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RESEARCH

Evaluation of FGF23, C-reactive Protein and Lipid Profile as Predictors for Cardiovascular Diseases in Patients with Type 2 Diabetes

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■ Abstract

Background: Cardiovascular illness is a prominent contributor to mortality, characterised by a lack of effective therapeutic interventions tailored to specific clinical requirements. Fibroblast growth factor 23 (FGF-23) is a protein hormone that was first characterised for its involvement in mineral metabolism. FGF-23 is mostly produced by osteocytes and has a pivotal function in suppressing the reabsorption of phosphate in the renal proximal convoluted tubule. **The aim:** Comparison between Cardiovascular Diseases in Patients With Type 2 Diabetes and without DM regarding serum level of FGF23. Study the relation between serum FGF23 level and insulin resistance in Cardiovascular Diseases in Patients With Type 2 Diabetes. **Material and Method:** The present study included 60 patients with Diabetes Mellitus (DM) with a mean age of 56.02±1.395 years and an age range of (40-80) years and 60 patients with cardiovascular diseases and diabetes (CVD and DM) with a mean age of 59.20±1.478 and an age range of (40-80) years, Who visited Al-Basrah Teaching Hospital in Basrah. in addition, the study included 60 healthy controls mean age of healthy control subjects was 54.72±1.405years. All patients in this study were diagnosed by specialized doctors and the diagnosis was verified by clinical and laboratory tests, during the period from September 2022 to September 2023. All Subjects signed a written informed consent form. The BMI was calculated as body weight (kg) and was divided by squared height in meters. **Results:** The results of this study showed an increase in the level of Triglycerides, Cholesterol, LDL Cholesterol and VLDL Cholesterol in CVD and DM patients as compared with DM and Control, while The results of this study showed a decrease in the level of HDL Cholesterol and there was a significant difference in concentrations among study groups (p-value <0.0001). Also, The results of this study showed an increase in the level of fibroblast growth factor 23 in CVD and DM patients as compared with DM and control and there was a significant difference in concentrations of FGF23 among study groups (p-value <0.0001). **Conclusion:** From this study, it could be concluded that FGF23, CRP, may be a clinically useful and simple index for predicting the concomitant presence of Cardiovascular Diseases insulin resistance and dysglycemia among apparently healthy, young (<50 years) Al-Basra populations.

Keywords: CRP, Cardiovascular Disease, Diabetes Mellitus, FGF23, Inflammation.

1. Introduction

Diabetes mellitus (DM) has been well-recognised as a significant risk factor for the development of cardiovascular disease (CVD). those diagnosed with type 2 diabetes mellitus (T2DM) have an increased incidence of cardiovascular morbidity and death and are disproportionately impacted by CVD as compared to those without diabetes [1]. Diabetic vascular disease has been identified as the underlying cause for the significant increase, ranging from two to four times, in the prevalence of coronary artery disease (CAD) and stroke. Additionally, it has been associated with a notable improvement, ranging from two to eight times, in the likelihood of developing heart failure [2]. Previous studies have shown that individuals diagnosed with T2DM who do not have a prior history of CAD are at a comparable risk for experiencing cardiac events as those who have previously suffered from a myocardial infarction [3]. Nevertheless, subsequent research has yielded inconsistent findings, suggesting that diabetes status may not universally serve as a CVD equivalent. This underscores the importance of employing a multivariate approach as a suitable foundation for risk stratification in CVD prevention among individuals with diabetes [4]. The risk of CVD exhibits a gradient, and the determination of this gradient is contingent upon the interplay of many risk factors [4]. The majority of this heightened risk is linked to an increased prevalence of established risk factors, including hypertension, dyslipidemia, and obesity, among these individuals. In

the last decade, substantial data has been accumulated to support the significant value of treating conventional risk factors in patients with T2DM to reduce the risk of CVD [5, 6]. The inadequate management of a significant proportion of cardiovascular risk factors in individuals with diabetes underscores the need for a more proactive approach to addressing modifiable cardiovascular risk factors, particularly in patients with a history of cardiovascular disease. Nevertheless, the enhanced occurrence of cardiovascular disease in individuals with type 2 diabetes mellitus cannot be simply ascribed to the increased incidence of conventional risk factors [7-9].

A lipid profile is a diagnostic procedure that involves the analysis of blood samples to assess the concentrations of various lipid molecules present in the bloodstream. Lipids, often referred to as fats, have limited solubility in the bloodstream. Elevated lipid levels are associated with an increased susceptibility to cardiovascular diseases, including heart disease, as well as an elevated chance of experiencing a heart attack or stroke. The lipid profile test includes the following components: The variables of interest in this study are total cholesterol, HDL-cholesterol, triglyceride levels, as well as computed LDL and VLDL [10, 11]. The results of this test can identify and determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other conditions [12]. Chronic inflammatory conditions, such as rheumatoid arthritis, systemic lupus erythematosus, and psoriasis, as well as infectious disorders including periodontal disease and HIV, are correlated with an elevated susceptibility to cardiovascular disease [13]. C-reactive protein (CRP) serves as a significant biomarker for systemic inflammation, eliciting a response to various inflammatory stimuli such as injury and infection. This protein plays a crucial role in inducing the systemic host response and contributing to the development of atherosclerosis by causing many abnormalities that heighten the associated risk [14]. Multiple studies have shown a consistent association between elevated levels of CRP and an increased susceptibility to coronary artery disease (CAD). Furthermore, baseline levels of CRP might potentially serve as a predictive factor for future occurrences of cardiovascular events [15], but can also predict the prognosis of patients with acute coronary syndrome [16]. The synthesis of the pentameric protein is mostly seen in liver hepatocytes, however, it has also been shown in many other cell types including smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes [17].

