

## Emerging Roles of Acid Ceramidase, Nitric Oxide Synthase, and C-Reactive Protein in Patients with Ischemic Heart Disease

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### Abstract

Myocardial infarction is one of the most frequently encountered reasons for hospital admission and is commonly seen in all populations worldwide. Myocardial infarction refers to the interruption of blood flow to a coronary artery of the heart. It usually occurs due to the occlusive thrombus formation at the site of rupture or erosion of an atheromatous plaque in the coronary artery causing the heart cell to die without treatment. The purpose of this study was to examine the effects of heart ischemia on some biological parameters. A total number of 120 participants were included in this study, 60 of whom were patients suffering from the acute coronary syndrome, and 60 were healthy individuals who served as control. The patients were admitted to Imam Al-Hussein Medical City, Karbala, Iraq, for cardiac diseases and surgery. Blood samples were obtained from all patients, and serum was separated after centrifugation of blood at 3000 rpm for 10 minutes to measure Human high sensitivity C-reactive protein (hs-CRP), Human acid ceramidase (AC), and Human nitric oxide synthase (eNOS3). The study findings revealed a significant increase of hs-CRP ( $P < 0.05$ ) in the acute coronary syndrome patients, compared to the healthy group with the means of  $36.56 \pm 7.18$  and  $16.21 \pm 1.09$ , for patients and the healthy group, respectively. There was a significant decrease ( $P < 0.05$ ) in AC of acute coronary syndrome patients when compared to the healthy group. Acute coronary syndrome patients indicated a significantly higher Serum eNOS3 concentration ( $P < 0.05$ ), compared to the healthy group. The high serum eNOS3 and hs-CRP concentrations as well as a decrease in AC, indicate a lesion in the heart, which necessitates good care of such patients.

**Keywords:** Ischemic heart disease, Acid ceramidase, Nitric oxide synthase

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### 1. Introduction

Myocardial infarction is commonly caused by coronary artery disease. According to a report by WHO, 32% of worldwide deaths originated from ischemic heart disease (IHD) in 2022 as the leading cause of death in high- or middle-income countries and second only to lower respiratory infections in lower-income countries [1]. The IHD death rates have declined in most high-income countries although cardiovascular disease still accounts for one in every three deaths in the USA [2]. In contrast, IHD is becoming a more common cause of death in the developing world [3]. The first diagnosis is to evaluate the symptoms and signs to identify patients with unstable angina or other acute coronary syndromes [4]. To diagnose angina, the medical history of patients plays a significant role. However, there is a need for the diagnosis to be accompanied by a physical examination, objective tests for

confirmation, and determination of the severity of the underlying disease [5]. Location, nature, and length, as well as other exacerbating or alleviating factors, are the four categories of discomfort associated with myocardial ischemia (angina pectoris). Myocardial ischemia causes pain in the chest, near the sternum, but it can also be felt anywhere in the body, from the epigastrium to the lower jaw or teeth, between the shoulder blades, or in either arm to the wrist and fingers [6]. Several factors may increase the probability of a person developing chronic heart disease (CHD). Individuals with CHD may be at risk of cardiac event until after 10 years, peripheral arterial disease, abdominal aortic aneurysm, or carotid artery disorder. Some people do not have common risk factors, but elevated low-density Lipoprotein (LDL) cholesterol is the primary goal of cholesterol-lowering therapy [7].

Age is considered a risk factor for acute heart disease. In developing countries, people over 48 years old are at a higher risk of cardiovascular disease [8]. In another study, only 4 to 5 cases of CHD death were recorded among a big community of elderly people over 65 years. Aging may affect heart function due to physiological changes such as the thickness of heart walls, stiffened arteries, and reduced blood pumping capacity [9].

