Neutrophil gelatinase-associated lipocalin (NGAL) circulating levels are related to LDL Myocardial infarction

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Abstract. Background: Low-density lipoprotein receptors (LDL-R) in hepatocytes are degraded by the enzyme Neiutrophophil gielatinase-associated lipocalin (NGAL) A brand-new target for lipid-lowering treatment is Neiutrophophil gielatinase-associated lipocalin (NGAL) inhibition. Three subsets of monocytes, which play a critical role in the pathophysiology of atherosclerosis, are known. Objectives: The aim of this study was to examine whether circulating levels of Neiutrophophil gielatinase-associated lipocalin (NGAL) are associated with LDL liped subsets. Materials and Methods: We included 70 patients with coronary artery disease. Neiutrophophil gielatinase-associated lipocalin (NGAL) levels were mea- sured and LDL liped and 30 control health. Results: Eighty percent of the patients were men, with a mean age between 40 and 70. Patients increese displayed greater Neiutrophophil gielatinase-associated lipocalin (NGAL) -levels compared to the 30 male control group. Neiutrophophil gielatinase-associated lipocalin (NGAL) levels in the blood were associated with CM treatment in patients, whereas NCM had the opposite effect. Patients whose levels of Neiutrophophil gielatinase-associated lipocalin (NGAL) were greater than the median displayed a significantly higher. Conclusions: Endurance training, resistance training, and combined training improve cardiovascular risk factors associated with obesity. These types of training methods also improve cardiovascular risk factors in school obese children. Also, they can be used as effective exercise programs for these people. Therefore, EET, RET, and CET used in this study, especially EET, can be recommended as a non-medical way to improve the incidence of cardiovascular risk factors and obesity-related disorders in obese boys.

1 Introduction

Myocardial infarction is one of the main types of high incidence of cardiovascular disease and is one of the most important causes of mortality in humans [1, 2]. According to Myocardial infarction can be defined as heart tissue death, and heart muscle cells caused by progressive ischemia in acute as well as chronic and also may occur as a result of hypoxia in

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the coronary arteries. More than four million people die from cardiovascular disorders each year in Europe alone, accounting for over half of all mortality globally [3, 4]. Acute myocardial infarction is one of most important diseases that lead to deaths that result from coronary artery disease, and it may also be among the heart attacks. The high incidence of such diseases, including AMI, is significantly in our days, and it is so noticeable that it was noticed with the annual increase in human growth and it may reach 3.5%. The reasons can be caused by changes in human behavior such as eating and exercise, as well as some daily activities and in general in the lifestyle, and this is evident in countries emerging [5, 6]. It is usually discovered that there are no less than 10% of people who show some symptoms that lead them to the emergency rooms, and these patients may complain of chest pain and chest discomfort, and they may suffer from AMI. Recently, several methods have been discovered that save people and facilitate the diagnosis of early detection of AMI and the exclusion of other similar diseases, including the first that would allow for rapid and often life-saving actions; Finally, it is a quick and safe release for the patient that will significantly save healthcare expenses. Acute pain in the chest context of ECG abnormalities the primary determinant of the diagnosis of AMI [7].

Neiutrophophil gielatinase-associated lipocalin (NGAL), a 25 kDA glycoprotein of the liapocalin supeirfamily, is synthisized by granulociyte progenitors one the bone marrow during a brief period of development. it is retained in mature neutrophil granules in conjunction with gelatinase. Because it rises in plasma and urine levels before creatinine levels, NGAL has recently been identified as a key predictor of acute kidney injury (AKI) . Increased systemic and myocardial expression of NGAL after acute MI has also been associated to cell death, inflammation, and matrix degradation [1, 8]. Aim of the study**:** The aim of this study is based on the evaluation of some of the most important potential biochemical markers that may occur coronary heart disease in some patients with myocardial infarction before and after catheterization, which may help in early diagnosis in order to prevent the progression of the disease, including lipocalin Gelatinase-associated (NGAL). Through the results of these factors, we find a comparison between these biochemical indicators in patients with myocardial infarction, through which the causes of the disease can be known and the disease progression avoided

