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Nitric Oxide Levels in Patients with Acute Myocardial Infarction

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ABSTRACT

Introduction:
L-Arginine derivative, Nitric Oxide (NO), is produced by endothelial cells of coronary arteries in the heart. NO is endogenously produced within the myocardium by nitric oxide synthases (NOS).Disturbances in NO's bio-availability result in changes in the regulation of cellular and critical physiological processes like apoptosis, angiogenesis, platelet function, vasodilatation, and cell proliferation of smooth muscles. One of the most common death causes is AMI (Acute myocardial infarction), estimated to be nearly 15% of fatalities globally. NO regulates coronary vasodilatation by acting directly on the smooth muscle cells of coronary blood vessels and is a potent anti-platelet aggregator. Procoagulant inflammatory responses have a role in the increased tendency for re-occlusion seen in coronary vessels afterPercutaneous Transluminal Coronary Angioplasty (PTCA) in AMI. Although it is uncertain whether lower NO bioavailability is a cause or an outcome of endothelial dysfunction, it is regarded as one of the central contributors to vascular diseases.

Objective:
This research is conducted to estimate NO levels changes and their connection with CK-MB in early stages of AMI as the CK-MB levels changes with its infarct size and is a critical prognosis predictor.

Materials And Methods:
In this research, 41 peoplewere suffering fromAcute Myocardial Infarction due to damage to the myocardium of the heart, because of occlusion of diseased coronary blood vessels, with CK -MB levels greater than 10IU/L. In the control group, 41 healthy blood donor volunteers were taken. NO levels were calculated with spectrophotometric technique using the cadmium reduction method. Standard procedures of clinical chemistry estimated serum CK-MB.

Results:
The NO serum levels significantly decreased in patients with AMI (19.6±4.5 mmol/L) compared to the controls (24±2.8mmol/L). The serum NO levels and CK-MB levels correlated in the AMI group ($p>0.05$)

Conclusion:
NO level is markedly decreased in patients with the early phase of AMI. This is due to damage to the lining endothelial cells of coronary blood vessels responsible for releasing endothelial-derived relaxing factor,nitric oxide (NO) . The primary cause of damage to the lining endothelium of coronary blood vessels is atherosclerotic plaque.This leads to less NO production and vascular dysfunction. Damage incardiac myocardium releases CK- MB in the bloodstream. Therefore, alterations of cardiac function reflected from changes in CK-MB values would be accomplished by changes in NO serum. Hence, determining the levels of NO in peripheral blood might be used as a vital marker in the follow-up process and prognosis.

I. INTRODUCTION

Cardiovascular disease is a broad term that refers to various medical conditions like peripheral vascular disease, stroke, and coronary heart disorders. Also,the underlying pathology for all these conditions is atherosclerosis.

Atherosclerosis pathology is highly complex and includes numerous structural elements like inflammatory cells like macrophages and monocytes, circulating cells like leukocytes and platelets, and the arterial wall[1.]AMI is a medical disorder that occurs with coronary artery disease and manifests when the atherosclerotic plaque ruptures and the thrombus blocks the coronary arteries partially or totally, which restricts regular blood assessment to the heart[2].The highest point of CK-MB reaches under 24 hours, which starts to rise 4-9 hours postmyocardial injuries, and it then decreases to its normal range within 48-72 hours.[3]The levels of CK-MBis proportional to itsinfarct size and is a vital prognosis predictor[4].

Endothelium induces vasodilation by releasing a substance that relaxes vascular smooth muscle cells; this was discovered in the 1970s[6]. During that time, the chemical structure of this

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substance was not known and named EDRF (Endothelium-Derived Relaxing Factor). Still, it was identified as an odorless and colorless Nitric Oxide (NO)[5]. After which, NO was recognized gradually as the gas signaling molecule, and its action and mechanism have been extensively researched.