Some forms of cardiovascular disease (CVD), including IHD, are more common in people with a positive family history [9]. Several studies have indicated that family history is an independent risk factor for IHD [10, 11, 12]. Smoking is another risk factor for CVD, which can be controlled. One year of smoking cessation may decrease the chance of CVD, compared to those who smoke. If a heart attack occurs, the possibility of death is more remarkable for smokers than non-smokers, so smoking cessation programs are effective for individuals with coronary artery diseases using available community resources [13]. Acid ceramidase (AC) is a N-acylsphingosine deacylase with a pH optimum in the acidic range. The enzyme is found in the lysosomal and endosomal compartments of the cell. The enzyme is largely involved in the breakdown of ceramide. In humans, a hereditary lack of lysosomal AC causes Farber disease, a lysosomal lipid storage condition [14]. Acid ceramidase regulates several cellular activities by hydrolyzing lysosomal membrane ceramide into sphingosine, the backbone of all sphingolipids. Ceramides are one of the most important bioactive membrane lipids, controlling signal transduction pathways that determine whether a cell lives or dies [15]. Ceramides accumulate in atheromatous plaques in the coronary arteries [16] and their glycosylated forms, glucosylceramides, and lactosylceramides, are higher in arterial tissue with apparent plaque formation [17]. Ceramides are produced by the myocardium in response to ischemia and reperfusion, and these ceramides induce mitochondrial autophagy and apoptosis. Since plasma ceramides are now easily measurable, it is possible to investigate the link between ceramides and cardiovascular death in stable and unstable CAD cohorts [18].

Endothelial Nitric Oxide synthase (eNOS3) produces nitric oxide (NO), which is vital for coronary blood flow modulation, vascular resistance reduction, and platelet aggregation and adhesion inhibition, all of which help to prevent coronary circulatory failure, thrombosis, and atherosclerosis [19]. Endothelial function can be affected by several pathologic factors, including smoking, chronic alcohol consumption, hypercholesterolemia, obesity, hyperglycemia, and hypertension. Reduced Nitric Oxide synthase (eNOS3) expression and activity, decreased NO bioavailability, increased generation of oxygen radicals, and endogenous (eNOS3) inhibitors are among the causes of endothelial dysfunction. Endothelial dysfunction appears to be linked to atrial fibrillation. In humans, endothelial dysfunction is a significant predictor of coronary artery disease. Ischemic heart disease is thought to be highly predictive of penile erectile dysfunction, linked to reduced bioavailability of NO generated by eNOS3 and neuronal NOS [20].

The NO inhibits smooth muscle contraction and platelet aggregation, as well as contributing to angiogenesis and cytoprotection in the heart, making it an essential modulator of coronary blood flow. The DASH approach to stop *Al-Atrakhey et al., 2023*

hypertension decreases blood pressure and significantly lowers coronary heart disease risk. The DASH diet improves endothelial function in hypertensive and obese hypertensive patients [21].

## 2. Materials and methods

The study was carried out on patients suffering from ACS admitted to Imam Al-Hussein Medical City, Karbala, Iraq, for cardiac diseases and surgery from March 2021 till the end of December 2021. The diagnosis of the symptomatic ACS was made by the cardiologists depending on the presence of typical symptoms, electrocardiogram (ECG), and biomarkers. A total of 60 patients were included in this study, among whom 20 were females and 40 were males within the age range of 24-80 years. The patients with ACS had a history of pain in the chest, one or both arms, the jaw, neck, back, or stomach, as well as shortness of breath, nausea, and sweating. The control group in the current study included 60 healthy individuals of both sexes. After approval by the Ethical Committee of the College of Medicine, Karbala, Iraq, blood samples were obtained from all patients. Venous blood, about (5 ml) was withdrawn from each patient who participated in this study. Then, blood was collected in gel tubes (EDTA-free) and used for serum separation after centrifugation at 3000 rpm for 10 minutes to measure Human high sensitivity C-reactive protein (hs-CRP), Human acid ceramidase (AC), and Human nitric oxide synthase (eNOS3).

## 3. Results and Discussions

### Smoking Status

Regarding the smoking history of IHD patients, 20 (33%) were smokers, and 40 (67%) were non-smokers (Figure 1).

### Human High Sensitivity C-reactive protein

Serum hs-CRP concentration significantly increased ( $P < 0.05$ ) in all IHD patients, compared to the control group (Figure 2).