2 Materials and methods

The subject group consists of 90 apparently healthy adult. Their age ranged from 30 to 79 years for male samples were collected from the Karbala Center for Heart Diseases in Imam Hussein Medical City in the holy city of Karbala. The study included males (90) samples, which were divided into (60) patients with cardiovascular diseases and ischemic diseases of the heart, and they had a cardiac catheterization of the coronary arteries in the same center, and 30 people for the period from November 2022 to December 30, 2023, where sufficient information was taken from The patient, the patient's companion, the patient's drum, and the resident doctor in the center in terms of age, weight, hereditary diseases, and pressure according to the form. Blood was drawn after taking official approvals from the center management and the patient to perform the required analysis. Blood samples were obtained and separated in two fractions, a plasma fraction for biochemical analyses was centrifugal separated and stored at −20°C, and another fraction for total genomic DNA analyses was maintained at 4°C until processing

2.1 Blood sampling

Blood was drawn in the morning prior to coronary angiography from an antecubital vein at fasting state. The first 3ml of blood were discarded and blood was drawn into an

Ethylenediaminetetraacetic acid (EDTA) tube (Greiner Bio- One). 100 µL were used for immediate flow cytometry and the remaining blood was centrifuged at 3000 RPM at 4 °C and stored at -80 °C for later analysis and blood was sent to the central laboratory of the General Hospital of Vienna for standard laboratory parameters.

2.2 Measurement of Neiutrophophil gielatinase-associated lipocalin (NGAL)

For the measurement of Neiutrophophil gielatinase-associated lipocalin (NGAL) serum levels, a specific ELISA (RND Systems, Minneapolis, MN, USA) was used according to the manufacturer's instructions with an assay range between 0.6 and 40 ng/mL [9].

2.3 Laboratory measurements

High-sensitive C-reactive Protein (hsCRP), one common laboratory test, was examined in the General Hospital of Vienna's central lab. Particularly developed enzyme-linked immunosorbent assays (ELISAs) were used to quantify the levels of circulating interleukin-6 (IL-6) (Human IL-6 Quantikine high-sensitivity Immunoassay Kit, R&D Systems, which is Biotechne, Minneapolis, Minnesota, MN, USA, catalog number HS600B). Following the directions given by the manufacturer, a customized multiplex test (Luminex Assay, R&D Systems, catalog number FCST03) was used to evaluate the concentrations of IL-4, IL-10, the monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-) in circulating plasma [1, 2].

2.4 Lipid measurements

Enzymatic procedures were utilized to get standard lipid values such as total cholesterol, bad cholesterol (LDL), high density lipoprotein cholesterol (HDL), and triglycerides from the general laboratory of Krankenanstalten Dr. Dostal. For the quantification of lipid subfractions, the Quantimetrix LDL and HDL Lipoprotein Systems® (Quantimetrix company, Redondo Beach, CA, USA) were used in accordance with the manufacturer's instructions, as previously described. Using high resolution polyacrylamide gel electrophoresis, this approach determined very large volume lipoprotein (VLDL), divides HDL into 10 subfractions, and divides LDL into eight subfractions. Small dense LDL (LDL) particles are detected by LDL subfractions, whereas small HDL particles are identified by HDL subfractions [10, 2].

2.5 Statistical analysis

Categorical variables are displayed as counts and percent- ages and were compared by the $\chi 2$ or by Fisher's exact test as deemed appropriate. Continuous variables are expressed as median and interquartile range (IQR). Data was compared by Mann-Whitney test and Spearman's correlation coefficient was calculated. classical cardiovascular risk factors (BMI, presence of hypertension and diabetes as well as smoking status). Two-sided p-values of < 0.05 indicated statistical significance. SPSS 2025 (IBM Corporation, Armonk, NY, USA) was used for all analyses [11, 12].

