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# Evaluation of osteonectin in cardiovascular patients

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**ABSTRACT**— Cardiovascular disease (CVD) is a non - communicable disease chronic disease that is one of the leading causes of death and disability. CVD incidents are becoming more common over the world. Atherosclerosis is the leading cause of CVD, which encompasses coronary heart disease, vascular disease, rheumatic heart disease, heart attack (MI), stable angina (SA), unstable angina (UA), as well as other disorders, according to the World Health (WHO). A total of 50 samples were used in this investigation, with 20 samples from the treatment group and 30 samples of patients with cardiovascular disorders. The levels of urea, creatinine, and osteonectin were measured in individuals with cardiovascular disease as well as a control group. That research reveals a link between osteonectin with age, as well as creatinine. Inside the current investigation, individuals with cardiovascular illnesses had a significant ($p<0.05$) increase in urea, creatinine, and osteonectin concentrations when compared with the control group. In addition, researchers discovered a link among osteonectin with age, and also creatinine, in individuals with cardiovascular disease. This study showed that patients suffering cardiovascular disorders have higher levels of bone biomarkers (osteonectin) and that cardiovascular diseases have an effect on kidney function.

**KEYWORDS:** Cardiovascular diseases, osteonectin, Urea, Creatinine

## 1. INTRODUCTION

Cardiovascular disease (CVD) is a non - communicable disease chronic illness which is one of the leading causes of mortality and morbidity. CVD incidents are more common over the world. It is also the biggest cause of death in Iran, accounting for 50% of all death including 79 percent of mortality for chronic diseases [1]. Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for 17.8 million premature deaths in 2012 [2]. CVD killed one in every five people in South Korea in 2014, while the total prevalence of atherosclerotic CVD was 101.11 per 1000 people in 2015. Individual CVD risk factors, such as health-related behaviors or biological variables, are very well understood. In the meantime, earlier research has sought to determine the risk factors for CVD at the social and community levels, in addition to the person level. In recent years, social capital has been identified as one of the most important social determinants of health among these community characteristics [3]. Physical inactivity, obesity, ageing, poor diet, smoking, abnormal lipids, and hypertension are all risk factors for cardiovascular disease [4]. In Greenland, more than half of the population smokes [5], while physical inactivity is far more prevalent. As a result of a social shift from a traditional active lifestyle to a modern sedentary lifestyle with availability to processed high-calorie foods, we now live in modern times [6]. Obesity is on the rise around the world, particularly in developed countries. It contributes to an increase in CVD morbidity and mortality. Obesity is caused by a sedentary lifestyle and poor eating habits. Obesity is linked to CVD mortality. Body mass index (BMI), obesity, or particularly abdominal thickness are all linked to total mortality. The lowest

BMI mortality rate is between 20 and 25 kg/m2 [7]. Lipids (cholesterol and triglycerides) travel in the bloodstream as lipoproteins, which are proteins (apolipoprotein). LDL cholesterol (atherogenic) accounts for the majority of circulating cholesterol and is directly linked to CVD risk. Hypercholesterolemia indicates a higher risk of heart disease. Total LDL cholesterol has a link to cardiovascular disease [8]. The function of triglyceride-rich lipoprotein is currently being investigated. Chylomicrons and very low-density lipoproteins (VLDL) are two types of chylomicrons and VLDL that can cause pancreatitis if present in excessive amounts [7]. Atherosclerosis is a chronic inflammatory disease that causes death in people all over the world. This clinical progression of atherosclerosis to myocardial infarction or stroke was attributed the thrombotic events associated to abrupt rupture or erosion of an unstable plaque, rather than narrowing the lumen [9]. Foam cell lesions are the first sign of atherosclerosis progressing. The retention of apo B-containing lipoproteins, especially low-density lipoprotein, to extracellular proteoglycans in the artery intima appears to be a significant starting process in atherosclerosis [10]. Angina pectoris is a clinical syndrome marked by a lack of blood supply to the coronary arteries, myocardial ischemia, and hypoxia, as well as episodic chest discomfort or pain [11]. This disease is most common in men over the age of 40 [12]. Angina pectoris is caused by a lack of blood flow to the heart, which can be caused by a variety of reasons including decreased myocardial blood (blood oxygen) supply (such as intravascular 6 thrombosis, vasospasm) and increased oxygen consumption (such as exercise, higher heart rate) [13].

