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Original Research Article

Exploring the Role of Human Endothelial Nitric Oxide Synthase (eNOS) in Ischemic Heart Disease (IHD)

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Abstract: ***Background*:** Ischemic heart disease, also called coronary artery disease, is the term given to heart problems caused by narrowed heart (coronary) arteries that supply blood to the heart muscle. ***Aim of the Study*:** The aim of the study is to determine the levels of Nitric oxide synthase (eNOS) in patients with coronary artery disease. ***Patients and Methods***: The current study is case-control study conducted in Tikrit city during the period between 10th of January to 10th of April 2023 on patients admitted to Coronary Care Unit of Salah Al-Din Teaching Hospital The study included 60 Iraqi patients with coronary heart disease with age range (37-66 years). The study also included 30 healthy individuals with same age range and from both sexes who apparently haven't any acute or chronic diseases. Blood was collected from each patients for determination Nitric oxide synthase by enzyme-linked immunosorbent assay (ELISA) and lactate dehydrogenase (LDH) by chemiluminence technique. ***Results*:** The study showed that lowest mean of Human Endothelial Nitric Oxide Synthase (eNOS) was detected among IHD patients (2.41±0.74 ng/ml) and the maximum mean was within the control group (5.15±1.24 ng/ml). The differences were highly significant (P-value: 0.0001). The study confirmed that the peak mean of lactate dehydrogenase was detected among IHD patients (257.8±94.9 IU/L) and the deepest mean was in the control group (168.9±16.8 IU/L). The differences were highly significant (P-value: 0.0001). The study showed that the mean eNOS was 2.17 ng/ml in patients with hypertension, which was significantly lower than that in non-hypertensive IHD patients (2.76 ng/ml), at a P value of 0.024. The study showed that the mean LDH was 284.6 IU/L in patients with hypertension, which was significantly more than that in non-hypertensive IHD patients (217.5 IU/L), at a P value of 0.001. ***Conclusions*:** In conclusion, the findings of the study suggest that patients with ischemic heart disease (IHD) have reduced levels of serum eNOS compared to healthy individuals**.** More researches are needed to fully understand the relationship among eNOS, cardiovascular disease and to determine the clinical utility of their measuring in the diagnosis and management of these conditions.

Keywords: Enos, LDH, IHD, Hypertension.

INTRODUCTION

Ischemia is defined as inadequate blood supply (circulation) to a local area due to blockage of the blood vessels supplying the area. Ischemic means that an organ (e.g., the heart) is not getting enough blood and oxygen. Ischemic heart disease, also called coronary heart disease (CHD) or coronary artery disease is the term given to heart problems caused by narrowed heart (coronary) arteries that supply blood to the heart muscle [1]. Coronary artery disease (CAD) is characterized by the occlusion or stenosis of coronary artery mostly caused by atherosclerosis, and is one of the leading causes of mortality in humans [2]. Patients with CAD are vulnerable in development of major cardiovascular events including nonfatal acute myocardial infarction, unstable angina, stroke, transient ischemic attack, peripheral arterial occlusive disorder, and death [3]. Although the narrowing can be caused by a blood clot or by constriction of the blood vessel, most often it is caused by buildup of plaque, called atherosclerosis. When the blood flow to the heart muscle is completely blocked, the heart muscle cells die, which is termed a heart attack or myocardial infarction (MI) [4]. Most people with early CHD do not experience symptoms or limitation of blood flow. However, as the atherosclerosis

progresses, especially if left untreated, symptoms may occur. They are most likely to occur during exercise or emotional stress, when the demand for the oxygen carried by the blood increases [5].

Nitric oxide is a soluble gas continuously synthesized by the endothelium. This substance has a wide range of biological properties that maintain vascular homeostasis, including modulation of vascular dilator tone, regulation of local cell growth, and protection of the vessel from injurious consequences of platelets and cells circulating in blood [6-8]. A growing list of conditions, including those commonly associated as risk factors for atherosclerosis such as hypertension and hypercholesterolemia, are associated with diminished release of nitric oxide into the arterial wall either because of impaired synthesis or excessive oxidative degradation [9]. The aim of the study is to determine the levels of Human Endothelial Nitric Oxide Synthase (eNOS) in patients with ischaemic heart disease (IHD) patients.

MATERIAL AND METHODS

The current study is case-control study conducted in Tikrit city during the period between 10th of January to 10th of April 2023 on patients admitted to Coronary Care Unit of Salah Al-Din Teaching Hospital.

The study included 60 Iraqi patients with coronary heart disease with age range (37-66 years). The diagnosis of ischemic heart disease was based on history and characteristic electrocardiographic changes. Hypertensions defined as a systolic blood pressure greater than 130 mmHg or a diastolic blood pressure greater than 80 mmHg or are taking medication for hypertension. Medical history was taken for patients including history of hypertension and/or diabetes mellitus in addition to drug history and smoking. The cases were collected from the Coronary Care Unit of Salah Al-Din Teaching Hospital.