### Human Acid Ceramidase

Serum Acid ceramidase concentration was significantly lower in IHD patients, compared to control group ( $P < 0.05$ , Figure 3).

## 4. Human Nitric Oxide Synthase

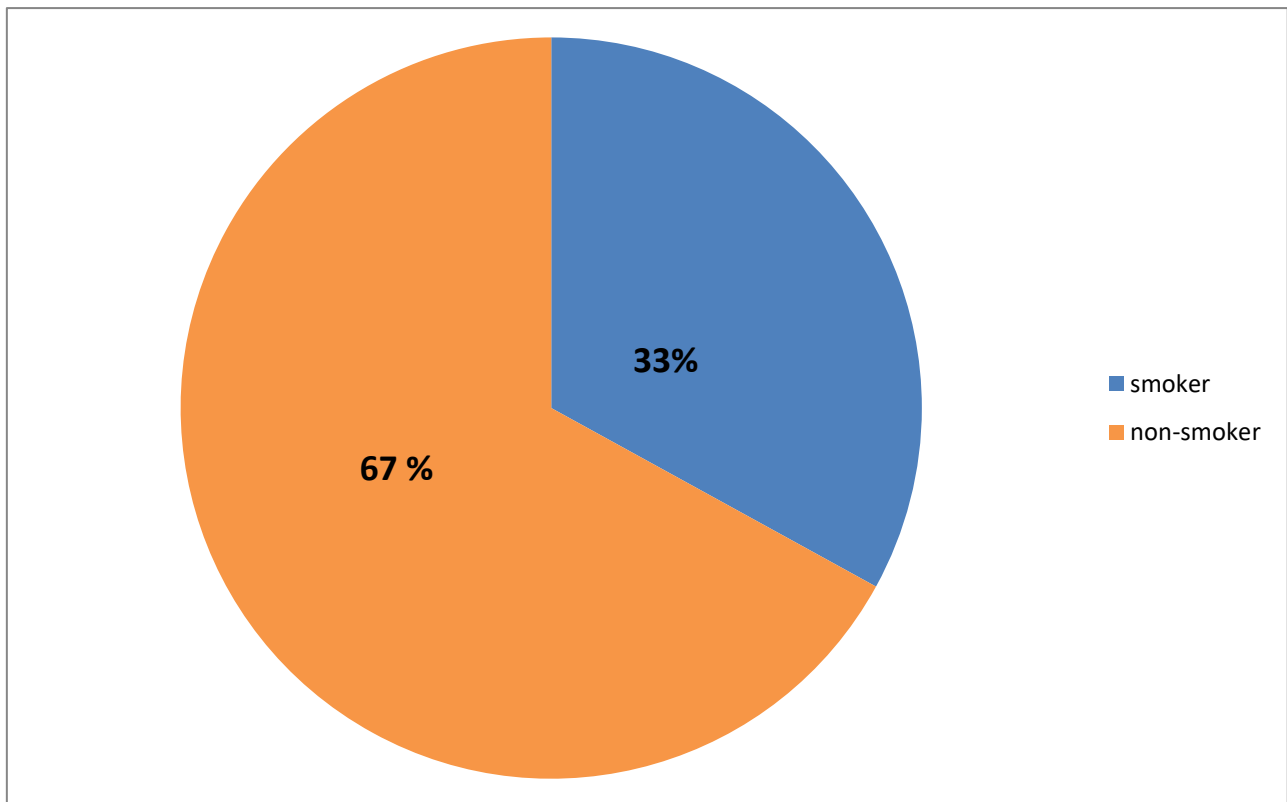
The Serum NOS3 concentration significantly increased ( $P < 0.05$ ) in IHD patients, compared to the control group (Figure 4).