C-reactive protein is first produced as individual monomers, which then undergo assembly into a pentameric structure inside the endoplasmic reticulum of the originating cell. The pentameric protein in hepatocytes is sequestered inside the endoplasmic reticulum via its interaction with two carboxylesterases, namely gp60a and gp50b [18]. During a quiescent (non-inflammatory) phase, CRP is gradually released from the endoplasmic reticulum. However, when there is an elevation in levels of inflammatory cytokines, the interaction between CRP and carboxylesterases diminishes, resulting in a fast secretion of CRP [19].

Fibroblast growth factor 23 (FGF-23) is a protein hormone that was first characterised due to its involvement in mineral metabolism [20]. FGF-23 is mostly produced by osteocytes and has a vital function in suppressing the reabsorption of phosphate in the renal proximal convoluted tubule. Furthermore, it impedes the process of converting 25-hydroxyvitamin D into its physiologically active form. The aforementioned effects serve to restrict the occurrence of hypophosphatemia in cases of renal failure, a condition characterised by heightened levels of FGF-23 [20, 21]. Previous studies have proposed a potential causative relationship between FGF-23 and unfavourable cardiovascular remodelling, including the development of left ventricular hypertrophy [22], alterations in myocyte calcium handling [23], renin-angiotensin system upregulation, and promotion of vascular calcification [7].

Considering the diverse impacts of FGF-23 on cardiovascular (CV) structure and function, it is plausible to anticipate that FGF-23 might provide prognostic significance that is not influenced by recognised biomarkers. Previous research has shown a correlation between elevated levels of FGF-23 and adverse cardiovascular events in persons without pre-existing cardiovascular disease, as well as in patients with stable coronary artery disease and normal left ventricular ejection fraction [24-28].

2. Materials and Methods

The Ethical Considerations: The research included the collecting of blood samples and the implementation of experimental methods, both of which received approval from the Ethical Committee of AL Basra Teaching Hospital in Iraq and Sousse University in Tunisia. Before sample collection, informed permission was acquired from all subjects included in the research. Furthermore, all methodologies and procedures were conducted by the standards and regulations set out by the Ethical Committee of the Faculty of Medicine Ibn Al Jazzar, Sousse, affiliated with Sousse University in Tunisia.

Study Design: This study is a case-control study which had been performed on patients who attended Al-Basrah Teaching Hospital.

The Subjects: The present study included 60 patients with Diabetes Mellitus (DM) with a mean age of 56.02±1.395 years and an age range of (40 - 80)years and 60 patients with cardiovascular diseases and diabetes (CVD and DM) with a mean age of 59.20±1.478and an age range of (40- 80) years, Who visited Al-Basrah Teaching Hospital in Basrah. in addition, the study included 60 healthy controls mean age of healthy control subjects was 54.72±1.405 years. All patients in this study were diagnosed by specialized doctors and the diagnosis was verified by clinical and laboratory tests, during the period from September 2022 to September 2023. All Subjects signed a written informed consent form. The BMI was calculated as body weight (kg) and was divided by squared height in meters.

Kits: The kits that had been used in this study are shown in Table 1.

Table 1: Kits (Companies and Countries of Origin) Used in the Present Study.

No	Kit	Company	Country
1	Glucose kit	Biosystem	Spain
2	Hemoglobin A1C-Direct	Biosystem	Spain
3	Tumor Necrosis Factor Alpha ELISA Assay	Elabscience	USA
4	Human Insulin ELISA Assay	Elabscience	USA
1	Triglycerides Kit	Biosystem	Spain
2	Cholesterol Kit	Biosystem	Spain
3	Cholesterol HDL Kit	Biosystem	Spain
4	C-reactive protein	i-chroma	Korea
5	Human Fibroblast Growth Factor 23 ELISA Assay	Elabscience	USA

Body Mass Index Measurement: The body weight and height were measured in all the subjects using the same scale. The body mass index (BMI) was calculated as the weight (kg) and divided by the height (meters) squared. The patients were classified according to BMI following the recommendation of the World Health Organization (WHO) as adopted by the American Diabetes Association (ADA). Accordingly, weight status was defined as normal (BMI 18.5-24.9), overweight (BMI 25-29.9), and severe obesity (BMI >30) [29].