Myocardial infraction (MI) is defined as the death of myocardial cells as a result of persistent ischemia. When there is evidence of myocardial necrosis in a clinical situation, consistent to acute myocardial ischemia and an increase in cardiac troponin, the term MI be employed [14]. Another definition for MI is occlusive coronary thrombosis, which occurs when blood gets exposed to a content of atherosclerotic plaques. Myocardial infarction is a common cardiovascular event that causes morbidity and mortality all around world [15]. Osteonectin is a 32-kDa calcium-binding matricellular protein also known as SPARC (Secreted protein acidic and rich in cysteine) or BM-40 (Basement membrane protein 40 [16]. SPARC expression is usually quite similar to that of fibrillar collagens such collagen type I. Collagen type I (94%) and a number of non-collagenous proteins make up the protein component of the pre-mineralized bone matrix (osteoid). The mineral content of the osteoid in the form of hydroxyapatite (HA) - a combination of calcium and phosphate that mineralizes the collagenous matrix - is largely responsible for the mechanical qualities of bone [17]. SPARC is a secreted, monomeric, glycosylated polypeptide that is expressed by a single gene. SPARC is divided into four distinct domains: 1) a mineral binding region in the N-terminal low-affinity, high-capacity calcium-binding domain, 2) a cysteine-rich domain, 3) a hydrophilic region, and 4) an extracellular Ca2+ (EC) domain with an E-F hand motif at the C-terminus that includes the collagen binding domain. Two alpha helices and one short loop region make up the helix-loop-helix structure (EF hand motif), which is seen in some calcium-binding proteins [18]. Other cell types found in mineralized tissues, such as endothelial cells and fibroblasts, produce SPARC [19].

## 2. Patients and Method

### *2.1 Subjects*

This research was carried out in Iraq's Karbala Center for Cardiac Diseases and Surgery. In addition to the control group, serum samples were taken from individuals with cardiovascular disorders. The samples tested were (50) samples, with (20) samples from of the treatment group and (30) samples from cardiovascular disease patients.

### *2.2 Collection of blood samples*

5 milliliters of blood were taken from the vein using sterilized synergies. The sample was placed in two

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labeled tubes, one of which contains EDTA as an anticoagulant to prevent blood clotting during physiological investigations. The second set of tubes were anticoagulant-free gel tubes for blood that would be used to prepare serum for biochemical and biomarker analysis. Blood was allowed to clot for 10 minutes at room temperature before being centrifuged at 6000 rpm for 10 minutes, after which serum was extracted and frozen at -80 C until the laboratory analysis for the study could be completed.

### *2.3 Determination of blood urea concentration*

The concentration of urea in the serum was determined using the colorimetric technique. Present urease converts urea to ammonium in the Berthelot reaction. This ammonium reacts with alkaline hypochlorite, sodium salicylate, and sodium nitroprusside to create indophenol, which is green in color (2.2-dicarboxylindophenol). The intensity of the hue is proportional to the amount of urea there in serum (Fawcett and Scott, 1960; Patton and Crouch, 1977)

### *2.4 Determination of serum creatinine concentration*

The concentration of creatinine in the serum was determined using a colorimetric reaction. Creatinine complexes with picric acid in an alkaline solution forms a yellow organ complex in the Jaffe reaction. Photo metrically, the colored complex is determined. The color intensity is proportional to the serum creatinine levels (Labbe et al., 1996; Fabiny, and Ertingshausen, 1971)

### *2.5 Instructions for the Human Osteonectin (ON) ELISA Kit*

Elabscience Biotechnology provided a specific kit for measuring human Osteonectin amounts in serum.