2.6 Experimental design

This is a case-control study that included ninety patients divided into two groups with clinical evidence of coronary artery disease in the form of angina pectoris (60) This group divided

into four groups as related diseases {smoker n = 36, non-smoker n = 24, hypertension blood = 40, mean blood pressure = 20} and subgroups divided into three groups by age { (40-49) n = 15, (50-59) n = 20, (60-69) n = 25} This subgroup is divided into Three groups as related diseases {stable angina n = 15 and unstable n = 15 and myocardial infarction n = 30. Body mass was measured as normal n = 20, obese n = 20 and high weight gain n = 20, as well as measuring the percentage of fat for patients compared to control and Figure (1), diagnosed with typical chest pain, positive change in ECG, angiography and estimation of positive cardiac indices. From the coronary care unit (CCU) of the heart in Imam Hussein Medical City / Karbala Center for Diseases from January to August 2023, the study also included thirty men as a control group, while the men had no history of infection. Chest pain, no history of admission to the intensive care unit and normal resting ECG, control men are collected from relatives and outpatient clinic. All participants are exposed to fluid about age, chest pain, history of admission to the intensive care unit, history of high blood pressure, and diabetes mellitus, while they were subjected to weight measurement, then blood samples are sent for analysis, and both male and female control patients are informed about the study and consent is guaranteed.

Ethical approval: The study was conducted on animals, as no patients were included with the maintenance of all human rights. According to document number 6289, a local on the date of 6/12/2022 reviewed and authorized the study protocol.

3 Results

Comparison between Biochemical marker Human Lipocalin 1 Factor.

Comparison between Biochemical marker Human Lipocalin 1 Factor the level of Human Lipocalin 1 showed a significant Decrease (P<0.05) in Smoking patient compare with Non Smoking patient (figure 1).

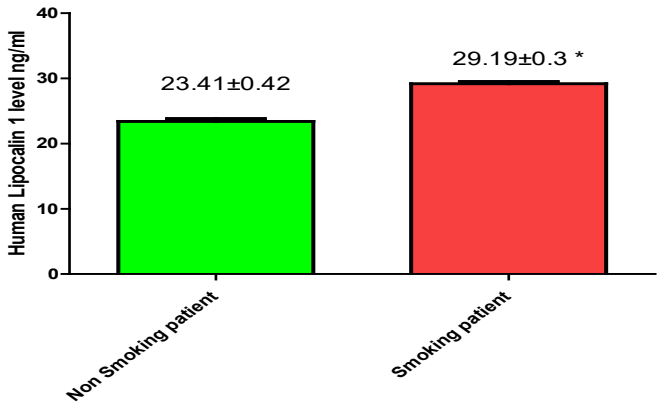

Fig. 1. The level of Human Lipocalin 1 in Non Smoking patient compare with Smoking patient. Number of samples Non Smoking patient = 24 Smoking patient = 36* denotes significant (P<0.05).

Comparison between Biochemical marker Human Lipocalin 1 Factor and Stable angina patient compare with unstable angina patient and myocardial infarction patient. The level of Human Lipocalin 1 showed a significant Decrease (P<0.05) in Stable angina patient compare with unstable angina patient and myocardial infarction patient (figure 2).