The Criteria of the Healthy Controls Group: The healthy control group samples were collected from the experimental after ensuring the adequacy of the criteria which is specified in this study. The selection of the healthy control group was based on several criteria, including:

- The healthy control participants should not have any medical history of heart disorders.
- They have not undergone any surgical intervention or illness requiring hospitalization.
- Subjective perception of good health is determined by a health questionnaire.
- With the approximate age range of the patient's group.

The Collection of the Samples: The human blood samples (patients and controls) are each divided into five millilitres. The samples were then put into anticoagulant tubes (containing sodium citrate), which were centrifuged for 15 minutes at 3000 rpm, and the serum was then separated and stored at -20 degrees Celsius until analysis.

The remaining samples are transferred to sterile test tubes and allowed to coagulate for 30 minutes at room temperature. The sample was then separated by centrifugation at 3000 rpm for 15 minutes, and the serum was extracted and stored at -20 (0C) until analysis.

The Biochemical Analysis: The principle of assay for measurement of cholesterol, triglycerides, and HDL using calorimetric methods. LDL-c calculated using the equation

[LDL-chol] = [total chol] - [HDL-chol] - [VLDL-chol], and VLDL measured using

VLDL-cholesterol=[TG]/5, all values are expressed in mg/dL.

Human Fibroblast Growth Factor-23: The experimental procedure outlined involves several steps for conducting an assay or experiment. Firstly, 100µL of either a standard or a sample is added to the wells. These wells are typically part of a microtiter plate or a similar platform. The plate is then incubated for 90 minutes at a temperature of 37°C, allowing the standard or sample to interact with the plate surface or any specific reagents present.

Moving on to the second step, once the incubation period is complete, the liquid in the wells is discarded. Immediately following this, 100µL of Biotinylated Detection Antibody working solution is added to each well. This solution contains antibodies that are labelled with biotin, a molecule that can bind specifically to certain target molecules or antigens. The plate is then incubated for a further 60 minutes at 37°C to allow the antibodies to bind to their respective targets. The third step involves aspirating and washing the plate. This is done to remove any unbound or non-specifically bound substances that may interfere with the subsequent steps. The plate is washed three times to ensure thorough removal of unwanted materials.

In the fourth step, 100µL of HRP (Horseradish Peroxidase) conjugate working solution is added to the wells. HRP is an enzyme commonly used in immunoassays. It is conjugated to another molecule, such as an antibody or a protein, and serves as a marker for the presence of the target molecule or antigen. The plate is then incubated for 30 minutes at 37°C. After the incubation, the liquid is aspirated, and the plate is washed five times to remove any unbound HRP conjugate.

Moving on to the fifth step, 90µL of Substrate Reagent is added to the wells. The substrate interacts with the HRP conjugate, leading to the production of a detectable signal. This step is typically carried out at 37°C and requires an incubation period of 15 minutes.

In the sixth step, 50µL of Stop Solution is added to halt the reaction. This prevents further signal generation and stabilizes the results obtained.

Finally, in the seventh step, the plate is read at a wavelength of 450nm, which corresponds to the absorbance of the generated signal. This measurement allows for the quantification or calculation of the results obtained from the assay. The absorbance reading at 450nm is typically proportional to the amount or concentration of the target molecule or antigen present in the original sample or standard.

Statistical Analysis: Data were summarized, analyzed, and presented using GraphPad Prism 9.2.0

and Microsoft Office Excel 2013. Numeric data were expressed as mean ± Standard Error of Mean, whereas categorical data were expressed as numbers. One-way ANOVA and unpaired t-test were used to compare the mean values among the different groups in the case of normally distributed variables. Chi-square was used to evaluate the qualitative data. Bivariate correlation was carried out using Pearson"s correlation coefficient. The P-value was considered significant at p-value ≤ 0.05.

3. Results

Demographic characteristics of the control group and other groups: The Demographic characteristics of control subjects and other groups (Table 2). The mean age of control subjects was 54.72±1.405years, that of diabetes mellitus groups was 56.02±1.395years and cardiovascular disease and diabetes groups was 59.20±1.478years mellitus and there was no significant difference in mean age among study groups (p=0.0760). The body mass index (BMI) (kg/m^2) of control was (24.58±0.2385) (kg/m^2), while that of DM and CVD and DM cases was (25.15±0.2875) (27.03±0.3339) (kg/m^2) respectively, and there was a significant difference in mean age among study groups (p-value<0.0001) (Table 2). The control group included 30 (50%) males and 30 (50.0 %) females. The diabetes mellitus group included 30 (50%) males and 30 (50%) females and the cardiovascular disease and diabetes mellitus group included 30 (50.0 %) males and 20 (50.0 %) females. There was no significant difference in the control subjects and other groups according to gender among the study groups (p=0.9723).

Age: The measurement of age showed a non-significant difference in mean values between the control and DM. (p-value=0.7958). In addition, a non-significant difference in mean values between control and CVD and DM (p-value=0.0702), and there was a non-significant difference in mean value between DM and CVD and DM (p-value=0.2579).

