Changes in Lactate and NUCB2/Nesfatin-1 Before, During and After Cardiopulmonary Bypass

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Abstract

Background: The main objective of this prospective study is to examine the effects of cardiopulmonary bypass on NUCB2/Nesfatin-1 hormone and its relation to hemodynamic parameters and to find out whether aorta, LIMA and saphena tissues synthesize NUCB2/Nesfatin-1.

Methods: From the 15 patients who underwent cardiopulmonary bypass surgery, LIMA and aortic tissues are harvested by making of hole for proximal anastomosis of the saphenous graft, and blood samples were collected before anesthesia induction (T1), before bypass (T2), before (T3) and after (T4) removing the cross clamp, upon transfer to intensive care (T5) and at postoperative 24 (T6) and 72 hours (T7). NUCB2/Nesfatin-1 amounts in biological samples were determined according to ELISA method and NUCB2/Nesfatin-1 immunoreactivity in the aorta, Left Internal Mammary Artery (LIMA) and leg vein (saphena) tissues was identified by immune histochemistry. Hemodynamic parameters and lactate levels of the patients were also measured.

Results: Circulating NUCB2/Nesfatin-1 levels of the patients steadily increased from T1 to T3, but showed a gradual decrease after T4 (including T4). Lactate levels displayed parallel changes to nesfatin-1. Additionally, synthesis of NUCB2/Nesfatin-1 on the tunica media layer of human aorta, saphena and LIMA tissues was reported for the first time.

Conclusions: considering that nesfatin-1 is synthesized in the human aorta, saphena and LIMA tissues and that the levels of this hormone have a parallel course with lactate levels in coronary artery bypass surgery, it can be a new parameter for use when monitoring coronary artery surgery.

Keywords: Lactate; NUCB2/Nesfatin-1; Cardiopulmonary bypass

Highlights

NUCB2/Nesfatin-1 is synthesized in the tunica media of human aorta, saphena and LIMA tissues. Since the vessel endothelium synthesizes NUCB2/Nesfatin-1, it should remain intact. Lactate and NUCB2/Nesfatin-1 show parallel increases or decreases. It can be a new parameter candidate in monitoring the Coronary Artery Bypass Grafting.

Introduction

In case that the vessels feeding the heart are constricted or blocked, coronary artery bypass surgery (open heart surgery) restores blood flow to coronary arteries without sufficient blood supply by bypassing them using vessels taken from another part of the body, such as Left Internal Mammary Artery (LIMA), vein of the leg (saphenous vein) or artery of the arm (radial artery) [1]. During this open heart surgery, a heart lung machine consisting of an oxygenator that fulfills the functions of the lungs and a pump serving the function of the heart is used [1-3]. Maintenance of circulation as such (non-physiologically) was reported to increase the synthesis and release of peptide hormones like Anti Diuretic Hormone (ADH) [2], and B-type Natriuretic Peptide (BNP) [3], which have effects on the vascular system. Another study where coronary artery bypass surgery was used for grafting investigated the changes in salusin alpha and beta and apelin 36, and showed that the levels of these peptides in blood samples dropped before anesthesia induction, before bypass, before and after removing the clamp and rose after admission to intensive care and at postoperative 24th and 72nd
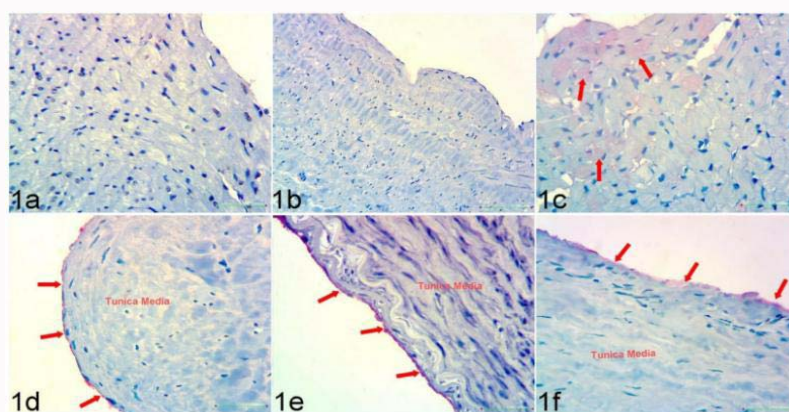
Figure 1: NUCB/Nesfatin-1 immunoreactivity (a, b, negative control; c, positive control) in the saphena (d) Left Internal Mammary Artery (LIMA) (e), and aorta (f). Presence of NUCB2/Nesfatin-1 in the tissues produces a reddish color with anti-human antibody (this immunoreactivity is shown with red arrows).
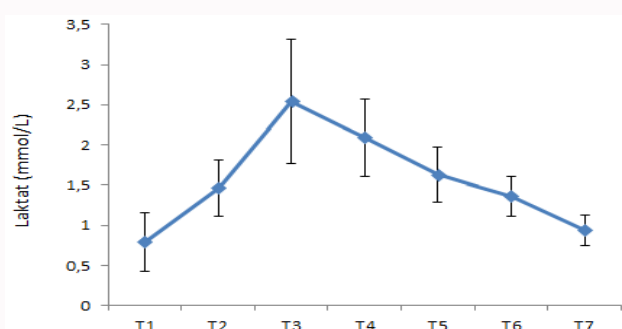

