Republic of Iraq
Ministry of Higher Education and Scientific Research
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Biochemical evaluation of patients with Cardiovascular Disease

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2024A.D **1445A.H**

بسم الله الرحمن الرحيم

﴿ يُؤْتِي الْحِكْمَةَ مَن يَشَاءُ وَمَن يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا وَمَا

يَذَّكَّرُ إِلَّا أُولُو الْأَلْبَابِ ﴾

سورة البقرة, 269الاية

الإهـــداء

الحمد لله اقصى مبلغ الحمد .. والشكر لله من قبل ومن بعد

أهدي هذا البحث إلى من قال الحق تعالى فيهما

(وَقُل رَّبِّ ٱرْحَمْهُمَا كَمَا رَبَّيَانِى صَغِيرًا)

الى رفاق الخطوة الاولى والخطوة ما قبل الاخيرة

الى كل استاذتي واخص منهم بالذكر مشرف البحث:د.دعاء

Acknowledgment

Owing to the blessing of The Almighty God, we finished this research project.

We want to express our gratitude towards our families for their encouragement.

We take the opportunity to record our thanks to our supervisor,

M.S.C doaa Nasser Wahab .

Further great thanks to all members in the department of biochemistry.

We also record our thank to all who directly or indirectly offer their helping in this venture.

CONTENT

Abstract

Cardiovascular diseases(CVD):A disease that effect the heart and blood vessels. include atherosclerosis, coronary artery disease, heart valve disease, arrhythmia, heart failure, hypertension , shock, diseases of the aorta and its branches, disorders of the peripheral vascular system, and Congenital heart disease

It was observed that calcium has an impact on cardiovascular disease, with a significant increase in phosphate levels in patients compared to the control group. Phosphorus is an essential mineral that regulates multiple metabolic metabolism Serum phosphorus concentrations are tightly regulated in healthy individuals through several mechanisms including dietary absorption, bone flux and renal excretion .phosphate play a crucial role in cardiovascular disease development when compared with calcium that exhibit minimal role.

CHAPTER ONE

1.1 Introduction

Cardiovascular disease (CVD) is literally disease of the heart and blood vessels, and covers all diseases that affect the heart and circulatory system of the body, including coronary heart disease (angina and heart attack), hypertension (high blood pressure), stroke and peripheral vascular disease (PVD) any disease or disorder of the circulatory system outside of the brain and heart [1].

When cardiovascular disease affects the heart, it can trigger angina attacks (chest pain) or a more serious heart attack. If CVD affects the brain, the result may be a stroke either a major stroke, or a mini stroke also known as a or transient ischemic attack (TIA). Peripheral vascular disease (PVD) most commonly affects the legs but it can also affect the arms and kidneys [2].

Calcium and phosphorous in the blood play an essential physiological role in cardiovascular disease(CVD), including atherosclerosis ,heart valve titration, and vascular calcification [3].

Vitamin D deficiency has been linked to several cardiovascular risk factors [4,5]Through increased renin and angiotensin II synthesis vitamin D deficiency can increase the production of reactive oxygen species and G protein RhoA, resulting in inhibition of the pathways necessary for intracellular glucose transporter and thus the development of insulin resistance and metabolic syndrome . In addition, direct

effects of vitamin D upon smooth muscle calcification and proliferation could contribute to their effects on cardiovascular health[6].

1.2.Cardiovascular Disease

Cardiovascular disease (CVD) is a class of diseases that involve the heart or blood vessels[7] It's usually associated with a build up of fatty deposits inside the arteries (atherosclerosis and an increased risk of blood clots).

Coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack) in general CVDs include stroke ,heart failure, hypertensive heart disease, rheumatic heart disease, congenital heart disease, valvular heart disease, peripheral artery disease, thromboembolic disease, and venous thrombosis[8] most frequent CVD are those of atherosclerotic origin, mainly Ischemic Heart Disease (IHD) and stroke. CVD clinically manifests itself in middle life and older age, after many years of exposure to unhealthy lifestyles (unhealthy diet, physical inactivity, and smoking habit) and risk factors (high blood pressure, high cholesterolemia, diabetes, obesity)[9] Some of these risk factors, such as age, sex or family history ,genetic predisposition, are immutable; however, many important cardiovascular risk factors are modifiable by lifestyle change, social change, drug treatment (for example prevention of hypertension, hyperlipidemia, and diabetes). People with obesity are at increased risk of atherosclerosis of the coronary arteries[10].