BMI: The measurement of BMI showed a non-significant difference in mean values between the control and DM. (p-value=0.0769). In addition, the a significant difference in mean values between the control and CVD and DM (p-value<0.0001), and there was a significant difference in mean value between DM and CVD and DM (p-value<0.0001).

Table 2: Demographic Characteristics of Control Subjects and other Groups.

Characteristic	Control	DM	CVD and DM	p-value
	n=60	***n=60***	***n=60***	
		Age(years)		
Range	40-80	40-80	40-80	0.076
Mean±SEM	54.72±1.405	56.02±1.395	59.20±1.478	ns
		BMI (kg/m²)		
Range	21.78-26.81	22.03-28.4	22.84-29.78	<0.0001
Mean±SEM	24.56±0.1985	25.28±0.2454	27.13±0.2501	****
		Gender		
Male, *n* (%)	30(50%)	30(50%)	30(50%)	0.9723
Female, *n* (%)	30(50%)	30(50%)	30(50%)	ns

n: number of cases; SEM: Standard Error of Mean; **ns**: not significant at p>0.05 ;********: significant at p <0.0001

Determination and comparison of serum levels studied biomarkers among the control group and other groups: The comparison of serum levels studied biomarkers among the control group and other groups (Table 3).

Triglycerides: The results of this study showed an increase in the level of triglycerides (248± 6.343) (mg/dL) in CVD and DM patients as compared with DM patients and control, (211.8±3.9), (110±3.098), (mg/dL) respectively, and there was a significant difference in concentrations of triglycerides among study groups (p-value<0.0001). The measurement of triglycerides showed a significant difference in mean values between the control and DM. (p-value<0.0001). In addition, the a significant difference in mean values between the control and CVD and DM (p-value<0.0001), and there was a significant difference in mean value between DM and CVD and DM (p-value<0.0001).

Cholesterol: The results of this study showed an increase in cholesterol level (284.5±5.216) (mg/dL) in CVD and DM patients as compared with DM patients and control, (211.1±3.569), (134.2±2.355), (mg/dL) respectively, and there was a significant difference in concentrations of cholesterol among study groups (p-value<0.0001). The measurement of cholesterol showed a significant difference in mean values between the control and DM (p-value<0.0001). In addition, the a significant difference in mean values between the control and CVD and DM (p-value<0.0001), and there was a significant difference in mean value between DM and CVD and DM (p-value<0.0001).

HDL Cholesterol: The results of this study showed a decrease in the level of high-density lipoprotein (HDL) (19.47±0.6245) (mg/dL) in CVD and DM patients compared to DM and control, (28.21±0.6183), (38.33±0.6059), (mg/dL) respectively, and there was a significant difference in concentrations of HDL among study groups (p-value<0.0001). The measurement of HDL showed a significant difference in mean values between the control and DM (p-value <0.0001). In addition, a significant difference in mean values between the control and CVD and DM (p-value<0.0001), and there was a significant difference in mean value between DM and CVD and DM (p-value<0.0001).

LDL Cholesterol: The results of this study showed an increase in the level of low-density lipoprotein (215.5±5.552) (mg/dL) in CVD and DM cases compared to DM and control, (140.5± 3.773), (73.87±2.455), (mg/dL) respectively, and there was a significant difference in concentrations of LDL among study groups (p-value<0.0001). The measurement of LDL cholesterol showed a significant difference in mean values between the control and DM (p-value <0.0001). In addition, the a significant difference in mean values between control and CVD and DM (p-value<0.0001), and there was a significant difference in mean value between DM and CVD and DM (p-value<0.0001) (p-value≤0.05).

VLDL Cholesterol: The results of this study showed an increase in very low-density lipoprotein cholesterol (49.60±1.269) (mg/dL) in CVD and DM cases compared to DM and Control (42.35± 0.78), (22.00±0.6197), (mg/dL) respectively, and there was a significant difference in concentrations of VLDL among study groups (p-value<0.0001). The measurement of very low-density lipoprotein cholesterol showed a significant difference in mean values between control and DM (p-value <0.0001). In addition, the a significant difference in mean values between the control and CVD and DM (p-value<0.0001), and there was a significant difference in mean value between DM and CVD and DM (p-value<0.0001).

C-reactive protein: The results of this study showed an increase in the level of C-reactive protein (CRP) (37.22±0.7506) (mg/dL) in CVD and DM patients as compared with DM and Control, (23.25±0.6724), (14.48±0.3199), (mg/dL) respectively and there was a significant difference in concentrations of CRP among study groups (p-value<0.0001). The measurement of CRP showed a significant difference in mean values between the control and DM (p-value<0.0001). In addition, the a significant difference in mean values between the control and CVD and DM (p-value<0.0001), and there was a significant difference in mean value between DM and CVD and DM (p-value<0.0001).