Although it is uncertain whether lower NO bioavailability is a cause of or an outcome of endothelial dysfunction, it is regarded as one of the central contributors to vascular disease. Several elements could influence NO production or its diffusion capacity to its cellular targets. Changes in bio-availability of NO result in disturbances in regulations of cellular and critical physiological processes like apoptosis, angiogenesis, platelet function, vasodilatation, and cell proliferation of smooth muscles

NO relaxes the vascular smooth muscles, which dilates the blood vessels. Acetylcholine is an agonist that binds to the receptors on endothelial cells, causing a brief spike in intracellular calcium. Calcium binds to calmodulin, and calcium calmodulin complex activates NOS (Nitric Oxide Synthase), formingnitric oxide[6].Nitric oxide diffuses from endothelial cells to neighbouring cell smooth muscles, where it binds to guanylate cyclase's heme groups.

Guanylate cyclase are activated for producing cGMP that induces relaxation in smooth muscles. Owing to the half-life of NO lying between 2-30secs, the impact of NO diminishes suddenly, and the vessel then constricts in case no more NO is generated.

NO produced can diffuse into the vessel lumen and enter the platelets. NOS found in platelets activates guanylate cyclase and adenylate cyclase[16]. It,inturn, inhibits platelet aggregation and prevents the clotting of blood[7].NO mediates vasorelaxation and inhibits leukocyte and platelet adhesion to vascular endothelium[8]. HOWEVER, the NO reacts readily with super-oxides, which generates a high-reacting molecule peroxy-nitrate (ONOO-),which further trigers a series of harmful events leading to cardiovascular complications.Oxygen presence detects if NO shows anyharmful or protective effects[9]. Procoagulant inflammatory responses have a role in the increased tendency for re-occlusion seen in coronary vessels afterPercutaneous Transluminal Coronary Angioplasty (PTCA) in AMI. Several lines of observation support this. For example, a higher leucocyte count is an essential risk for adverse and subsequent cardiac events in AMI [10]. NO also has a vital function forischemia-reperfusion injury and IHD.

These conclusions exhibit a cardio-protective role of eNOS derived NO in ischemia-reperfusion injury in the heartof a mouse [11]. Whereas, research conducted by Wang et al.[12] states that NO derived from the iNOS contributes to some of the myocardial injuries following IHD.

Thus, it appears that the issue of whether NO is beneficial or not is large model dependent, dependent upon the quantity of NO present in the system, the type of NOS that is being studied and may also partly depend on how much of the free radical, peroxy-nitrite, is formed.

II. MATERIAL AND METHODS

The research was conducted in 2006 in "M.S. Ramaiah Medical college Teaching hospital", Bangalore, India, considering 41 patients of any gender aged 35 years or above. Patients clinically diagnosed with acute myocardial infarction with CK-MB > 10 IU/L were considered for this research. Patients suffering from congestive heart failure andnitrate with diabetes mellitus and kidney conditions were not included in the study. Age-matched healthy controls belong to either sex in the 25-50 age group. The study was carried out after the patients gave their informed consent and the institution's ethical committee authorized the study.

For the aseptic precautions, 5ml venous blood was takenin plain tubes. Clotted blood is subjected to centrifugation. A clear serum was separated and is used for biochemical investigations of Serum CK-MB and Serum Nitric oxide. The chemicals used were of high analytic standard,readily available to work within India.

Greiss reaction was used to determine the Nitric Oxide Serum similar to Cortas and Wakid[13]. NO is a diffusible and labile molecule that forms stable nitrate/nitride metabolites, and this is detected using the Greiss reaction. As per the Kinetic technique, nitrates are reduced to nitrite using the cadmium granules coated with copper. The produced Nitride is estimated with sulfanilamide diazotization and Napthyl-ethylenediamine coupling to form a purple complex, measured on 545nm with the help of a spectrophotometer. Serum CK-MB was calculated using an autoanalyzer by standard clinical chemistry methods[14].

III. STATISTICAL ANALYSIS

SPSS 10.0 and Systat 8.0 statistical software evaluated the data collected.[15]

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IV. RESULTS

This study found that serum NO levels of patients with AMI were significantly lower than the average healthy controls (p<0.01),table1. The NO serum showed a positive and significant relation with CK-MB serum in people suffering from AMI (r =0.219) (p <0.01),table 2. A quadratic link exists in CK-MB and NO serum, which shows that Serum NO steeply decreases after specific values of CK-MB (12U/L), which could be due to the damage to the myocardium.

