Journal of Trace Elements in Medicine and Biology 51 (2019) 86–90

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

The comparison of zinc, copper and iron levels in serum, aorta and left internal mammarian artery tissues in coronary by-pass graft surgery patients☆

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ARTICLE INFO

Keywords:
Zinc
Copper
Iron
Atherosclerosis
Coronary artery by-pass graft operation
Left internal mammary artery

ABSTRACT

Trace elements are crucial for vital enzymatic reactions in all metabolic processes. Zinc (Zn) acts as a co-factor for many enzymes. Copper (Cu) and iron (Fe) have pro-atherogenic effects resulting in atherosclerosis. Aorta exposing high pulsatile pressure is sensitive for atherosclerosis because of its fast metabolism and poor nutrition by diffusion from vasa vasorum. We aimed to determine the relationship between serum Zn, Cu and Fe levels with aortic and left internal mammary artery (LIMA) tissues in 33 atherosclerotic individuals who inevitably underwent coronary artery by-pass graft (CBAG) surgery that is an end-point treatment for atherosclerosis. Trace elements in serum and tissues were measured using inductively coupled plasma-optical emission spectrophotometer. Pre-operative (Pre-op) serum Fe levels were statistically 46% higher than post-operative (Post-op) values ($p = 0.009$). Aortic Fe level was 49.8% higher than LIMA Fe ($p = 0.0001$). Our study points out the tendency of aortic tissue to atherosclerosis via pro- atherogenic effect of Fe. LIMA, being a potential graft for CBAG, is resistant to atherosclerosis with its intimal specialty of graft patency. In conclusion, serum Zn, Cu and Fe levels in atherosclerotic CBAG patients might be monitored to reveal minor alterations pre-operatively and post-operatively for ameliorating the treatment and life quality.

1. Introduction

Cardiovascular diseases (CVD) are the leading global cause of death, accounting 1 of every 7 deaths in the United States and 45% of all deaths in Europe. The financial cost of CVD in Europe is estimated €210 billion a year, moreover the estimated global cost of CVD would reach $1044 billion by 2030 [1,2]. Turkey had a 6.7% prevalence of CVD in 1990 that gradually increased to 12.7% in recent years. The mortality rates of CVD in Turkey that was 42% in 1990 reached a value of 39.8% by the year 2010 [3,4]. CVD are related with several risk factors including hypertension, diabetes mellitus, dyslipidemia and atherosclerosis [5–8].

Atherosclerosis is a chronic inflammatory process that includes the thickening of arterial intima and proliferation of fibrous tissue [9]. Dyslipidemia is one of the most crucial leading pathologies among the other atherosclerotic risk factors. The increase in low density lipoprotein (LDL) triggers an inflammatory site in vessel wall simplifying cell adhesion and leukocyte infiltration towards intimal space [10]. Monocytes adhere to endothelial surface and differentiate to macrophages in sub-endothelial space promoting atherosclerotic plaque growth. Tissue macrophages have the ability to endocytose modified lipids resulting in foam cell formation [11]. The modification of LDL by intimal vascular cells are accepted to be dependent on low levels of trace elements like copper (Cu) and iron (Fe), whereas zinc (Zn) governs an important role in defense mechanisms [12].

Trace elements that constitute 0.02% of total body weight have vital functions at biological, chemical and molecular levels whether as active centers of enzymes or as being a cofactor for metabolic reactions [13,14]. Zn is an antioxidant essential trace element for physiologic membrane structure and enzymatic reactions. Zn plays a protective role in atherosclerosis via inhibiting the formation of modified LDL, displacing Cu and Fe from sensitive sites located on erythrocyte membranes and LDL, and protecting the cell against oxidative stress [15–17]. Zn deficiency is revealed to be associated with pro-

☆ The study was carried at Trace Element Analysis Laboratory at Biophysics Department of Cerrahpasa Medical Faculty.

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https://doi.org/10.1016/j.jtemb.2018.10.003
Received 15 January 2018; Received in revised form 12 September 2018; Accepted 2 October 2018

atherogenic events and induction of inflammatory markers resulting in CVD [18]. Cu is a transition trace element that is essential for various enzymatic reactions carrying a dual effect of being both pro- and anti-atherogenic property due to its level in serum and tissues. Cu promotes LDL modification and foam cell formation in pro-atherogenic process. Zn antagonizes Cu driven pro-atherogenic effects via balancing the bioavailability and metabolism of Cu [10,17,19,20]. Fe is involved in several metabolic processes, including oxygen transport, DNA synthesis, and electron transport [21]. Besides, Fe induces modification of LDL and initiates the atherosclerotic plaque via pro-inflammatory cytokines [9,22]. Increased Fe level in serum and tissues promote lipid peroxidation in artery wall gradually resulting in atherosclerosis [5–8].

The structure of aorta differentiates due to its proximity to heart and its response to unidirectional flow with high pressure. Aorta is prone to atherosclerosis based on its mechanical and metabolic factors related with the multiple fenestrations among its lumen [23–25]. Left internal mammary artery (LIMA) has been used as an important potential graft since 1970s for coronary artery by-pass graft (CBAG) surgery. LIMA is a branch of subclavian artery located proximally to heart and supplies oxygen and nutrients directly from its lumen differently from other arterial grafts. LIMA has a potent biological function with its production of vasodilatatory and platelet inhibiting factors. The specialty of LIMA having fewer fenestrations, lower tendency of intimal hyperplasia and less permeability at intercellular junctions prevents lipoproteins from entering subendothelial space [26,27].

