

Serum oxidizability potential of ischemic heart disease patients predicts exercise test results and disease severity.

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Citation

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Abstract

Background: Little is known about the relationship between exercise test indices and serum oxidizability potential in chronic ischemic heart disease (IHD) patients.

Aim: to find out whether serum oxidizability potential correlates with exercise test parameters and predicts its result in chronic IHD patients.

Methods: 54 chronic IHD patients and 11 normal controls underwent a symptom limited exercise test (EXT) upon initiation of a cardiac rehabilitation program; prior to the test a thermo-chemiluminiscence (TCL) assay of serum oxidizability potential was performed. This assay is based on heat-induced oxidation of the serum which leads to the formation electronically excited species in the form of unstable carbonyls, which further decompose into stable carbonyls and light energy (low chemiluminiscence). Measured photons emission is represented by a kinetic curve which is described by its amplitude and slope (= ratio). Correlations of TCL ratio with exercise duration (ED), metabolic equivalents (METS), maximal heart rate (mHR), maximal systolic BP (mSBP), >1mm S-T depression, Diabetes, Hypertension, Smoking, LV ejection fraction (LVEF) > or < 40%, previous myocardial infarction, and aorto-coronary by pass surgery were assessed and compared to the TCL ratio of 11 normal controls.

Results:

High TCL ratio (%) correlated well with METS, $r = 0.84$, with mHR, $r = 0.79$, and with exercise induced S-T segment shift, $r = 0.87$, $p < .05$. A lower serum oxidizability potential expressed as a low TCL ratio, thus, a previous high oxidative stress, was found in IHD patients compared to normal controls, and in patients with low LVEF% in particular. TCL ratio (%) in IHD patients was 193 ± 21 compared to 215 ± 13 in controls, $p < .05$, in patients with LVEF < 40% = 188 ± 14.7 vs 200 ± 11.9 in patients with LVEF > 40%, $p < .01$.

A trend for lower TCL ratio (%) was found in diabetic, hypertensive, and post- CABG patients. A paradoxically low TCL ratio (low oxidizability potential) was observed in patients without S-T depression compared to patients with S-T depression (189 ± 22 vs 201 ± 15 , $p = ns$) which is due to the fact these patients had a much lower LVEF% as well as a lower exercise capacity.

Conclusion: Serum oxidizability potential is associated with exercise test parameters, results, and IHD severity. TCL ratio is an "easy to measure marker" that might be incorporated into risk assessment and prediction in chronic ischemic heart disease patients.

INTRODUCTION

Oxidative stress reflects a condition in which the balance between reactive oxygen species production and the subsequent response of the antioxidant defense system is lost becoming skewed in favor of free radical expression [1,2,3]. Although a multitude of free radicals exist (hydrogen atoms, transition metal ions, carbon centered radicals, sulfur centered radicals etc.), those derived from oxygen are referred to as reactive oxygen species (ROS). Reactive oxygen species are highly reactive very unstable molecules

which tend to initiate chain reactions that result in irreversible chemical changes in lipids or proteins. These potentially deleterious reactions can result in profound cellular dysfunction and even cytotoxicity (4).

Recent data imply that measurement of oxidizability is a key to kinetic evaluation of oxidative processes of LDL, blood serum and other body fluids, and can be used for monitoring the oxidative stress in different diseases and antioxidant drug therapy (5,6). The oxidizability of a biological sample is a measure of its susceptibility to oxidation. The

Thermochemiluminescence (TCL) assay which was used in our study is one of accepted, validated and reproducible methods (7,8,9) for measurement of oxidative stress and serum oxidizability potential. This assay is based on the heat-induced oxidation of a sample which leads to the formation of electronically excited species, triplet excited carbonyls in particular, and to the formation of light energy, low-level chemiluminescence.

Growing evidence indicates that chronic and acute overproduction of reactive oxygen species (ROS) under pathophysiologic conditions is integral in the development of cardiovascular diseases (CVD). ROS mediate various signaling pathways that underlie vascular inflammation in atherogenesis: from the initiation of fatty streak development through lesion progress to ultimate plaque rupture. Oxidative stress is the unifying mechanism for many CVD risk factors, which additionally supports its central role in CVD (10).

Evidence for increased oxidative stress was found in plasma of patients with ischemic and nonischemic dilated cardiomyopathy and correlates directly with the severity and chronicity of symptoms, and inversely with left ventricular ejection fraction (LVEF) (11, 12).

Free radical injury has as well been implicated in the pathogenesis, evolution and progression of heart failure (9,13-15). Furthermore, with the evolution of heart failure there is a progressive increase in free radical injury and encroachment on antioxidant reserves with an impact on prognosis.