## 3. Statistical Analysis

Using the SPSS software, the data was statistically evaluated (SPSS, Version 23). The descriptive analyses of means and standard Error between the patients and control groups were conducted using the T-test. The correlation coefficient was calculated to estimate the correlation between markers and parameters, and Pearson correlation and one-way ANOVA by LSD were used to compare the subdivided groups in the measured parameters. The graphs were created with Microsoft Office 2016's excel software. All of these were statistically significant at the P>0.05 level.

## 4. Results

### *4.1 Biochemical studies*

#### *4.1.1 Comparison the concentration of Urea in patients with cardiovascular diseases and control group*

The results of current study, that indicated a significant increase ($p< 0.05$) in the urea (42.27±3.2368) in patients with cardiovascular diseases relative to the control groups (26.79±0.61), shown in figure (1).

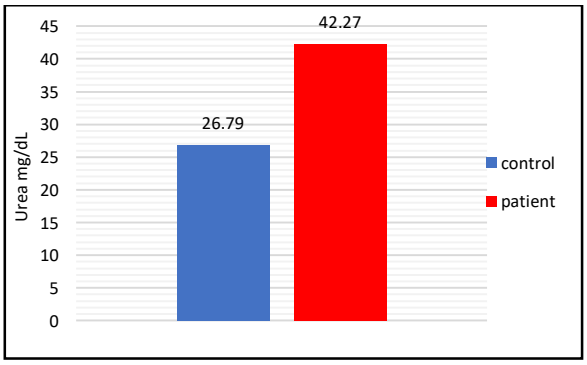


**Figure 1:** Comparison of urea level between patients with cardiovascular diseases and control group

### *4.1.2 Comparison the concentration of Creatinine in patients with cardiovascular diseases and control group*

The results of this study, that revealed a significant increase ($p< 0.05$) in creatinine concentration (0.956±0.043) in patients with cardiovascular diseases compared to the control group (0.68 ±0.023), shown in figure (2).

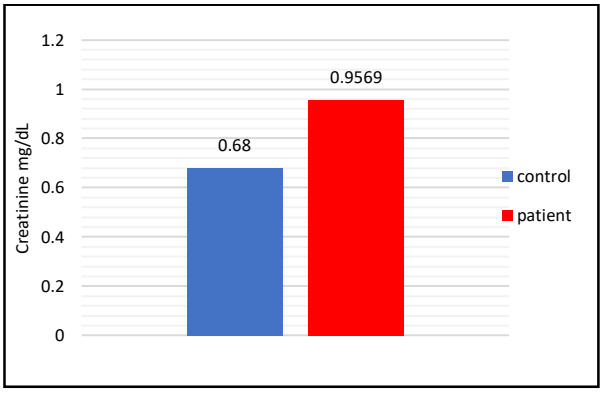


**Figure 2:** Comparison of creatinine concentration between patients with cardiovascular diseases and control group

### *4.1.3 Comparison the concentration of osteonectin in patients with cardiovascular diseases and control group*

The results of current study, that indicated a significant increase ($p< 0.05$) in the osteonectin concentration (0.962 ± 0.046) in patients with cardiovascular diseases compared to the control groups (0.806± 0.075), shown in figure (3-3).

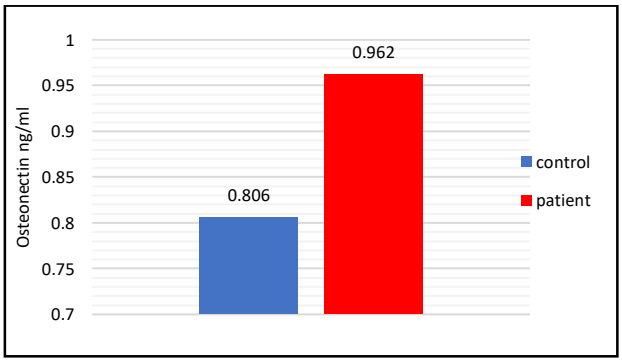


**Figure 3:** Comparison of osteonectin concentration between patients with cardiovascular diseases and control group

## 5. Correlation

### *5.1 Correlation between the Age and Osteonectin*

The result in current study indicated the non-significant positive correlation (r=0.098) between the osteonectin concentration and Age in patients with cardiovascular diseases, shown in figure (4).