Figure 2: Changes in lactate levels before, during and after coronary artery bypass operation.

hours [4]. How ADH, responsible for re-absorption of water, changes in coronary artery bypass surgery is known [2], although the changes in the fate of NUCB2/Nesfatin-1, which has been reported to be involved in the feeling of thirst [5], during and after coronary artery bypass, still remain to be clarified. It has been suggested that NUCB2/ Nesfatin-1 modulates cardiovascular functions in both hemorrhagic and normotensive conditions [6], as besides inhibiting thirstiness; it elevates to systemic vascular resistance and blood pressure. Also, NUCB2/Nesfatin-1 leads to an increase in body temperature [7]. NUCB2/ Nesfatin-1, an 82 Amino Acid (AA) length polypeptide derived from calcium and DNA binding protein NUCB2, was for the first time discovered by Oh-I and colleagues in 2006. Nesfatin-1 can pass through the blood brain barrier in both directions after secretion. Nesfatin-1 is stable in blood for 20 minutes after intravenous injection. NUCB2/Nesfatin-1 is mainly secreted from the hypothalamus and brain stem, and its secretion decreases during fasting [8]. It is also secreted by peripheral adipose tissue, pancreatic endocrine beta cells, gastric mucosa, testis tissue and heart cells [9]. NUCB2/ Nesfatin-1 to be associated with Body Mass Index (BMI) [10] and hypertension [11], and also age affects NUCB2/ Nesfatin-1 level [12]. However, it is not known if it is synthesized in the Left Internal Mammary Artery (LIMA), leg vein (saphena) and heart tissue.

Based on the foregoing information, our objectives in designing this study are 1) to determine the changes in nesfatin-1 concentrations in the blood samples collected before anesthesia induction, before bypass, before and after removing the cross clamp, upon transfer to intensive care and at postoperative 24th and 72nd hours using the ELISA method; 2) to find out whether nesfatin-1 is synthesized in the mammary artery (LIMA), leg vein (saphena) and aorta tissues using immunohistochemical methods; and 3) to reveal if there is a connection between NUCB2/Nesfatin-1 and lactate, which is used in hemodynamic monitoring of coronary artery bypass surgery, and if NUCB2/Nesfatin-1 is related to other hemodynamic parameters that are affected by coronary artery bypass surgery (cardiac output, mean arterial pressure, central venous pressure, stroke volume index).

Materials and Methods

This study was conducted retrospectively upon the approval number 02, meeting issue 12, and date 06.17.2014 of the Clinical Studies Ethics Board of Fırat University. All patients admitted to the clinic to undergo an open heart surgery were informed of the study to obtain their written and oral consent. Inclusion (having similar age and BMI) and exclusion criteria (conditions including previous myocardial infarction, unstable angina, infarction presence, diabetes mellitus, hypertension and chronic obstructive pulmonary disease, abnormal renal and hepatic functions, cardiac valve disease) for patients were explained in detail in a previous study authored by our team [4]. The New York Heart Association (NYHA) classification and the Cleveland Clinical Severity Scoring System (CSSS) were used for the evaluation of all patients [13].