The study also included 30 healthy individuals with same age range and from both sexes who apparently haven't any acute or chronic diseases.

Exclusion Criteria

Patients suffering from:

- Kidney disease,
- Liver disease,
- Cancer,
- Pregnancy and lactating mothers,
- Cerebrovascular accidents,
- Alcoholics,
- Rheumatoid arthritis,
- Autoimmune disease.

Methods

After at least 12 hours of fasting ,blood was collected by vein puncture with plastic disposable syringes took up to 5ml of venous blood from each healthy control and patient and added to the gel tube ,which was then left at room temperature for 30minutes in order to initiate the clotting process ,the sample was then centrifuged to separate the serum at 3,000xg for 15 minutes and the obtained serum were aspirate using mechanical micropipette and transferred in to eppendrof tubes and stored at-20C for determination of cholesterol, triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein(LDL),very low density lipoprotein by bio chemical colorimetric methods and determination of human von Willebrand Factor by enzyme-linked immunosorbent assay (ELISA).

RESULTS

The study confirmed that the lowest mean of Human Endothelial Nitric Oxide Synthase (eNOS) was detected among IHD patients (2.41±0.74 ng/ml) and the maximum mean was within the control group (5.15±1.24 ng/ml). The differences was highly significant (P-value: 0.0001), Table 1.

Table 1: Mean level of eNOS in IHD patients and the control group

eNOS (ng/ml)	Mean	SD	Minimum	Median	Maximum
IHD patients	2.41	0.74	1.1700	2.2640	3.9200
Control group	5.15	1.24	2.864	3.128	6.849

P-value: 0.0001

The study confirmed that the peak mean of lactate dehydrogenase was detected among IHD patients (257.8±94.9 IU/L) and the deepest mean was in the control group (168.9±16.8 IU/L). The differences was highly significant (P-value: 0.0001), Table 2.

Table 2: Mean level of LDH in IHD patients and the control group

LDH (IU/L)	Mean	SD	Minimum	Median	Maximum
IHD patients	257.8	94.9	86.9	257.5	488.0
Control group	168.9	16.8	140.20	169.25	198.60

P-value: 0.0001

The study showed that the mean eNOS was 2.17 ng/ml in patients with hypertension, which was significantly lower than that in non-hypertensive IHD patients (2.76 ng/ml), at a P value of 0.024 (Table 3).

Table 3: Comparison between hypertensive and non-hypertensive IHD patients regarding the level of eNOS

Variable	Hypertension	No.	Mean	SD	P-value
eNOS (ng/ml)	Absent	24	2.76	0.73	0.024
	Present	36	2.17	0.77	

The study showed that the mean LDH was 284.6 IU/L in patients with hypertension, which was significantly more than in non-hypertensive IHD patients (217.5 IU/L), at a P value of 0.001 (Table 4).

Table 4: Comparison between hypertensive and non-hypertensive IHD patients regarding the level of LDH

Variable	Hypertension	No.	Mean	SD	P-value
LDH (IU/L)	Absent	24	217.5	69.8	0.001
	Present	36	284.6	100.6	

DISCUSSIONS

The current study mentioned found that the mean level of human endothelial nitric oxide synthase (eNOS) was significantly lower in patients with Ischemic Heart Disease (IHD) compared to the control group. Specifically, the study showed that the lowest mean level of eNOS was detected among IHD patients, while the maximum mean level was observed in the control group. The present study highlights the importance of eNOS measurements in in IHD patients. Some studies show a reduced level of eNOS activity among patients with heart disease [1, 2].

The major decrease levels of eNOS expression in various cardiovascular disorders, such as heart failure, cardiomyopathies, and arteriosclerosis has been well documented [3]. Specifically, Zakula *et al.*, [4] found an increase in iNOS expression and a decrease in eNOS expression is associated with atherosclerosis. In results of different studies indicated and reduced eNOS level in CAD patients was accompanied by elevated iNOS level in the same group, which is a well-known predictor of cardiovascular disorders [5]. Moreover, Liu *et al.,* [6] focused on the reduction of eNOS polymorphism among CAD, and this polymorphism might be a marker for the risk evaluation of CAD. The expression of eNOS lead to endothelial NO plays a protective role exerted in the vascular wall against oxidative stress [7].

The eNOS is an enzyme that plays a critical role in the production of nitric oxide (NO) in endothelial cells, which helps to regulate blood flow and blood pressure. Nitric oxide also has anti-inflammatory properties and can help prevent the formation of blood clots. The finding of lower eNOS levels among IHD patients is significant, as it suggests that there may be a dysfunction in the nitric oxide pathway that contributes to the development of IHD. Previous studies have also reported lower levels of eNOS in patients with various forms of cardiovascular disease, including hypertension, coronary artery disease, and heart failure [8-10]. The lower eNOS levels in IHD patients could be due to various factors, including genetic factors, lifestyle factors, and environmental factors. For example, genetic polymorphisms in the eNOS gene have been associated with reduced eNOS activity and an increased risk of cardiovascular disease [11, 12]. Lifestyle factors such as smoking, physical inactivity, and a poor diet can also contribute to endothelial dysfunction and reduced eNOS activity [13]. The finding of higher eNOS levels in the control group is expected, as the control group was composed of individuals without known cardiovascular diseases or risk factors. This suggests that a normal functioning nitric oxide pathway may be protective against the development of IHD.