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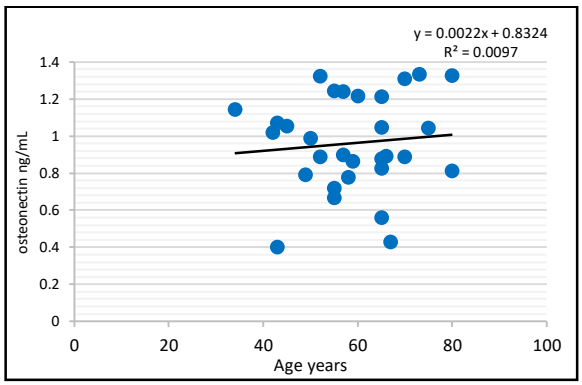


**Figure 4:** Correlation between the Osteonectin and Age

### *5.2 Correlation between the creatinine and Osteonectin*

The result in figure (3-5) indicated the significant positive correlation (r=0.375) between the Osteonectin concentration and creatinine concentration in patients with cardiovascular diseases.

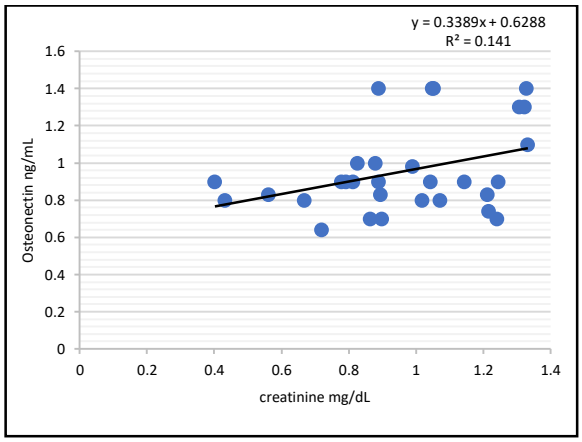


**Figure 5:** Correlation between the Osteonectin and Creatinine

## 6. Discussion

The current study's findings, which showed a significant increase ($p < 0.05$) in urea in patients with cardiovascular diseases compared to control groups, had been supported by a study [20], which found that BUN levels are high in patients with CVD and this biomarker is just an important independent predictor of CVD outcomes when compared to other markers of renal function. BUN levels are influenced not just by kidney function but also by endocrine diseases, according to researchers. BUN is a neurohumoral activity and renal function marker that can thus represent the pathophysiology of CVD. Higher BUN is related with worse outcomes for patients with CVD, including acute decompensating and chronic stable CVD, indicating that the intricate interplay between the heart and kidneys plays a key role in the occurrence and development of CVD. Researchers believe BUN is a measure of neurohormone-activated "renal response," and so plays a role in the pathophysiology of CVD [21]. The kidneys expel waste nitrogen mostly as urea (90%), and there are numerous ways for enhancing urea reabsorption in heart failure patients [21]. Increased activity of the renin-angiotensin-aldosterone system and the sympathetic nervous system improves salt and water absorption while also causing passive ("concentration dependent") urea reabsorption in the proximal tubules. Additionally, these activities facilitate "flow dependent" urea reabsorption in the collecting duct [22], which is greatly enhanced in the presence of arginine vasopressin (AVP) via the urea transporter, and AVP-mediated up regulation of such transporters will enhance this process. BUN is not a good indicator of renal function since it is regulated by protein intake, catabolism,