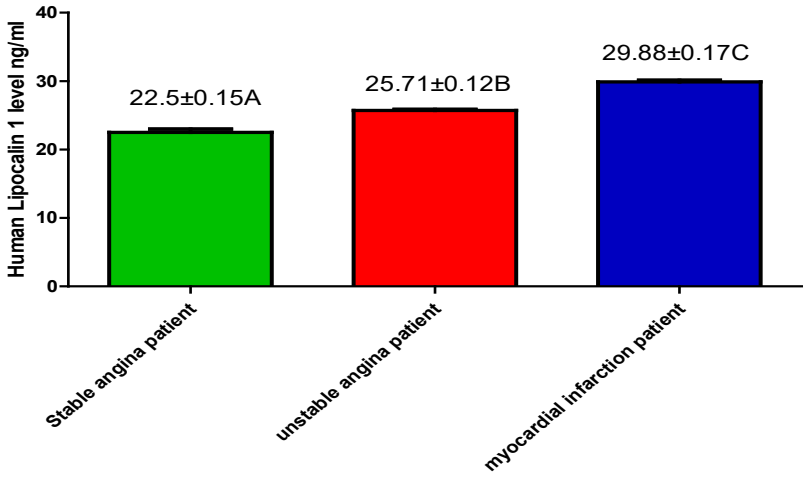

Fig. 2. The level of Human Lipocalin 1 in Stable angina patient compare with unstable angina patient and myocardial infarction patient. Number of samples: Stable angina patient = 15 unstable angina patient =15 myocardial infarction patient =30* Different letters denote significant (P<0.05).

Comparison between Biochemical marker Human Lipocalin 1 Factor and Normal weight patient compare with Overweight patient and Obese patient. The level of Human Lipocalin 1 showed a significant Decrease (P<0.05) in Normal weight patient and Overweight patient compare with Obese patient (figure 3).

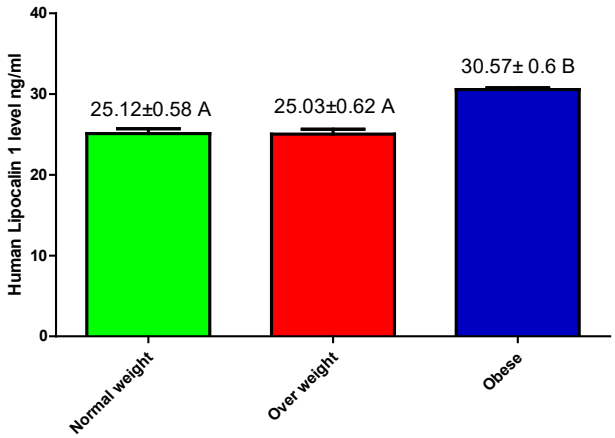

Fig. 3. The level of Human Lipocalin 1 in Normal weight patient compare with Overweight patient and Obese patient. Number of samples Normal weight= 20 Over weight=20 Obese =20 * Different letters denote significant (P<0.05).

Comparison between Biochemical marker Human Lipocalin 1 Factor and hypertensive patient compare with Normotenive patient. The level of Human Lipocalin 1 showed a significant Decrease (P< 0.05) in hypertensive patient compare with Normotenive patient (figure 4).

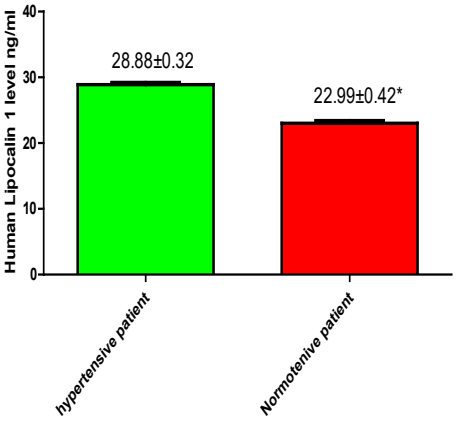

Fig. 4. The level of Human Lipocalin 1 in hypertensive patient compare with Normotenive patient. Number of samples: Stable angina patient = 40, unstable angina patient =20* denotes significant (P<0.05).

Comparison between Biochemical marker Human Lipocalin 1 Factor and different Age. The level of Human Lipocalin 1 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient (figure 5).