Operation

Patients to undergo coronary artery bypass (on pump) were transferred to the operating room in supine position. The standard bypass surgery procedure consisting of median sternotomy, aorta-aorto-atrial cannulation, blood cardioplegia warm induction, cold blood cardioplegia, complemented by warm blood "hot shot" hyperkalemic, reperfusion, membrane oxygenator, and mild hypothermia was followed. Saphenous veins and internal thoracic arteries of the patients included in the study were used for coronary artery bypass grafting, with a mean graft number of 3.2 for each patient. Additionally, arterial oxygen saturation (SaO2), Cardiac Index (CI), Central Venous Pressure (CVP), Cardiac Output (CO), Electrocardiography (ECG), Heart Rate (HR), Left Cardiac Work (LCW), Right Cardiac Work (RCW), Mean Arterial Pressure (MAP), Mean Pulmonary Artery Pressure (MPAP), Systemic Vascular Resistance (SVR) and Pulmonary Capillary Wedge Pressure (PCWP) hemodynamic parameters were monitored, as described previously [4,14].
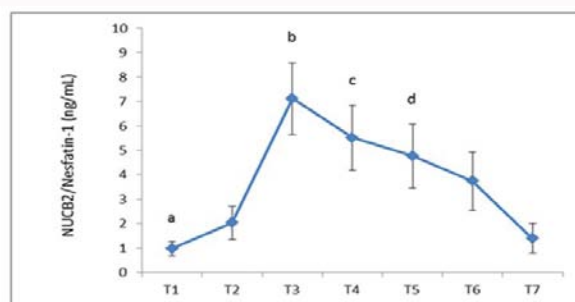
a: T1 vs T2 (p< 0.05). b: T3 vs T4 (p< 0.05). c: T4 vs T5 (p< 0.005). d: T5 vs T6 (p< 0.05).

Figure 3: Changes in NUCB2/Nesfatin-1 levels before, during and after coronary bypass operation.

Collection of biological samples

A total of 105 blood samples from 15 patients, of whom 4 were females (26.6%) and 11 were males (73.4%), were collected before anesthesia induction (T1), before bypass (T2), before (T3) and after (T4) removing the cross clamp, upon transfer to intensive care (T5) and at postoperative 24th (T6) and 72nd hours (T7). The 105 samples collected as previously described were centrifuged at 4000 rpm (1870 g) and then stored at -80°C until the time of analysis [4]. In addition; the number of bypasses and cross-clamping times during the operation were recorded. Nineteen healthy volunteers (8 females and 11 males) who came to hospital to make annual checks, and if they did not have any health problems were included as control subjects for this study.

NUCB2/Nesfatin-1 and lactate analysis

Blood NUCB2/Nesfatin-1 concentrations were quantified following the Enzyme-Linked Immuno Sorbent Assay (ELISA) method as instructed on the producer firm's catalogue (EK-003-26). The minimum detection limit of the kit was established to be 0.78 ng/mL. The intra- and inter-assay Coefficients of Variation (CV) values were found <10% and <15%, respectively. All these validation procedures were conducted following the previously described method [15]. Blood NUSB2/Nesfatin-1 concentrations were measured on the ChroMate, Microplate Reader P4300 equipment (Awareness Technology Instruments, Florida, USA). Lactate levels were determined using a lactate apparatus.

Immunohistochemical NUCB2/Nesfatin-1 analysis in the aorta, left internal mammary artery (LIMA) and leg vein (saphenous vein) tissues

The aortic and biological tissues harvested by making of hole for proximal anastomosis of the saphenous graft were put into 10% formaldehyde and fixed for immunohistochemical NUCB2/Nesfatin-1 analysis. All tissues were stained following the previously described method [16]. The biological tissues in 10% formaldehyde were buried into paraffin blocks. Then, cross-sections of 4 μm to 6 μm, obtained from the paraffin blocks, were transferred to poly-l-lysine-coated slides. After being deparaffinized, the tissues were hydrated with graded alcohol series. For antigen retrieval, they were put into a citrate buffer solution of pH: 6 and boiled for 7+5 minutes in a microwave oven (750W). They were then kept at room temperature for about 20 minutes and washed with PBS (Phosphate Buffered Saline, P4417, Sigma-Aldrich, USA) solution for 3 × 5 minutes. In order to prevent endogenous peroxidase activity, they were incubated with a hydrogen peroxide block solution for 5 minutes (Hydrogen

Table 1: Some biochemical parameters of experiment groups before and after Cardiopulmonary Bypass the surgery.