Patients with IHD were divided into two groups, including non-smokers (33%) and smokers (67%). This finding was supported by other studies indicating a relationship between alcohol and tobacco use and high blood fat level leading to CHD [22, 23]. Smoking cigarette is a significant health risk factor for CHD, stroke, peripheral vascular disease, and congestive heart failure [24]. For both men and women, smoking is a significant risk factor for stroke, particularly cerebral infarction [25]. Cigarette smoke induces

cerebrovascular and cardiovascular disorders through various synergistic mechanisms [26], including thrombosis, endothelial dysfunction, atherosclerosis, and hemodynamic consequences [27]. The results indicated that the serum Hs-CRP concentration increased significantly in all IHD patients, compared to the control group. Coronary artery disease is one of the leading causes of morbidity and mortality worldwide, accounting for approximately one-third of all cases. Activation and aggregation of platelets, thrombus formation, and subsequent infarction are all characteristics of this condition [28]. According to various studies, at least 250 factors are associated with the development of coronary artery disease, including aging, elevated hs-CRP smoking, HbA1c, diabetes, hypertension, and hyperuricemia. [29]. There was a significant difference between the IHD patients and the control group in terms of serum acid ceramidase concentration ( $P < 0.05$ ). Cardiac disease, diabetes, heart failure, and other cardiometabolic disorders root in a global pandemic of calorie consumption and inactivity. They raise circulating lipid levels, which can accumulate in non-adipose

tissues, such as blood vessel walls and the heart. Deficiencies in lipid metabolism lead to cellular dysfunction and death. Ceramides, for example, are thought to be responsible for much of the tissue damage in these cardiometabolic disorders. Human serum ceramide levels are reliable indicators of cardiovascular disease. One of the most important reasons for the low concentration of enzymes in patients is inhibiting or depleting enzymes involved in ceramide production, which is protected against diabetes, atherosclerosis, hypertension, and heart failure in humans [16].

A significant difference was found between IHD patients and the control group regarding serum eNOS3 levels ( $P < 0.05$ ). The release of mediators, such as nitric oxide, by the vascular endothelium plays a critical role in the progression of the atherosclerotic process in the body. Endothelial homeostasis is influenced by nitric oxide, which suppresses platelet aggregation, leukocyte adhesion, smooth muscle cell migration, and proliferation [30].