1.3 Types of Cardiovascular Disease

There are several different types of cardiovascular disease that related with affect the heart in different ways [16] .Fig (1.1)

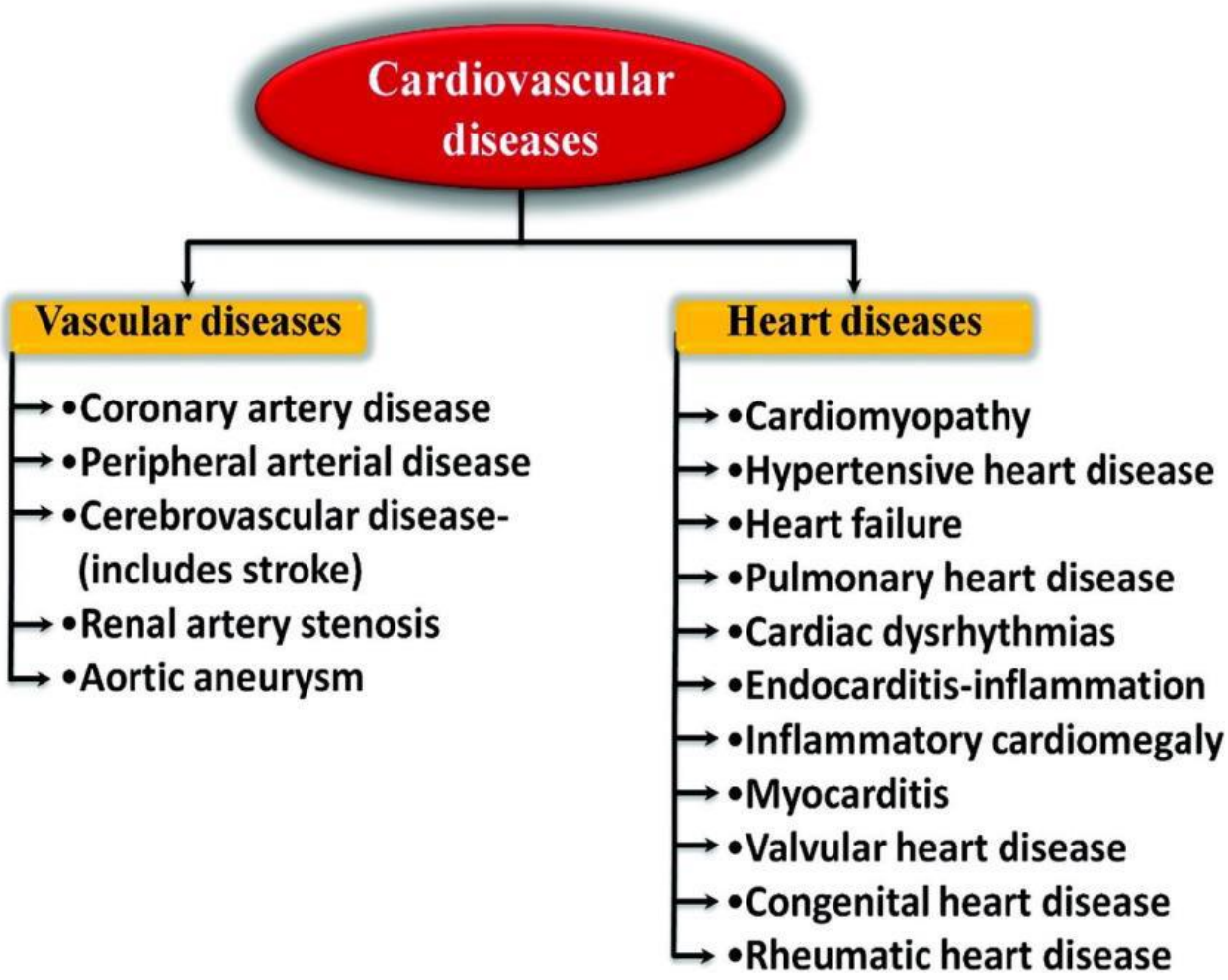


Figure (1.1) Types of cardiovascular disease[11]

Myocardial infarction (MI) refers to tissue death (infarction) of the heart muscle (myocardium) caused by ischemia, that is lack of oxygen delivery to myocardial tissue. It is a type of acute coronary syndrome, which describes a sudden or short term change in symptoms related to blood flow to the heart .Unlike the other type of acute coronary syndrome, unstable angina .a myocardial infarction occurs when there is cell death[12].