Parameters	Pre-operation	Post-operation
Glucose (mg/dl)	89 ± 8.1	92 ± 7.6
Calcium (mg/dl)	94.4 ± 0.7	96.4 ± 0.9
Potassium (mEq/ml)	4.4 ± 0.3	4.3 ± 0.2
Sodium (mEq/ml)	143 ± 2.3	1412 ± 2.8

Peroxide Block, TA-125-HP, Lab Vision Corporation, USA). An Ultra V Block (TA-125-UB, Lab Vision Corporation, USA) solution was applied for 5 minutes to rule out background staining. Then, Nesfatin-1 was diluted at a rate of 1/200 and incubated with primary antibody (Rabbit Nesfatin-1 primary antibody (cat no: H-003-25, Phoenix Pharmaceuticals Inc., California, USA) for one hour in a humid environment at room temperature. After being washed in PBS for 3x5 minutes, it was incubated with secondary antibody (biotinylated Goat Anti-Polyvalent (anti-mouse/rabbit IgG), TP-125-BN, Lab Vision Corporation, USA) for 30 minutes in a humid environment at room temperature. Following this step, the tissues were washed in PBS for 3 × 5 minutes and incubated with Streptavidin Peroxidase (TS-125-HR, Lab Vision Corporation, USA) for 30 minutes in a humid environment at room temperature. After being transferred into the PBS solution, the tissues were added 3-Amino-9-Ethylcarbazole (AEC) Substrate + AEC Chromogen (AEC Substrate, TA-015 and HAS, AEC Chromogen, TA-002-HAC, Lab Vision Corporation, USA). When an image signal was received on the light microscope, they were taken to the PBS bath. Rabbit IgG was used for negative control. Tissues stained with Mayer's hematoxylin for contrast staining were then rinsed with PBS and distilled water and closed with the appropriate closing solution (Large Volume Vision Mount, TA-125-UG, Lab Vision Corporation, USA). Preparations obtained as such were evaluated under a Leica DM500 microscope and photographed (Leica DFC295). Scale bar was 20 μm. The staining was used to calculate a histoscore based on the diffuseness (0.1: <25%, 0.4:26% to 50%, 0.6: 51% to 75%, 0.9: 76% to 100%) and intensity (0: no staining, +0.5: very weak, +1: weak, +2: median, +3: strong) of immune reactivity.

Statistical analyses

The data were presented as mean ± standard deviation. SPSS version 22 software was used in the statistical analyses. Wilcox on matched-pairs signed rank test was employed in the comparison between Nesfatin-1 values measured at different times. Correlations between hemodynamic or clinical or echocardiographic parameters and NUCB2/Nesfatin-1 values were studied with Spearman rank correlation test. Comparisons between multiple groups were made with Kruskal-Wallis test. Values for which $p < 0.05$ were accepted as significant.

Results

The mean age of bypass patients included in the study was 65.7 ± 4.9 years and their mean BMI was 27.7 ± 2.3. The groups of healthy volunteers were of similar age (66.9 ± 6.3 years) and had similar BMI (26.9 ± 2.6). There was not any statistically noteworthy difference between the biochemical parameters of experiment groups before and after the surgery, as presented in Table 1. Graft number was 3.5 ± 0.6, ejection fraction percentage was 45.8 ± 4.8, cardiopulmonary bypass duration 156 ± 11 min., cross clamp duration 99 ± 6, time spent in the intensive care 2.8 ± 0.9 days and time spent in the hospital was 8.4 ± 1.8 days. All surgery parameters data points presented here were

average of 13-15 subjects. Details about hemodynamic parameters are also presented in our previous work [14].

When immunohistochemical staining performed to find out NUCB2/Nesfatin-1 immunoreactivity was examined with light microscopy, it was seen that heart and vessel tissues used as negative controls did not have NUCB2/Nesfatin-1 immunoreactivity (Figure 1A and 1B). However, strong NUCB2/Nesfatin-1 immunoreactivity was observed in the heart tissue used as positive control (red arrow) (Figure 1C). There was NUCB/Nesfatin-1 immunoreactivity only on the tunica intima of the aorta, saphena, and Left Internal Mammary Artery (LIMA) (red arrow) (Figure 1D, 1E, and 1F).