**Figure 1.** Smoking status of ischemic heart disease patients

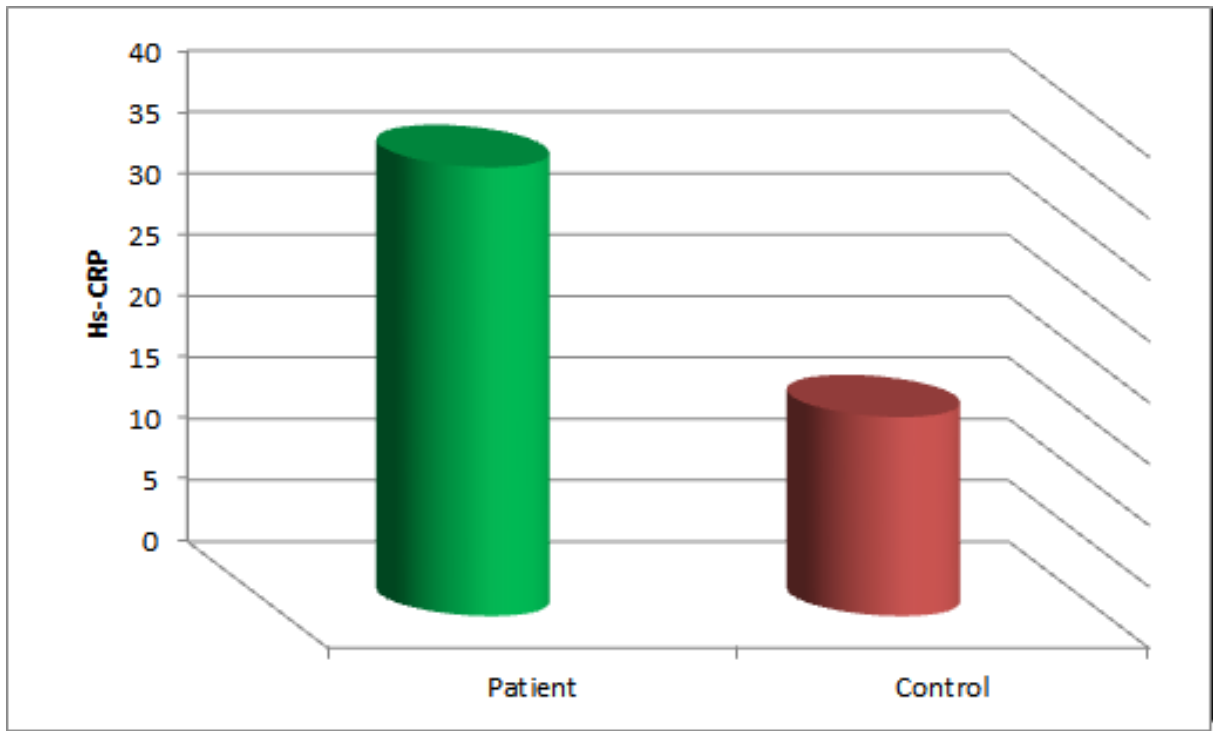


Figure 2. Serum levels of Hs-CRP in ischemic heart disease patients and control group