Fibroblast Growth Factor 23: The results of this study showed an increase in the level of fibroblast growth factor 23 (FGF23) (440.8±19.32) (pg/mL) in CVD and DM patients as compared with DM and control, (403.8±4.076), (360.6±3.260), (pg/mL) respectively, and there was a significant difference in concentrations of FGF23 among study groups (p-value<0.0001). The measurement of FGF 23 showed a significant difference in mean values between the control and DM (p-value = 0.0244). In addition, a significant difference in mean values between the control and CVD and DM (p-value<0.0001), and there was a non-significant in mean value between DM and CVD and DM (p-value=0.0633).

Table 3: Comparison of Mean Values of the Serum Levels of Studied Biomarkers between the Control Group and other Groups.

Characteristic	Control	DM	CVD and DM	P Value
	n=60	*n=60*	*n=60*	
Triglycerides (mg/dL)				
Range	70.64-165.6	170.5-269.7	185-358.6	<0.0001
Mean±SEM	110±3.098	211.8±3.900	248±6.343	****
Cholesterol (mg/dL)				
Range	111-190.4	141.3-250.6	200.5-366	<0.0001
Mean±SEM	134.2±2.355	211.1±3.569	284.5±5.216	****
HDL Cholesterol (mg/dL)				
Range	28.59-51.64	18.59-41.64	13.87-37.7	<0.0001
Mean±SEM	38.33±0.6059	28.21±0.6183	19.47±0.6245	****
LDL Cholesterol (mg/dL)				
Range	43.68-133.8	64.7-183.1	112.8-313.1	<0.0001
Mean±SEM	73.87±2.455	140.5±3.773	215.5±5.552	****
VLDL Cholesterol (mg/dL)				
Range	14.13-33.13	34.1-53.94	37-71.72	<0.0001
Mean±SEM	22±0.6197	42.35±0.78	49.6±1.269	****
CRP (mg/dL)				
Range	11.15-18.48	16.25-32.32	27.41-49.62	<0.0001
Mean±SEM	14.48±0.3199	23.25±0.6724	37.22±0.7506	****
FGF23 (pg/mL)				
Range	300.7-396.4	385.7-547.9	385.7-1219	<0.0001
Mean±SEM	360.6±3.260	403.8±4.076	440.8±19.32	****

n: number of cases; SD: Standard Definition; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; VLDL: Very Low-Density Lipoprotein; CRP: C-reactive protein; FGF23: Fibroblast Growth Factor 23; ****: significant at p <0.0001

4. Discussion

One of the leading causes of mortality in the majority of industrialised nations is cardiovascular disease (CVD), which is mostly brought on by coronary artery atherosclerosis. Age, gender, economic status, and other factors all affect CVD incidence. Highly correlated processes seen in atherosclerosis include lipid abnormalities, thrombosis, inflammation, activation of vascular smooth cells and platelets, endothelial dysfunction, oxidative stress, and hereditary variables [30, 31]. The mean age of control subjects was 54.72±1.405 years, that of diabetes mellitus groups was 56.02±1.395 years and cardiovascular disease and diabetes groups were 59.20± 1.478years mellitus and there was no significant

difference in mean age among study groups; this result agrees with [32], who found that the mean age of patients with CVD was(55) years. Also, the results came in agreement with [33]. who observed that the mean age of patients with CVD was 64.72 years (55.6-73.8 years).

The findings of our investigation concurred with those of [34], which discovered that the elderly and ageing populations are more vulnerable to CVD. High age-related CVD incidence is associated with several wider ageing processes, including morbidity accumulation, a decline in homeostasis, and persistently harmful effects of CVD risk factors. Insidious ageing-related alterations in CVD morphology and function are another cause of heart disease [35]. For instance, vascular stiffening of the central vasculature is a constant ageing phenomenon that typically begins by middle age and progresses to increased afterload stress, increased myocardial workload, and changes in diastolic perfusion that predispose to functional declines and ultimately lead to ischemia, heart failure, arrhythmia, and other CVD disorders [36].

Moreover, the senior demographic has a higher susceptibility to sarcopenic obesity and heightened insulin resistance compared to middle-aged individuals, mostly as a result of advancing age, hormonal fluctuations, and a predominantly inactive way of life. Consequently, this population faces an elevated likelihood of developing diabetes [37]. The process of ageing is associated with an increase in blood triglyceride (TG) levels and alterations in the metabolism of triglycerides inside the body. The aforementioned circumstances have been seen to increase the susceptibility of older individuals to the development of metabolic-related disorders, such as diabetes and metabolic syndrome, in comparison to younger individuals [38, 39]. Although there is a general rise in cardiovascular disease (CVD) with advancing age, cardiologists encounter the task of customising preventative and treatment strategies based on the unique circumstances of each person [35]. The outcomes of our study have disagreed with [40], which did not contribute to explaining the higher prevalence and extent of CVD observed in elderly patients.

The current research found that, in terms of body mass index (BMI), patients with CVD and DM had mean BMIs that were considerably higher than those of the DM and controls. These findings support [41] they noted that the average BMI of CVD patients was below 25, and they proposed that obesity is a separate risk factor for CVD in both sexes. The results also support [42], they discovered that a greater risk profile and BMI are both strongly connected with an increased risk of cardiovascular risk factors [43]. It has been found that individuals with a higher BMI have a greater likelihood of being linked to cardiovascular risk factors such as hypertension, hypercholesterolemia, and diabetes mellitus.

