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SCIENCE DIRECT

Microvascular Research 70 (2005) 84 – 89

Microvascular Research

www.elsevier.com/locate/ymvre

Cold stimulus-fingertip lacticemy: Standardization of the test in normal volunteers and diagnostic application for systemic sclerosis

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Received 21 January 2005
Available online 31 May 2005

Abstract

Objectives: To standardize the cold stimulus-fingertip lacticemy test (CS-FTL) in normal individuals and to establish reference levels for discrimination of normal individuals and patients with systemic sclerosis (SSc).
Methods: FTL was determined before (pre-CS) and 3, 8, and 13 min after cold stimulus (post-CS) in 94 normal controls according to gender and age. Diagnostic performance of ΔCS-FTL (percentage difference between post- and pre-CS-FTL) was tested comparing 25 SSc patients and the 94 normal individuals by ROC curve analysis.
Results: Successive FTL determinations in the same fingertip yielded consistent results and the whole CS-FTL test proved to be reproducible. Pre-CS-FTL in SSc patients was higher than in normal controls ($P < 0.001$). FTL decreased after cold stimulus (negative ΔCS-FTL) in normal controls while SSc patients presented positive ΔCS-FTL. Gender did not influence pre-CS-FTL and post-CS-FTL in normal controls. The decrease in FTL after cold stimulus was more prominent for normal individuals between 25 and 34 years old in comparison to other age subgroups, with statistical significance for females at 3 min post-CS ($P < 0.05$). ROC curve analysis showed better diagnostic performance with post-CS-FTL at 8 and 13 min.
Conclusion: CS-FTL test provides a reproducible quantitative biochemical parameter that reflects fingertip microcirculation status and was able to discriminate patients with SSc and normal controls, with optimal performance with post-CS-FTL at 8 to 13 min.

Keywords: Raynaud's disease; Systemic sclerosis; Lacticemy; Microcirculation

Introduction

Raynaud's phenomenon (RP) is a relatively common clinical event characterized by paroxysmal ischemic episodes mainly in fingers and toes involving the digital arteries, precapillary arterioles, and cutaneous arteriovenous shunts (Wigley, 2002). Classical RP is usually triggered by cold exposure or emotional stress and has three successive stages, represented by pallor, cyanosis, and hyperemia. Alternatively, patients may present only intermittent pallor and/or cyanosis. The frequency of RP in the general population is estimated as 3 to 17% (Maricq et al., 1993; Weinrich et al., 1991). Pure RP with no known associated systemic or local disease is designated as primary RP. Conversely, the existence of an associated systemic or local disease defines secondary RP. It is prevalent in autoimmune rheumatic diseases, especially in systemic sclerosis (SSc) and related overlap syndromes in which it is present in over 90% of the patients (Maricq et al., 1990).

In order to quantify microcirculatory disturbances and to improve the understanding of the rheological, biophysical, and morphological aspects of Raynaud's phenomenon (RP) plethysmography, digital thermography, finger systolic blood pressure, laser Doppler fluxometry, and nailfold capillaroscopy have been applied to study these patients (Andrade et al., 1990; Baxter et al., 1990; Carter et al., 1988; Cleophas et al., 1982; Liedl et al., 1996; Maricq and LeRoy, 1973; Wouda, 1977). In order to increase sensitivity,

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0026-2862/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.mvr.2005.04.004

provocative tests (e.g., immersion of the patient's hand in ice or cold water) are recommended. None of the above-mentioned methods evaluate the biochemical consequences of RP-induced ischemia.

Lactate analysis is a common procedure in exercise physiology laboratories to measure endurance performance (Dassonville et al., 1998; Foxdal et al., 1990). For this purpose, handy disposable test strip lactate meters have been developed to measure with high accuracy the lactate concentration in small blood samples (Fell et al., 1998). The determination of lactic acid concentration in blood obtained from fingertips [fingertip lacticemy (FTL)] brings accurate information on the degree of local anaerobiosis. The glycolytic pathway is the main energy source of the cell. In conditions of normal oxygen supply, glucose is transformed in pyruvic acid in the cytosol, enters the Krebs cycle and is metabolized to CO_2 + H_2O by oxidative phosphorylation, yielding enough energy to support the synthesis of 36 molecules of ATP per each glucose molecule (Toffaletti, 1991). Tissue hypoperfusion or excessive energy demand, such as in intense muscle exercise, results in oxygen deficit that ensues activation of an alternative biochemical pathway characterized by conversion of pyruvic acid into lactic acid, leading to increase in blood lactic acid concentration (Gollnick et al., 1986; Gutierrez and Wulf, 1996; Karlsson, 1986).