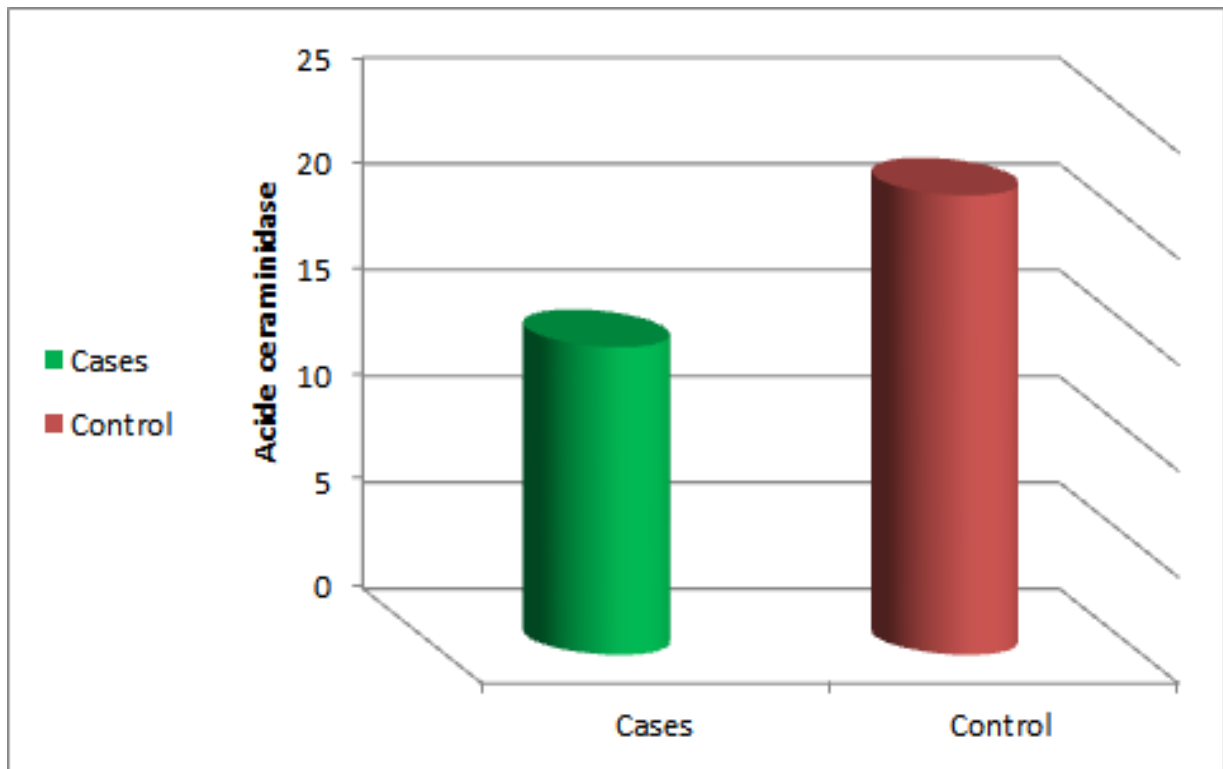
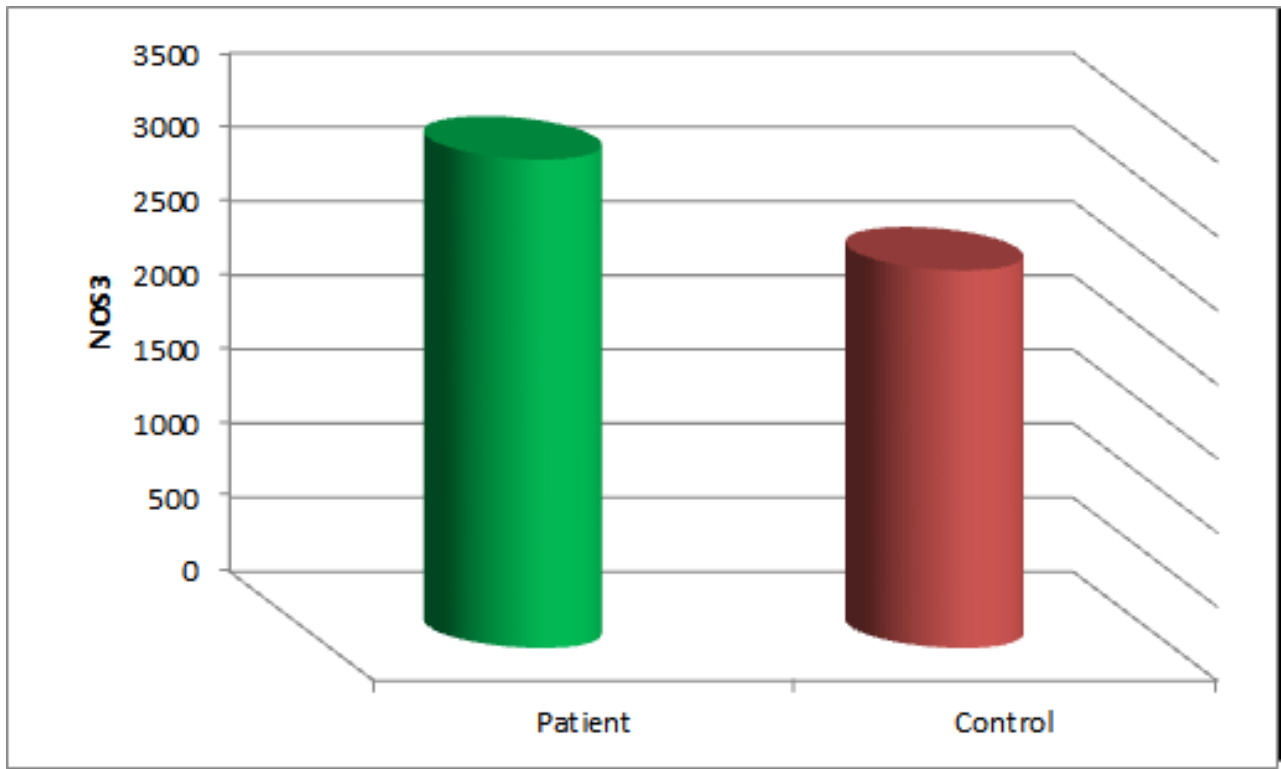


Figure 3. Serum levels of acid ceramidase in ischemic heart disease patients and control group



**Figure 4.** Serum levels of eNOS3 in ischemic heart disease patients and control group

#### 4. Conclusions

In conclusion, nitric oxide synthetase, serum acid ceramidase, and C- reactive protein are the most common indicators for patients with ischemic heart disease. There was a significant decrease in AC of acute coronary syndrome patients. Acute coronary syndrome patients indicated a significantly higher Serum eNOS3 concentration compared to the healthy group. The high serum eNOS3 and hs-CRP concentrations as well as a decrease in AC, indicate a lesion in the heart, which necessitates good care of such patients.

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