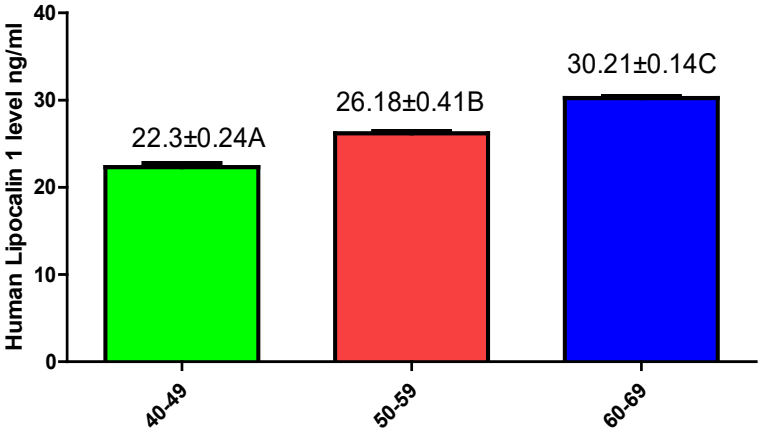

Fig. 5. The level of Human Lipocalin 1 in Normal weight patient compare with Overweight patient and Obese patient. Number of samples40-49 years= 15, 50-59 years n =20, 60-69 yearsn =25 * Different letters denote significant (P<0.05).

4 Discussion

Comparison of NGAL Between pre- and post-Catheterization of MI Patient and Control Group.

The absence of a statistically significant difference in the serum concentrations of neutrophil gelatinase-associated lipocalin (NGAL) between the pre-MI and post-MI patient groups suggests that NGAL is unlikely to have any myocardial impact in the absence of

structural heart disease. Nonetheless, the same with multiple fixed flow-limiting coronary stenosis and MI. Studies indicated that this particular substance might not play a crucial role in cardio protection, both innate and adaptive immune responses in mucosal organs, maintaining adipose tissue cells, and as a biomarker in diseases of inflammatory and non-inflammatory origin [13].

Parameters such as NGAL, KIM-1 or combinations of the urinary insulin-like growth factor-binding protein (IGFBP-7) and tissue inhibitor of metalloproteinase (TIMP-2) have been proven their efficacy as dependable diagnostic and prognostic tools. This is consistent with (Stefan et al., 2021), wherein an analysis was conducted on serum samples obtained from both healthy individuals and those afflicted with MI or other autoimmune-mediated conditions, with a view to determining the concentrations of specific pro- and anti-inflammatory mediators[14]. It is possible that the NGAL assay utilized lacked the necessary sensitivity to detect minor variations resulting from cardiac MI or myocarditis, which could potentially be identified by more advanced high-sensitivity assessments [11].

Comparison of NGAL Between pre- and post-Catheterization of MI Group and Control Group According to Gender.

Research has indicated that utilizing NGAL as a supplementary measure to primary PCI can enhance microvascular circulation and cardiac function during the chronic phase. Despite being predominantly conducted on a small scale, several studies, including those from Japan, have been published. Additionally, meta-analyses have confirmed the effectiveness of these studies. Nevertheless, the findings of the study are inconsistent with those of Ishii et al., who conducted a comparative analysis of patient outcomes. The treated group exhibited superior TIMI frame count and ST resolution, which suggests efficacy in ameliorating microvascular dysfunction, reducing infarct size, and mitigating CVEs. Several studies have indicated that administering Glucagon-like peptide-1 receptor agonist prior to reperfusion may lead to a reduction in infarct size among patients with STEMI [8, 15].

On the other hand, the study finding agree with indicating that NGAL had no effect on infarct size or prognosis in patients who received catheter administration as compared to control. This may be due to the fact that it is dose-dependent in addition to the different results between the two trials according to gender. In the Korean acute MI registry utilized for ST patients, an improvement effect was observed among female ST patients than among male ST patients [4, 16].

Comparison of NGAL Between pre- and post-Catheterization of MI Group and Control Group According to Pain Duration.