Lactate and NUCB2/Nesfatin-1 concentrations displayed a gradual increase from T1 to T3. The concentrations of both parameters reached a peak at T3 (Figure 2 and 3). However, after T3, concentrations of both parameters showed a statistically significant, gradual drop until T7. Lactate and NUCB/Nesfatin-1 concentrations at T7 (three days after the operation) were similar to those at T1 (onset blood).

Discussion

The heart does not only serve in pumping blood to the body, but also functions as an endocrine organ synthesizing Atrial Natriuretic Peptide (ANP) and brain natriuretic peptide, as well as peptide hormones such as apelin, ADH, salusins and adropin [2,4]. These peptides released from myocytes are involved in maintaining cardiovascular homeostasis by mediating the harmonic progression of certain physiological and biochemical events such as natriuresis, diuresis, vasodilation and vasoconstriction. During cardiopulmonary bypass, which is a non-physiological event where circulation takes place outside the body, the molecules synthesized by myocytes are altered [1,4]. NUCB2/Nesfatin-1, another peptide hormone, mediates water-food intake, body weight, and glucose homeostasis [5,17,18]. The mRNA of this peptide hormone was shown in numerous biological tissues, including the heart tissue. Additionally, rat heart was reported to synthesize NUCB2/Nesfatin-1 [9]. However, the changes in NUCB2/Nesfatin-1 quantities during coronary artery bypass grafting have not been studied until now, and neither is there any literature study examining whether it is synthesized in saphena and LIMA tissues, which are coronary artery bypass grafting materials. With the present study, it is shown for the first time that there is NUCB2/Nesfatin-1 immunoreactivity in the human saphena, Left Internal Mammary Artery, and tunica intima of aorta. It is assumed that, besides contributing to water intake and glucose homeostasis through paracrine and autocrine functions, NUCB2-Nesfatin-1 present in the concerned tissues is involved in reducing Ischemia/Reperfusion (I/R) injury [19]. In an ex vivo rat heart tissue study, it was reported that Nesfatin-1 directly depressed contractility, promoted relaxation via cGMP, PKG and ERK1/2, and limited ischemia/reperfusion damage [19], also acting in post-conditioning protection [20]. In this study first time NUCB2/Nesfatin-1 immunoreactivity found in the human saphena, left internal mammary artery and tunica intima of the aorta indicates that these tissues also function as endocrine organs. Therefore, intact removal of saphena and Left Internal Mammary Artery, which are known to synthesize NUCB2/Nesfatin-1, will not only prolong the life of the grafts, but also help the regulation of the heart's energy metabolism, with Nesfatin-1 contributing to glucose homeostasis. Besides, it was reported that NUCB2/Nesfatin-1 was synthesized in the atrium tissues of rats [21] and its synthesis increased in obesity [18,19]. It is already known from previous studies

that human saphena, left internal mammary artery and aorta contain apelin and salusins [4]. NUCB2/Nesfatin-1 levels of CABG patients before anesthesia induction (T1) were statistically significantly higher than those of healthy controls. After the operation started, Nesfatin-1 levels in blood samples were still higher before bypass (T2) and before the removal of cross clamp. However, Nesfatin-1 concentrations dropped after the clamp was removed (T4), when the patients were transferred to the intensive care unit (T5), and at post-operative 24th (T6) and 72nd hours (T7), and were similar to the levels in healthy controls at 72 hours after the operation (T7). Higher NUCB2/Nesfatin-1 in circulation can be an indicator that these individuals are potential CABG candidates. We think that the elevation of NUCB2/Nesfatin-1 in the pre-operative blood samples of CABG patient's results from the cardiac tissue's increasing its capacity in an attempt to keep cardiac glucose under strict control. A similar mechanism was reported by Banding et al. who compared Nesfatin-1 levels of coronary heart disease patients and controls and found higher levels of mRNA in the former [21]. Although not all mRNAs end up as proteins, the central dogma is that the information on mRNA will be translated into protein, that is, NUCB2/Nesfatin-1 whose mRNA is elevated will form the basis of increased Nesfatin-1 protein in circulation. Therefore, the finding of elevated NUCB2/Nesfatin-1 in the blood of coronary artery bypass patients in the preoperative period in this study is consistent with increased Nesfatin-1 mRNA reported in coronary heart tissues [21]. NUCB2/Nesfatin-1 levels correlated with preoperative left ventricular volumes (both end-systolic and end-diastolic volume), but did not correlate with ventricle diameter. This means NUCB2/Nesfatin-1 level is a direct indicator of volume changes, but is not related to ventricle diameter. A similar situation was reported in BNP measurements of CABG patients. In this study, a positive correlation was found between the aortic cross clamp duration and NUCB2/Nesfatin-1 levels. It was seen that as myocardial interstitial fluid (edema) increased, NUCB2/Nesfatin-1 levels increased as well. In a previous study, it was reported that despite the use of cardioplegic protection against intra-operative cardiac ischemia damage, there was an increase in the myocardial interstitial fluid [22-24]. However, with the exception of mean arterial pressure, there was not any strong correlation between hemodynamic parameters and NUCB2/Nesfatin-1.