and tubular reabsorption. However, BUN increase may be a symptom of severe HF, which includes reduced cardiac output and an active neurohormone system [23]. This study's findings, which revealed a significant increase (p 0.05) in creatinine concentration in patients with cardiovascular diseases compared to the control group, were also consistent to [24], which suggested the etiology of acute kidney injury (AKI) through patients with congestive heart failure (ADHF), Apart from reduced cardiac output, is multifactorial and complex. AKI development has been linked to heart failure medicines, according to several studies. Diuretics are still the most common treatment for ADHF patients; however, they can cause a considerable reduction in renal perfusion, worsening renal function. Furthermore, given the etiology of HF, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers are crucial for treating ADHF patients, however the link between these medications and increased Cr levels has been widely described. Clinically, there are five different forms of cardio renal syndrome (CRS). Acute kidney damage is the result of a rapid decrease of cardiac function in CRS type 1 [25]. The incidence of AHF following an acute myocardial infraction (AMI) was found to be 32.4 percent [26]. The BUN, Cr, and BUN/Cr levels are now considered as markers of renal function [27]. Studies have revealed that even when the Cr level has increased significantly, continuing decongestion therapy for patients with AMI complicated by AHF for diuresis and other reasons is advised for patients with lower BUN/Cr [28].

Furthermore, the findings of this study, which showed a significant increase ($p< 0.05$) in osteonectin concentration in patients with cardiovascular diseases compared to control groups, were consistent with the findings of another study by [29] which found that increased osteonectin concentration was linked to a higher prevalence of intimal arterial calcification (IAC). IAC and d medial arterial calcification (MAC) have yet to be fully understood mechanisms. IAC represents atherosclerotic plaque burden, which might be caused by vascular inflammation, whereas MAC could be caused by mechanisms comparable to bone formation but without the inflammatory component [30]. That role of osteonectin is yet unknown, however our findings suggest that it could be used as an indication of IAC. Vascular smooth muscle cells (VSMC) make up the majority of the artery's medial layer [31]. [32] proposed that osteonectin can regulate the calcification process and has a potentiating effect on mitosis and cell differentiation regulation [33]. The findings show a link between circulating levels of the SPARC family member OSN and higher 3-year combined cardiovascular events (CHF) related death, all-cause mortality, and readmission owing to CHF) in patients with ischemic-induced symptomatic heart failure. OSN was recently not defined as a biological marker with potential predictive value in heart failure patients, despite its well-established role as a surrogate biomarker of atherosclerosis and cardiovascular remodeling. According to the aforementioned study, patients with cardiovascular problems have higher levels of osteonectin than those without. The correlation of this study found the non-significant positive correlation ($r=0.098$) However, there was a substantial positive association between osteonectin concentration and creatinine concentration in individuals with cardiovascular illnesses, which was not found in healthy people. The extracellular matrix glycoprotein osteonectin is involved in cell–matrix interaction. Overexpression of osteonectin prevents C2C12 muscle cells from differentiating [34]. As a result, people with myopathies have higher levels of osteonectin expression [35]. Reduced muscle mass in sarcopenia is negatively associated to the level of circulating osteonectin, suggesting a link with aging [36]. There is evidence that OSN levels rise with age [37]. [38], SPARC family member osteonectin (OSN) causes myocardial hypertrophy, increased fibrillar collagen content, stimulates cell signaling, adhesion, survival, proliferation, and migration in several cell types, mediates vascular wall calcification, coagulation, and endothelial dysfunction, according to the findings of this study. According to a study, even when the Cr level has increased significantly, continuing decongestion therapy for patients with AMI complicated by AHF for diuresis and other reasons is advised for patients with lowered BUN/Cr [28]. According to this research, osteonectin and creatinine have a beneficial association in cardiovascular patients.

## 7. Conclusion and Recommendation

The present study shows an increase in osteonectin in patients with cardiovascular disorders, which can be used to detect calcification in patients with cardiovascular diseases, which can lead to disease progression. The osteonectin effect by age was also discovered, which increased with older individuals. Kidney damage is a complication of cardiovascular disease. The study's recommendations include looking at osteonectin levels in a large number of individuals with cardiovascular illnesses and in patients with kidney failure.

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