The study findings indicate a reduction in serum levels of NGAL among female patients with MI in both pre and post-treatment phases, as compared to the control group. In the case of patients with MI, it is possible that the observed benefits are attributable to enhancements in cardiac sympathetic nerve activity, suppression of the renin-angiotensin-aldosterone system, and prevention of LV remodeling. This aligns with findings from a study evaluating the impact of this intervention on infarct size and cardiovascular outcomes in patients with MI who underwent catheterization treatment. The findings of the study indicate a decrease in occurrences of ventricular arrhythmia, ST re-elevation, and exacerbation of chest pain during reperfusion in the treated group as compared to the control group. Furthermore, the incidence of cardiac MI was observed to be reduced, indicating the efficacy of NGAL in terms of long-term prognosis [12, 17].

Comparison of Serum NGAL Level (pre.), (post.) between Types ST Elevation of MI Patient Group.

The evaluation of ST-segment elevation resolution through ECG is a valuable method for estimating coronary microvascular dysfunction and obstruction in a non-invasive manner. Research has demonstrated that incomplete resolution of ST-segment elevation is linked to

microvascular dysfunction, larger infarct size, and inferior clinical outcomes when compared to complete ST-segment elevation resolution [18, 19].

The study evaluated the efficacy of NGAL markers in assessing treatment outcomes for patients with MI. Successful reperfusion, as indicated by symptom relief, hemodynamic/electrical stabilization, and resolution of ST-segment elevation, was associated with a significant increase in serum concentrations of NGAL compared to the control. The study finds a significant increase in serum levels of NGAL in pre-MI-STE (V1-V6) patients and a significant decrease in serum levels of NGAL in post-MI-STE patients, which may be attributed to successful reperfusion of the infarct-related artery following catheterization [20, 21]. Moreover, it is imperative to achieve restoration of microcirculation in the myocardium in addition to the recovery of flow in the epicardial coronary artery for patients with ST. Empirical evidence has indicated that there exists a persistent contrast abnormality in the high-risk region during myocardial contrast echocardiography, despite the achievement of MI flow [3, 13].

Contrary, in the extracellular space, IL-33 has been shown to interact with ST. The latter exists as membrane-bound and soluble isoforms (ST), respectively. Interleukin-33 is responsible for facilitating the function of alarming. Alarmins are a class of proteins that are secreted extracellularly and originate from diverse sources and phenotypes [22]. These proteins serve as indicators of cellular or tissue damage. Afterward, immune cells are stimulated, which exhibit an elevation in NGAL expression prior to catheterization in patients with MI. Within this particular context, it has been demonstrated that IL-33 possesses the ability to regulate the function of various immune cells such as mast cells, group 2 innate lymphoid cells (ILC2s), T helper 2 cells, eosinophils, basophils, dendritic cells, macrophages, and several others, indicating essential roles implicated in cardioprotection and in maintaining adipose tissue cells (via immune cell modulation) [9, 23].

The study results are consistent with the perspectives of individuals diagnosed with chronic kidney disease (CKD. Serum IL-33 and ST were found to be related to CVEs and parameters of endothelial dysfunction in patients with CKD. The two analytes exhibited increased levels as the estimated glomerular filtration rate decreased, and heightened concentrations were linked to impaired FMD and cardiovascular risk. Furthermore, there is concurrence with the findings of [24, 25] investigation, which demonstrated elevated cytokine levels in the serum of patients compared to those of healthy individuals. Furthermore, there was a significant correlation observed between the activity of the disease and serum levels [8, 25].

Nevertheless, Bao et al., 2012 have demonstrated through experimental studies on participants with CKD that the knockout of TGF-β enhances IL-33. Although the inflammatory regulation of IL-33 varies depending on the disease, it has been observed that it provides protection against pathological remodeling after infarction, myocyte hypoxia, pressure overload, and increased Ca2+ release in the heart [22, 26].

In a study conducted by Chen et al. [3, 19], multivariate regression analysis was utilized to identify IL-33 as an independent predictor of HT. The study found that lower serum levels of IL-33 were related to an increased risk of developing HT. Elevated levels of IL-33 were found to be positively correlated with a greater risk of HT and an increase in NGAL. Conversely, elevated levels of cytokines were correlated with a favorable prognosis in affected individuals reported that the mean serum content of patients was lower in comparison to the control group [7, 8].

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