The study confirmed that the peak mean of lactate dehydrogenase was detected among IHD patients and the deepest mean was in the control group. Lactate dehydrogenase (LDH) is an enzyme that plays a key role in the body's energy production process. When there is damage or injury to cells, the LDH levels in the blood can increase. The study findings suggest that LDH levels are significantly higher in patients with ischemic heart disease (IHD) than in healthy individuals. The peak mean of LDH observed in IHD patients in the study was 257.8±94.9 IU/L, which was significantly

higher than the deepest mean of 168.9±16.8 IU/L observed in the control group. The difference in mean LDH levels between the two groups was highly significant with a p-value of 0.0001. This suggests that elevated LDH levels may be a marker of IHD and could potentially be used to diagnose or monitor the condition. For instance, a study by Kopel *et al.*, [6] found that LDH levels were significantly higher in patients with acute coronary syndrome (ACS) compared to healthy controls. Similarly, a study by Koseler *et al.*, [15] found that LDH levels were significantly higher in patients with coronary artery disease (CAD) compared to healthy controls. The elevated levels of LDH in IHD patients may be due to the damage to heart tissue caused by reduced blood supply and oxygen, leading to the release of LDH into the bloodstream. This damage can cause LDH to leak from the heart cells into the circulation, leading to an increase in serum LDH levels [16]. LDH levels can rise in response to a variety of factors such as tissue damage, inflammation, and stress. In the case of IHD, the higher LDH levels observed may be due to the damage to heart tissue caused by reduced blood supply and oxygen. This damage can lead to the release of LDH into the bloodstream, resulting in elevated levels [17]. Monitoring LDH levels in patients with IHD could be useful in assessing the severity of the condition and evaluating treatment effectiveness. However, it is important to note that LDH levels can also be elevated in other conditions such as liver disease, kidney disease, and certain types of cancer. The study showed that the mean eNOS was 2.17 ng/ml in patients with hypertension, which was significantly lower than that in non-hypertensive IHD patients (2.76 ng/ml), at a P value of 0.024. Endothelial nitric oxide synthase (eNOS) is an enzyme that plays a critical role in regulating vascular tone and blood pressure. It produces nitric oxide (NO), which acts as a vasodilator and helps to maintain normal blood flow. Previous studies have suggested that reduced eNOS activity was associated with hypertension and other cardiovascular diseases [13, 14]. This finding suggests that there may be a link between eNOS levels and hypertension. One potential explanation is that reduced eNOS activity leads to impaired NO production, which may contribute to the development of hypertension. NO helps to dilate blood vessels, which in turn reduce blood pressure. When eNOS activity is reduced, NO production may also be reduced, leading to impaired blood vessel function and an increase in blood pressure [2]. The studies mentioned by the prompt provide evidence for the relationship between hypertension and reduced levels of eNOS. Firoenza *et al.*, [16], Pan *et al.*, [17], Nyberg *et al.*, [18] and Hansen *et al.*, [19] all found that hypertensive individuals had lower levels of muscle eNOS and plasma NO compared to normotensive individuals. However, it is important to note that the relationship between eNOS levels and hypertension is complex and may involve multiple factors. For example, oxidative stress and inflammation may also contribute to the development of hypertension, and these factors can also impact eNOS activity [20]. However, one study found that patients with acute myocardial infarction (AMI), a type of IHD, had lower eNOS levels compared to healthy individuals. However, there was no significant difference in eNOS levels between hypertensive and non-hypertensive AMI patients [21].

In agreement with our research, one study has shown that the LDH correlates positively in the male hypertensives [1]. Sakao *et al.*, [22] and Cottrill *et al.*, [23] showed that pulmonary arterial hypertension have high levels of LDH than healthy individuals and which suggested that pulmonary smooth muscle and endothelial cells may be the sources of the increased LDH. The higher levels of LDH in hypertensive IHD patients may indicate greater damage or inflammation to the heart muscle. Hypertension can lead to increased stress on the heart and blood vessels, which can contribute to the development and progression of IHD. The resulting tissue damage or inflammation may lead to increased LDH levels in hypertensive IHD patients [24].

CONCLUSIONS

In conclusion, the findings of the study suggest that patients with ischemic heart disease (IHD) have reduced levels of eNOS**.** More researches are needed to fully understand the relationship among eNOS and cardiovascular disease and to determine the clinical utility of its measuring in the diagnosis and management of these conditions.

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