The condition of obesity is correlated with a heightened likelihood of developing cardiovascular disease, and there is a possibility of a stronger manifestation of CVD in individuals with obesity. Inflammatory responses have been observed in individuals with obesity, resulting in elevated levels of clotting factors such as fibrinogen, von Willebrand factor, and factors VII and VIII. Additionally, there is an increase in plasminogen activator inhibitor type-I, which is associated with reduced fibrinolysis. These factors collectively contribute to the progression of cardiovascular disease [44]. The weight of each individual was measured by personal balance without heavy clothes and shoes. The height also was measured by tape measure, and then the body mass index was estimated according to the World Health Organization (WHO) classification [45].

The results of our study agreed with the survey [46], the literature indicates that an elevated BMI is linked to many noteworthy health consequences, such as hypertension (HTN), DM, metabolic syndrome (MetS), and dyslipidemia. These conditions are recognised as independent risk factors for CVD. The findings of our investigation were in alignment with the results obtained. Labounty*et al.* [47], The study shows that persons with a higher BMI have a higher prevalence, extent, and severity of CVD. The findings of our investigation are incongruous with the existing body of literature. [48, 49] which reported an inverse relationship between BMI and CVD.

Hyperlipidemia is a comprehensive term that comprises a range of inherited and acquired illnesses characterised by increased amounts of lipids in the body. Hyperlipidemia is characterised by elevated levels of low-density lipoprotein (LDL), total cholesterol, triglycerides, or lipoproteins that exceed the 90th percentile of the general population. Additionally, it may be identified by high-density lipoprotein (HDL) levels that fall below the 10th percentile when compared to the general population [50].

The current study results have shown an elevation in cholesterol, Triglyceride, and lipoprotein levels compared with positive and negative control. Our findings were consistent with [51, 52], which revealed an increase in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C) levels in patients with CVD.

Hypercholesterolemia is a significant risk factor and has been associated with exacerbated production of reactive oxygen species (ROS), which significantly contributes to the initiation and development of atherosclerotic lesions in patients with CVD [53]. The outcomes of the current study were agreed with the study [54]. This showed that abnormal blood lipid metabolism is a risk factor for CVD. Patients with high triglyceride (TG) levels and low HDL cholesterol (HDL-C) levels are risk factors for cardiovascular disease.

Elevated levels of Low-Density Lipoprotein-cholesterol (LDL-C) are a substantial risk factor for the development of cardiovascular disease. The levels of low-density lipoprotein cholesterol (LDL-C) have a significant role in determining endothelial function and oxidative stress

in individuals diagnosed with severe cardiovascular disease (CVD). LDL and oxidised LDL-cholesterols have been shown to elevate the formation of superoxide, as well as activate xanthine oxidase and NAD(P)H oxidoreductase. These processes have been identified as contributors to the cholesterol-dependent synthesis of superoxide. Besides the excessive creation of superoxide, mounting evidence suggests that the mitochondria's overproduction of reactive oxygen species may have a role in the pathogenesis of atherosclerotic disease [55, 56].

The results of our study have disagreed with the analysis, which has suggested that elevated LDL cholesterol is not associated with an increased risk of CVD and atherosclerotic cardiovascular disease. Still, the results of our data were consistent with the study [57], which showed similar findings.

High-density lipoprotein-cholesterol (HDL-C) functions to eliminate surplus cholesterol from peripheral cells, averting the possibility of its detrimental buildup, and then conveying it to the liver for excretion via bile. The aforementioned process assumes a pivotal function in the prevention of atherosclerosis by facilitating the effective removal of cholesterol from arterial walls, which have a propensity for accumulation [58]. The results of this study showed a decrease in the level of high-density lipoprotein (HDL) (20.16±0.8736) (mg/dL) in CVD and DM patients compared to DM and control, (28.69±0.7147), (38.09±0.7086). These consequences were agreed with [59], which showed that CVD patients had higher levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and apolipoprotein B (APOB), but lower high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-1 (APOA-1) as compared with control. The relationship between high-density lipoprotein cholesterol and CVD remains controversial. Circulating monocytes as a source of various cytokines interact with platelets and endothelial cells and leading to aggravation of inflammatory, pro-thrombotic pathways. HDL-C defuse these pro-inflammatory and pro-oxidant effects of monocytes by inhibiting the migration of macrophages and oxidation of the LDL-C molecules as well as promoting the efflux of cholesterol from these cells [60, 61].