The hypoxic condition during coronary artery surgery leads to hyperlactatemia [14]. For this reason, lactate changes were also investigated whenever NUCB2/Nesfatin-1 was studied. Lactate levels which measured 0.9 mmol/L to 1.2 mmol/L before anesthesia started increasing with the placement of the cross clamp and reached a peak before the cross clamp was removed (T3), although the peak value was still close to the normal physiological limits. A possible reason why patients did not develop hyperlactatemia may be the short coronary artery bypass grafting times in our study. If coronary artery bypass grafting time is longer, oxygen level may drop below the critical threshold value and lactic acidosis may result. Besides, beta-2 agonists were not used in our study. Beta-2 agonists are agents that cause hyperlactatemia [25]. The most important conclusion of this study is that NUCB2/Nesfatin-1 and lactate levels show parallel increases and decreases. Thus, measurement of NUCB2/Nesfatin-1 can be an alternative parameter for lactate measurement while monitoring coronary artery bypass grafting in the future. Under normal physiological conditions, production and destruction of lactate are equal. However, when there is not adequate oxygen in the medium, pyruvate that is not used in oxidative reactions is converted

to lactate at a rate of 80% and used in gluconeogenesis at a rate of 20% [14]. Since NUCB2/Nesfatin-1 serves in the supervision of glucose use, the results of this study suggest that, lactate may be assuming mediating role(s), based on the amounts of lactate produced in the body, in maintaining homeostasis under hypoxic conditions [14,25].

Conclusion

In conclusion, it was shown for the first time using the immunohistochemical method in this study that human saphenous vein, Left Internal Mammary Artery (LIMA) and tunica intima tissue of the aorta synthesize NUCB2/Nesfatin-1. NUCB2/Nesfatin-1levels of patients who underwent coronary artery bypass grafting were lower than the control levels, and this decrease was found in the blood samples collected before anesthesia was induced (T1), before bypass (T2), and before the cross clamp was removed (T3). However, NUCB2/Nesfatin-1 concentrations showed a statistically significant increase in the blood samples taken after the removal of the cross clamp (T4), upon transfer to the intensive care unit (T5), and at postoperative 24th (T6) and 72nd hours (T7), reaching the levels found in healthy controls. Therefore, these changes observed in NUCB2/Nesfatin-1levels during and after coronary artery bypass grafting can be accepted as critical markers of cardiac performance. In brief, we think that just like norepinephrine and vasopressin [26], adrenomedullin [27], renin activity [28], endothelin-1 [29], tumor necrosis factor-α [30], atrial natriuretic factor [31], ADH [2], brain natriuretic factor [3], apelin and salusins [4], which are recognized as indicators of cardiac performance, NUCB2/Nesfatin-1 is a regulatory player in the cardiovascular system and can give an idea when monitoring the physiology of the cardiovascular system during and after coronary artery bypass grafting. One limitation of our study is the low number of cases. We anticipate that after being tested by an independent clinic and laboratory with a higher number of patients in the future, NUCB2/Nesfatin-1 measurement may prove useful in monitoring bypass surgery.

Acknowledgments

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