values of markers (MDA and VEGF-A) are shown in Table 2. Baseline MDA and VEGF-A showed a trend to increase after angioplasty, suggesting that these markers are accumulated within the atheroma plaque, and they are released to systemic circulation after the angioplasty procedure.

Elevation of VEGF-A was similar to other reports and might be interpreted as an angiogenic response [9-11]. Interestingly, the relative quantity (evaluated as the percentage of change) of VEGF-A released post-angioplasty was lower in the group that underwent further major limb amputation, suggesting its role as predictive marker joined to FMD.

Consistently, VEGFα response was lower in amputated patients, suggesting that an impaired response of angiogenic factors may influence amputation. Likewise, we can consider that MDA elevation after 30 minutes post-angioplasty was not harmful enough to determine "which patient is to be amputated or not" Figure 1.



Group	One	Two
Limb Status	Amputated	Not Amputated
MDA (% change)	6.2 (2.7 - 13.4)	8.3 (5.9 - 13.0)
VEGF-A (% change)	17.9 (15.7 - 22.0)*	35.9 (33.3 - 38.9)

Figure 1 Pre-angioplasty FMD and post-angioplasty biomarkers modification. The graphic shows the distribution of pre-angioplasty FMD values according to further requirement of lower limb amputation during 30-days post-angioplasty follow-up. The percentage of change of biomarkers, expressed as median (p25-p75), is also shown in the table below the graphic. Abbreviations: FMD, Flow Mediated Dilation; MDA, Malondialdehyde; VEGF-A, Vascular Endothelial Growth Factor - A. (*) T-test, $p < 0.05$.

On the other hand, pre-angioplasty FMD values were within a range considered as severe PAD. To assess potential clinical predictive impact, an estimated pre-angioplasty FMD cut off (-7.8%) was used to divide the population in 2 groups: Group 1 (FMD ranging from -7.8 to -11.3%) and Group 2 (FMD from -5.4 to 16.2%). Group 1 showed a higher incidence of major amputation after 30-days follow up compared to Group 2 (n=67% Vs 14%, Fisher exacts test=0.03). Figure 1 **s**hows significant differences in brachial reactivity (FMD) according to lower limb amputation status. Our results indicate that FMD was more predictive of a beneficial vascular outcome, according to its association with major lower limb amputation. This would support the standard use of FMD in the preoperative evaluation of patients with CLI submitted to endovascular procedures.

Some limitations of the present study should be considered: 1) We do not know whether baseline pre-angioplasty levels of MDA and VEGF may be considered normal, since we did not compare with concentrations in healthy subjects; 2) Serial consecutive determination of biomarkers after angioplasty procedure would have shown a more reliable behavior of the study biomarkers than only one measure; and 3) Our results should be replicated by other studies with a larger number of patients, and additional biomarkers of endothelial damage and vascular remodeling. This would enrich vascular research field by characterizing the predicting ability of vascular biomarkers, because currently there is scarce number of studies exploring whether vascular biomarkers may predict major lower limb amputation.

CONCLUSION

Significantly abnormal pre-angioplasty FMD value was associated with 67% of major amputation; while markers like VEGF−A and MDA increase with endovascular procedure, probably as a reflect of endothelial repair and damage.

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