We have previously adapted the FTL approach largely used in sports medicine to study the ischemic stage of RP by measuring FTL before and after cold stimulus (CS-FTL) and tested this method in a small group of normal individuals and in patients with SSc and primary RP (Pucinelli et al., 2002). In brief, a drop of fingertip blood for lactic acid determination was obtained in resting conditions and 10 min after a cold stimulus. SSc patients presented basal FTL higher than normal individuals. Moreover, FTL decreased or remained unchanged after cold stimulus in controls and increased in most SSc patients. Our preliminary data suggested that the CS-FTL test might be useful for pathophysiologic investigation as well as for diagnosis and clinical staging of RP.

In our pioneer study, we arbitrarily chose the interval of 10 min for FTL sampling after cold stimulus and studied a limited number of normal individuals. The kinetics of FTL following a cold stimulus has not been determined in the normal individuals. Therefore, a formal standardization of this phenomenon is necessary. The present study aimed at standardizing and optimizing the CS-FTL test by evaluating its performance in three different time points after the cold stimulus regarding: (1) elucidation of CS-FTL behavior in normal individuals according to gender and age; and (2) comparing the ability of CS-FTL in discriminating normal individuals and SSc patients at these three different time points. Additionally, we tested the intra-individual variability of the CS-FTL test and the intrinsic variability of the FTL measurement.

Materials and methods

The study involved 94 healthy normal adults and 25 patients with SSc meeting the American College of Rheumatology criteria (Masi et al., 1980). Normal controls comprised students, nurses, and physicians at UNIFESP Medical School Hospital. All SSc patients had a positive history of RP and the characteristic microangiopathic SD pattern at nailfold capillaroscopy (Maricq and LeRoy, 1973). Patients were consecutively selected from the Scleroderma Spectrum Outpatient Clinic at UNIFESP Medical School Hospital. Exclusion criteria were: (1) existence of active fingertip ulceration; (2) smoking; (3) age under 18 years old; (4) occupational exposure to cold environment and vibratory agents; (5) existence of systemic arterial hypertension, diabetes mellitus, heart failure, renal insufficiency, severe respiratory insufficiency, carpal tunnel syndrome, and clinical evidence of proximal arterial disease; and (6) prior digital or proximal sympathectomy. Patients that stopped smoking within the last 6 months were also excluded. Presence of severe skin involvement and history of previous digital ulcers that were well healed were not exclusion criteria in the study. All individuals filled out an informed consent form approved by the UNIFESP Ethics Committee and underwent a thorough rheumatologic examination. Those receiving oral vasodilators had the drug discontinued for 3 days in advance to the procedure. No patients were in use of prozac, angiotensin converting enzyme inhibitors, prostanoids, bosentan, or phosphodiesterase inhibitors.

The CS-FTL test consisted of four phases. In phase I (stabilization), patients rested in sitting position for 30 min at 24 ± 1°C, making sure hands and fingers were warm. Next, phase II, a brisk puncture was performed at the volar surface of the left fourth fingertip with an automatic device (Softclix, Boehringer-Mannheim, Germany) and the first blood drop obtained without milking was adsorbed onto a lactate strip (Boehringer-Mannheim, Germany). The strip was processed immediately in a portable spectrophotometer (Accusport, Boehringer-Mannheim, Germany). In the third phase (cold stimulus—CS), patients submerged both hands in water at 10°C for 1 min (Unitemp 116, Fanem, Brazil). Phase IV comprised additional FTL determinations at 3, 8, and 13 min after cold stimulus. All punctures were performed at distinct sites in the same fingertip.

The difference between post- and pre-CS-FTL (designated ΔCS-FTL) was expressed as the percent variation relative to pre-CS-FTL and was defined by the formula below.

$$\Delta\mathrm{CS-FTL} = \frac{\text{post-CS-FTL} - \text{pre-CS-FTL}}{\text{pre-CS-FTL}} \times 100$$

Therefore, ΔCS-FTL3, ΔCS-FTL8, and ΔCS-FTL13 corresponded to the variation of FTL at 3, 8, and 13 min, respectively, in relation to pre-CS-FTL.