Single bouts of aerobic and anaerobic exercise can induce an acute state of oxidative stress. This is indicated by an increased presence of oxidized molecules in a variety of tissues. Exercise mode, intensity, and duration, as well as the subject population tested, all can impact the extent of oxidation (16-22). Exercise induced oxidative stress has been investigated in during and after exercise testing in chronic heart disease and chronic heart failure patients (13,23,24). Most of studies have shown increased oxidative stress pre and post exercise, however no study assessed the relationship between pre-exercise test oxidizability potential, exercise test parameters and results.

To the best of our knowledge no study has yet addressed the question whether the pre exercise oxidative status may have an impact on exercise test results.

METHODS

54 chronic ischemic heart disease (IHD) patients, 13 females and 41 males, age – 63 years \pm 5 s.d. and 11 normal age matched controls were included. In the ischemic heart disease group patients. 47 patients (87%) had a previous myocardial infarction, 19 (35.2%) had an aorto coronary bypass surgery (CABG), and 35 patients (64.8%) had a previous percutaneous intervention (PCI). 15 patients (27.7%) had diabetes mellitus (DM), 31 (57.4%) had hypertension, and 34 (62.9%) had dyslipidemia. 39 patients (72.2%) were in New York Heart Association (NYHA) class I-II, and 15 (27.8%) in class III; patients in NYHA class were not included. 28 (51.8%) had left ventricular ejection fraction (EF) < 40%, and 26 (48.2%) had an EF \geq 40%, including 4 patients who had normal EF (\geq 55%).

Patients with an acute or recent febrile disease, significant liver dysfunction, or renal failure (serum creatinine \geq 2.0 mg/dl) were excluded from the study. Subjects using regularly anti-oxidant supplements (vitamins A, C, E, or Co-Enzyme Q-10) or drugs with presumed anti oxidant properties (statins, carvedilol) were required to stop these medications 7 days prior to the test.

All subjects underwent a symptom limited exercise test (EXT) upon initiation of a cardiac rehabilitation program; prior to the EXT a 2 cc venous blood sample was drawn for TCL assay.

Photons emission during heating was measured by TCL Analyzer (manufactured by Lumitest Ltd., Caesarea, Israel) using a photomultiplier model R265P (Hamamatsu Photonics Co. Ltd. Ichino-cho, Higashi-ku, Hamamatsu City, Japan) with a spectral response range of 280-650 nm. The computer program of the device has two main functions: a) analysis of the sample preparation (0.05 ml of serum required for the test), b) Data processing, display and storage. The examined serum was distributed over the surface of aluminum tray (a kind of miniature Petri dish) inside the sample preparation block and then was vacuum-dried. Then the dish was mounted on a constant heater with heating temperature $80 \pm 0.5^\circ$ in the analysis block and the photons emission was measured each second for 300 sec. The obtained TCL curve was described mathematically as the amplitude of the kinetic curve of the photons emission and slope of the curve. The obtained curve is described mathematically as the amplitude of the kinetic curve and its slope (= ratio) which reflects the heat-induced susceptibility to oxidative modification of the tested sample, a ratio which

reflects residual oxidative capacity due to prior in vivo molecular oxidation (i.e. lower curve slope, lower oxidative potential, indicating higher oxidative activity before test).

Data are presented as means \pm s.d.; Student's t-test was used due to the normal distribution of laboratory results, and correlations were determined by Pearson's correlation coefficient computation. Regression analysis was performed in order to find out the independent variable with the most evident impact on TCL ratio being the dependent variable.

RESULTS

A lower serum oxidizability potential expressed as a low TCL ratio, thus, a previous high oxidative stress, was found in patients with IHD compared to normal controls, and in patients with low LVEF% in particular (examples in table 1, figures 1 & 2).

The TCL ratio (%) in IHD patients was 193 ± 21 compared to 215 ± 13 in the control group, $p < 0.05$, and in patients with low LVEF ($< 40\%$) = 188 ± 14.7 compared to 200 ± 11.9 in patients with a better LVEF ($> 40\%$), $p < 0.01$. The TCL ratio correlated well with exercise tolerance expressed in metabolic equivalents (METs) as well with exercise duration, $r = 0.89$ and 0.91 , $p < 0.01$ respectively. Similarly, TCL ratio correlated with exercise maximal heart (maxHR), $r = 0.79$ and with exercise induced ≥ 1 mm ST segment shift, $r = 0.77$.

Figure 1

Fig. 1 TCL ratios (and trendlines) of a normal subject (TCL 1) and a patient suffering from congestive heart Failure (TCL 2).