CBAG surgery is an end-point treatment for atherosclerotic patients, thus the patency of the graft used in CBAG surgery is of great importance [27]. The aim of our study is to analyse the effect of surgical trauma on the serum levels of Zn, Cu and Fe and the resistance of LIMA to atherosclerosis by means of tissue levels of Zn, Cu and Fe in 33 male individuals inevitably underwent CBAG surgery. The patient group was standardized by means of gender and biochemical parameters in order to reveal the absolute effect of Zn, Cu and Fe over serum, aortic tissue and LIMA tissue.

2. Materials and methods

2.1. Patient population

Thirty three male patients attended to the Outpatient Clinic of Department of Cardiovascular Surgery at Kartal Kosuyolu State Hospital in Istanbul were enrolled in the study. The patients were grouped as Pre-op (24 h before the surgery) and Post-op (7 days after the surgery in the clinic) prospectively after written informed consent had been obtained. The study group was operated electively for CBAG surgery due to coronary artery disease. The mean age of the study group was 61.69 ± 5.65 years. Patients with congenital valvular disease (bicuspid, unicuspid aortic valve), inherited connective tissue disease (Marfan, Ehlers Danlos syndrome, Loeys-Dietz syndrome, and so on.), redo cases, dissecting aortic aneurysm, ruptured ascending aorta aneurysms and patients with the diagnosis of malignancy and inflammatory diseases were not included to the study. Five patients were excluded from the study with diagnosis of systemic infection (3), acute renal failure (1) and acute myocardial infarction (1). The protocol was approved by the local ethics committee (Kartal Kosuyolu Training and Research Hospital, Ethics Committee; 2013.1/23) and conducted in accordance with the Declaration of Helsinki.

2.2. Blood sample collection

Blood was drawn from the antecubital vein of each subject 24 h before the surgery after twelve hours overnight fasting (Pre-op) and 7 days after the surgery (Post-op). Blood samples were collected in test tubes containing citrate, ethylenediamine-tetraacetic acid (EDTA) and test tubes without anticoagulant for biochemical analysis. Serum samples were obtained from Eppendorf tubes after centrifugation at 4000 rpm for 10 min for trace element analysis. All serum samples were kept in a −80 °C until the analysis.

2.3. Biochemical parameters assay

Biochemical parameters were analysed at the Biochemistry Department of Kartal Kosuyolu Education & Research Hospital. Hemogram was studied using electro impedance method with Coulter LH 780 device of Beckman Coulter Company. Serum glucose, total cholesterol, triglyceride (TG), high density lipoprotein (HDL) cholesterol and creatinine were determined on a Hitachi Modular P analyser using commercial kits (Roche Diagnostics, GmbH, Mannheim). LDL was calculated using the Friedewald's formula if the TGs were less than 4.5 mmol/L. Other biochemical parameters were studied using spectrophotometric method with Advia 2400 device of Siemens Company.

2.4. Premedication and anesthesia

Patients were premedicated with diazepam 10 mg orally before the night from surgery. The left radial artery was catheterized for arterial monitorization. Electrocardiogram, systolic arterial pressure, central venous pressure and pulse oximetry were monitozied throughout the operation for hemodynamic stability. Urine output was monitorized utilizing a Foley catheter in every patient intra-operatively and post-operatively. 0.2 mg/kg midazolam and 20 μg /kg of fentanyl were given for the induction of anesthesia. 0.1 mg/kg rocuronium was given to provide muscular relaxation before endotracheal intubation. Right internal jugular vein catheter was inserted for central venous catheterization. The use of activated clotting time was held between 400–600 s to optimize heparinization during the operation. Blood sugar level was measured in half-hour intervals to keep blood glucose level below 200 mg/dL.

2.5. Surgery technique

Cardiac arrest was supplied by antegrade cold blood cardioplegia following the sternotomy and cardiac exploration procedure. LIMA with its pedicule was resected up to subclavian artery in proximal segment and up to the bifurcation of epigastrica superior artery and musculophrenica artery in distal segment. The sub-branches of LIMA were cut by utilizing metallic clips. LIMA accompanied with its peripheral surroundings were protected in pleural space in a sterile gause embedded with papaverin until the anostomosis. The myocardium was protected continuously by retrograde blood cardioplegia with potassium during the operation. Systemic perfusion output and systemic perfusion pressure were maintained between 2.4 L/min/m^2 and 50 mmHg, respectively.

Aorta punch biopsy samples and LIMA samples were prepared from the ***identical individual*** undergone CBAG surgery and were stored at −80 °C until trace element analysis.

2.6. Tissue preparation

The tissue samples were weighed and transferred into metal-free glass tubes for digestion. All the glassware was kept in 10% (v/v) nitric acid solution before use. The samples were first digested with 2 mL of concentrated nitric acid at 100 °C in the furnace (Heraeus W.C. Heraeus GmbH, Hanau, Germany) and 2 mL of perchloric acid was added to the cooled materials. The materials were then completely digested at 120 °C until the materials diminished to half of the original total volume. Digested materials were diluted with deionized water to 10 mL. The last dilutions of the samples were mixed in a shaker for 15 min just before measurement. Results were calculated as μg/g wet weight (μg/g_{tissue}) [28]. 1 mL of serum was diluted in deionized water to 10 mL and then vortexed before the analysis [29]. Serum Zn, Cu and Fe levels of serum samples were indicated in microgram per milliliter (ppm = μg/mL).