Very Low-Density Lipoprotein (VLDL) is a lipoprotein synthesised by the hepatic cells. The process of hepatic assembly involves the synthesis of lipoproteins by incorporating triglycerides, cholesterol, and apolipoproteins inside the liver. VLDL undergoes conversion inside the circulatory system, resulting in the formation of low-density lipoprotein (LDL) and intermediate-density lipoprotein (IDL)(63) [62]. The results of this study showed an increase in very low-density lipoprotein cholesterol (50.12±1.586) (mg/dL) in CVD and DM cases compared to DM and Control (41.34±0.9098), (22.15±0.8037). VLDL is considered a marker of atherogenic lipoprotein remnants. Most TG in the bloodstream is carried by very low-density lipoproteins (VLDL) and chylomicrons (64) [63]. Due to their huge size, both very low-density lipoproteins (VLDL) and chylomicrons are unable to permeate the artery wall. Hence, it may be inferred that TG does not have a direct causal relationship with the development of atherosclerosis. Nevertheless, heightened levels of triglycerides (TG) lead to a rise in triglyceride-rich lipoproteins (TRLs) such as very low-density lipoproteins (VLDL) and chylomicrons [64]. The consequences of our study agreed with the study (66) [65], which revealed that elevated VLDL cholesterol levels were found to increase CVD risk by 2.1-3.3 fold. Our results were consistent with Xie*et al.* [66] found that higher VLDL-C levels were associated with increased CVD.

The liver is responsible for the production of C-reactive protein (CRP). The amount of [specific component] increases in the presence of bodily inflammation. Low-density lipoprotein (LDL) cholesterol not only forms a layer on the inner walls of arteries but also has detrimental effects on their structural integrity [67]. The resulting harm induces an inflammatory response in which the body initiates a reparative process by mobilising a group of proteins known as "acute phase reactants." CRP is among the proteins in question. Research conducted has shown that the assessment of CRP levels proves to be a more reliable and accurate predictor of the presence of CVD compared to the measurement of LDL levels [68]. However, it is crucial to note that the CRP test does not serve as a diagnostic tool for heart disease. Rather, it is used to detect and measure levels of inflammation inside the body [69].

The results of this study showed an increase in the level of C-reactive protein in CVD and DM patients as compared with DM and Control, respectively and there was a significant difference in concentrations of CRP among study groups (p-value <0.0001). This finding suggests that there is significant public interest in the association between CRP and CVD, even among those who already have CVD. Therefore, it is essential to allocate more emphasis towards the prevention of CVD on a population-wide scale.

This research provides evidence that a higher level of CRP is associated with a considerably increased risk of developing CVD in a large group of individuals in middle age. Furthermore, the incorporation of CRP into the conventional risk factors for CVD has the potential to enhance the categorization of risk. In comparison to participants without CVD, individuals with DM had elevated levels of high-sensitivity C-reactive protein (hs-CRP), whereas those without DM displayed lower CRP levels. Furthermore, after controlling for factors such as age, gender, smoking status, alcohol consumption, systolic blood pressure, blood pressure treatment, total cholesterol, triglycerides, and high-density lipoprotein, those with DM had a 62% increased chance of developing CVD. This discovery aligns with the recommendation established by the European Society of Cardiology, which advises those with a moderate risk of CVD to monitor their CRP levels, as opposed to those with a low risk [70].

Increased cardiovascular risk is related to elevated baseline levels of C-reactive protein (CRP) [71]. Consequently, it may be more suitable to put patients with such elevated levels in the high-risk group, warranting consideration for pharmacological intervention to control risk factors [72, 73]. The significant occurrence of increased CRP levels in patients categorised as having intermediate risk suggests that evaluating CRP levels in these individuals is likely to detect a substantial proportion of patients who have a higher risk than anticipated based solely on their traditional risk factors.

Elevated levels of Fibroblast growth factor 23 (FGF23) have been linked to several cardiovascular diseases, such as hypertension, subclinical atherosclerotic disease, left ventricular hypertrophy, atrial fibrillation, and other cardiovascular events [74, 75]. Animal studies have shown that the administration of FGF-23 results in adverse effects such as myocyte enlargement and left ventricular hypertrophy (LVH). The numerical value provided by the user is 8. Prior research has shown a correlation between elevated levels of fibroblast growth factor-23 (FGF-23) and left ventricular hypertrophy (LVH), as well as increased risk of cardiovascular events and death from all causes [75, 76]. The occurrence of hyperglycemia results in the generation of advanced glycation end products (AGEs), which subsequently contribute to an elevation in the level of fibroblast growth factor 23 (FGF23) [77, 78]. Individuals diagnosed with type 2 diabetes mellitus (T2DM), particularly those who are obese, have been seen to exhibit a condition of heightened inflammation. It is commonly recognised that inflammation plays a significant role in the elevation of fibroblast growth factor 23 (FGF23) levels [79, 80].

There have been identified correlations between elevated levels of FGF-23 and the development of atherosclerosis, specifically coronary artery calcification and carotid artery intima-media thickness. The findings of a research conducted on a cohort of 282 African American individuals diagnosed with type 2 diabetes mellitus revealed a correlation between elevated levels of FGF-23 and the presence of coronary artery calcified atherosclerotic plaque. However, no such link was seen with the carotid artery or aorta-iliac calcified plaque [81]. In a separate investigation including diverse ethnic cohorts, with a predominant representation of Hispanic individuals (68% of the sample), a correlation was indeed seen between elevated levels of FGF-23 and the presence of carotid calcification [82]. There exists a correlation between elevated levels of FGF-23 and scores indicating the presence of coronary artery calcification [83, 84]. The research conducted in China further revealed that those exhibiting elevated levels of FGF-23 and extensive coronary calcification had a significantly heightened susceptibility to all-cause death [83].