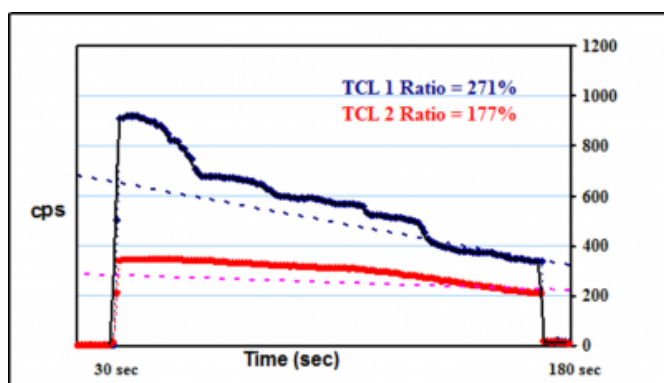
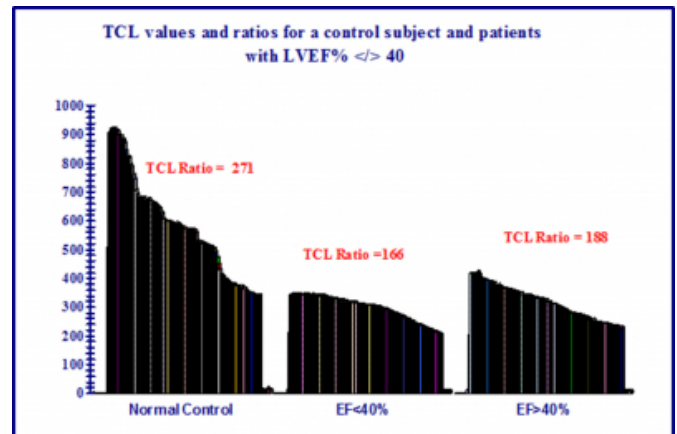


Figure 2

Fig. 2 TCL ratio of a normal control, a patient with EF 40%



A trend for lower TCL ratio (%) was found in diabetic, hypertensive, and post-CABG patients (194 ± 13 , 195 ± 17 , and 197 ± 13 , respectively, $p = ns$).

A paradoxically low TCL ratio (low oxidizability potential) was observed in patients without S-T depression compared to patients with S-T depression (189 ± 22 vs 201 ± 15 , $p = ns$) which is due to the fact these patients had a much lower LVEF% as well as a lower exercise capacity.

Regression analysis has shown that left ventricular EF% being the independent variable was the best predictor for the TCL ratio being the dependent variable, $R^2 = 0.87208$, $R = 0.9338$, St Err = 17.76, Adj F = 0.87118 (No. of observations = 144), $p < 0.001$

DISCUSSION

Oxidative stress, which may result in oxidative tissue damage, occurs when there is an imbalance between ROS production and antioxidant defenses, such that either ROS production is increased and/or defense mechanisms are impaired. The net result may be assessed by the oxidizability potential, the method used in our study.

It is well known that atherosclerosis and ischemic heart disease are associated with increased lipid peroxidation, and exaggerated free radical production is often observed in patients with congestive heart failure (CHF). Increased ROS production has been shown to impair endothelium-dependent vasorelaxation, to cause myocyte apoptosis, to increase monocyte adhesion, inflammatory gene expression, thus, contributing to myocardial and skeletal muscle contractile dysfunction and deterioration in CHF patients (13).

In most studies involving chronic IHD and CHF patients, a

significant increase in exercise-induced plasma oxidative stress was found. Exercise mode, intensity, and duration, as well as the subject population tested, all can certainly impact the extent of oxidation (16-21). However, these studies disagree when pre exercise oxidative status is examined, Sayar in his study (13), did not find a significant difference in resting plasma oxidative stress in CHF patients as compared with controls, the reason for these unexpected findings is probably because their control group did not contain healthy controls but, in fact, patients with many cardiovascular risk factors, therefore, they suggested that the underlying risk factors are associated with an increase in resting pre-exercise plasma malondialdehyde (MDA) levels. Diaz-Velez et al. (26) have similarly found that the resting plasma oxidative stress in symptomatic CHF patients (LVEF <40%), and asymptomatic patients with LVEF >40% without clinical evidence of CHF but with hypertension, diabetes mellitus or a history of myocardial infarction, had similar findings. On the other hand, in the study of Belch et al. (27), there was a significant negative correlation between left ventricular ejection fraction (EF) and oxidative stress.

Our findings suggest that previous, chronic, recurrent oxidative stress in IHD patients practically reduces, depletes residual oxidative capacity. In other words, residual oxidative capacity is reduced due to prior recurrent in vivo molecular oxidation in chronic IHD patients.

No study so far, has addressed the question whether the pre-exercise oxidative status may have an impact on exercise test results or whether it can contribute to the risk assessment of these patients. The findings of our study show that the sicker the patient is, with lower EF and lower exercise capacity, the lower is his serum oxidizability potential, as reflected by the lower TCL ratio.

Assessment of TCL ratio at resting conditions may predict exercise test results, thus, may support risk assessment in chronic IHD patients.

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