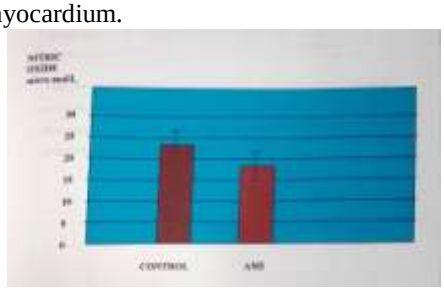

Fig 1.Comaprison of serum NO levels between controls and cases

COMPARISON OF SERUM NITRIC OXIDE LEVELS BETWEEN THE HEALTHY CONTROLS AND THE STUDY GROUP

PARAMETER	CONTROLS (n= 41) Mean ± S. D	STUDY GROUP (n = 41) Mean ± S. D	COMPARISON
SERUM NITRIC OXIDE (μ mol / L)	24± 2. 8	19.6 ± 4.5	p < 0.01 Highly Significant

Table 1 . Significantly decreased mean levelsinserum NO levels (p<0.01) in the study groupas compared to controls.

Magnitude of study effect reflects that a variance explained by Serum Nitric Oxide was around 68% variance

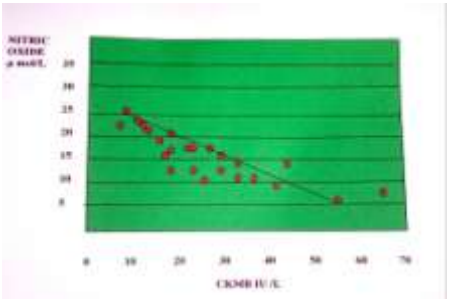

Fig 2.Correlation between NO and CK-MB in acute myocardial infarction

Table 2.Correlation between NO and CK-MB in acute myocardial infarction

V. DISCUSSION

It has been found that the low serum nitric oxide levels in AMI patients may be due to the damage to the lining endothelium of the coronary blood vessels. A positive correlation between serum NO and CK-MB suggests alterations in myocardial function. Thus, whenever there is damage to the myocardium, there are increased CK-MB levels, and damage to the lining endothelium causes less NO production.The study of NO synthase activity regulation could lead to a better understanding of its function.The creation of NOS inhibitors that block specific isoforms of NO and stable molecules that release it would be a key challenge in the therapeutic development process. Comparison in two vital parameters proposed CK-MB serum and NO serum can be conducted as the prognostic follow-up indicator in people suffering from AMI. The study can further estimate the serum nitric oxide levels in the latter stages of AMI and correlate with the reperfusion injury.

Since our studies showed that the nitric oxide levels were low in AMI, further studies can be carried on for therapeutic uses of NO.Reperfusion after the ischemic period can be linked to reduced NO bioavailability, probably because of NO activation by super-oxide, resulting in reduced NO production. NO, and L-Arginine donors protect from any myocardial injuries when this drug is administered right before or on reperfusion onset, which indicates that NO helps safeguard against reperfusion injuries[17]. In mouse with lack of genes for endothelial NO synthase, its infarct size was more extensive, and the post-ischemic cardiac functional recovery was slower than in wild mice[18,19], which supports a notion that the endogenous NO protects against reperfusion/ischemia injuries.Administrating NO (Nitric Oxide), the NO drugs or donors increase NO release (dexamethasone, ACE-inhibitors, calcium antagonists, statins) before ischemia protecting myocardium against reperfusion/ischemia injuries in myocardial infarction and reduce infarct size.There also have been many studies that indicate that NO might exert anti-arrhythmic actions during

reperfusion/ischemia. NO donor was studied to control arrhythmias among pigs, which subjects coronary artery occlusion[20]. NO-donor chemicals widen systemic arteries and reduce blood pressure has hampered their therapeutic application after MI.

Further studies can be conducted on NO importance in reperfusion/ischemia injuries, hypotension,anti-arrhythmia actions, minimize infarct size and prevent cardiac failure after AMIand focus mainly on standardization and identification of potential confounding factors which have an effect on synthesis, interaction, and transport of NO with different targets in tissues and blood.

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