The precise processes behind the relationship between FGF-23 and subclinical atherosclerosis have yet to be fully elucidated. If there is a correlation between increased levels of FGF-23 and hypertension, and hypertension is a known risk factor for atherosclerosis, it is plausible to hypothesise that raised FGF-23 levels may be related to the development of atherosclerosis and vascular dysfunction, maybe mediated by hypertension. An alternative theory posits that FGF-23 has a role in promoting vascular calcification caused by phosphate [85].

Previous research has shown correlations between elevated levels of FGF-23 and occurrences of coronary and other ischemic cardiovascular events. In a comprehensive analysis including a cohort of 704 individuals diagnosed with CVD, it was shown that elevated levels of FGF-23 were significantly correlated with an amalgamated outcome encompassing acute coronary syndrome, stroke or transient ischemic attack, heart failure, and mortality among patients with T2DM at the commencement of the trial [86].

The findings of the Cardiovascular Health Study indicate a positive correlation between elevated levels of FGF-23 and an increased likelihood of experiencing non-abrupt cardiac death, including cardiovascular fatalities that are not characterised by sudden events such as myocardial infarctions and strokes [87]. Elevated levels of FGF-23 have also been shown to be correlated with a heightened risk of death in individuals diagnosed with heart failure characterised by lower ejection fraction [88]. Therefore, elevated levels of FGF-23 have been linked to various cardiovascular events across diverse groups.

In their study, Kestenbaum *et a*l. evaluated a total of 6547 individuals who were originally free of CVD. These participants were then followed up for 7.5 years [89]. It was ultimately shown that an elevated concentration of FGF23 exhibited a correlation with preclinical cardiac pathology, the onset of heart failure (HF), and a 14% higher likelihood of developing ischemic heart disease (IHD). Udell and colleagues successfully acquired consistent findings, demonstrating an independent association between the amount of FGF23 and an increased risk of cardiovascular mortality or heart failure in individuals with stable IHD Udell*et al.* [24]. A subsequent extensive investigation demonstrated a strong correlation between the concentration of FGF23, as assessed in individuals having coronary angiography, and the risk of death from all causes and cardiovascular-related causes. Multiple studies have shown a distinct correlation between the amount of FGF-23 and cardiovascular morbidity and death in individuals diagnosed with HF as opposed to MI. A study done by researchers from Germany revealed a correlation between FGF23 and one-year mortality in patients diagnosed with MI and concurrent acute HF [91].

Thorsen*et al.* [92] conducted a study that revealed a drop in the level of FGF23 during the acute phase of myocardial infarction (MI), followed by its subsequent normalisation at seven days after revascularization. During one year of observation, a progressive increase in FGF23 concentrations was seen in individuals diagnosed with overt heart failure [92].

The available evidence suggests that persons diagnosed with type 2 diabetes, especially those with

cardiovascular disease and diabetes mellitus, often have elevated levels of fibroblast growth factor 23 (FGF23) in comparison to those without diabetes. The observed outcome is likely attributed to a multifaceted interaction of many dysregulated mechanisms that concurrently manifest in cardiovascular disease (CVD). Significantly, it is worth noting that although the precise mechanisms have yet to be completely elucidated, there exists a robust and persistent association between elevated levels of FGF23 and an increased susceptibility to adverse cardiovascular outcomes, including morbidity and death. Notably, similar correlations have been shown in individuals diagnosed with cardiovascular disease (CVD) and diabetes mellitus (DM), underscoring the significance of fibroblast growth factor 23 (FGF23) as a risk determinant in diabetic patients.

5. Conclusion

The findings of this study suggest that FGF23 and CRP may serve as valuable indicators in predicting the simultaneous occurrence of cardiovascular diseases, insulin resistance, and dysglycemia among seemingly healthy individuals below the age of 50 in the Al-Basra population. FGF23, also known as fibroblast growth factor 23, has been linked to various cardiovascular conditions and has emerged as a potential biomarker for assessing cardiovascular risk. Its involvement in mineral metabolism and phosphate regulation has garnered attention in recent research. Moreover, C-reactive protein (CRP), an inflammatory marker, has been widely studied as a risk indicator for cardiovascular diseases. Its elevation in the blood has been associated with systemic inflammation and has been shown to correlate with the occurrence of insulin resistance and dysglycemia. The study implies that the measurement of FGF23 and CRP levels could be a clinically useful and straightforward approach to identifying individuals who are at a higher risk of developing cardiovascular diseases, insulin resistance, and dysglycemia. Implementing these biomarkers as part of routine screenings may aid in early detection and prompt intervention to mitigate the adverse effects of these health conditions.

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Ethical Clearance: The project was approved by the local ethical committee at the University of Baghdad.

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