Stroke is a neurological disorder characterized by blockage of blood vessels. Clots form in the brain and interrupt blood flow, clogging arteries and causing blood vessels to break, leading to bleeding. Rupture of the arteries leading to the brain during stroke results in the sudden death of brain cells owing to a lack of oxygen. Stroke can also lead to depression and dementia [13].

Three main types of stroke are:

- **Ischemic Stroke**

Most strokes (87%) are ischemic strokes. An ischemic stroke happens when blood flow through the artery that supplies oxygen-rich blood to the brain becomes blocked. Blood clots often cause the blockages that lead to ischemic strokes [14].

- **Hemorrhagic Stroke**

A hemorrhagic stroke happens when an artery in the brain leaks blood or ruptures (breaks open). The leaked blood puts too much pressure on brain cells, which damages them [15].

❖ **Transient Ischemic Attack (TIA)**

A transient ischemic attack (TIA) is sometimes called a “mini-stroke.” It is different from the major types of stroke because blood flow to the brain is blocked for only a short time usually no more than 5 minutes[16].

Ischemic Heart Disease, also known as coronary heart disease, occurs when the blood flow to the heart muscle is reduced because of a partial or complete blockage of the arteries supplying it with blood.

coronary arteries is a system similar to tubes , as they progressively become blocked it means that the liquid flowing through them in this case blood, does not reach its destination, the heart, correctly[17].

1.4Epidemiology

Chronic cardiovascular diseases (CVDs) are considered the leading cause of mortality worldwide [18]. Mortalities by CVDs occur mainly due to coronary heart disease (CHD), stroke, rheumatic heart, and myocardial infarction (MI). In 2017 CVD caused an estimated 17.8 million deaths worldwide, corresponding to 330 million years of life lost and another 35.6 million years lived with disability[19]. According to epidemiological reports by the WHO in 2016, the highest prevalence rate of ischemic heart disease was observed in Saudi Arabia (46%) and Kuwait (41%)[20], In Iraq the epidemiological data on the incidence and prevalence of CAD as evidence of awareness are limited due to the unavailability of evidence-

based national guidelines for the management of cardiovascular disease and surveillance studies as compared to other Eastern Mediterranean countries [21].Study in 2014, cardiovascular disease mortality was estimated to account for 33% in Iraq. A better understanding of the burden of cardiovascular disease and associated risk factors in this region and increasing the public knowledge and awareness of (CAD) symptoms and its risk factors are highly imperative to control and prevent this disease[22].

1.5.Etiology and Risk Factors

Although CVD may directly arise from different etiologies include personal habits (smoking ,sleep ,fitness ,drinking alcohol) and clinical conditions(diabetes ,dyslipidemia and hypertension have been shown to be strongly associated with the etiology of CVD. Epidemiological studies have shown poor adherence of people to healthy lifestyle and lack of knowledge in adopting healthy alternatives In general [23].

1.5.1 Hypertension

The heart problems associated with high blood pressure relate to the heart's arteries and muscles. The types of hypertensive heart disease include[24].

Narrowing of the arteries. Coronary arteries transport blood to heart muscle. When high blood pressure causes the blood vessels to become narrow, blood flow to the

heart can slow or stop. This condition is known as coronary heart disease (CHD), also called coronary artery disease[25].

Coronary Heart Disease makes which difficult for heart to function and supply the rest of organs with blood, causes risk for heart attack from a blood clot that gets stuck in one of the narrowed arteries and cuts off blood flow to heart Thickening and enlargement of the heart[26].

1.5.2 Diabetes

Diabetes is a condition that causes high levels of glucose in the blood, is a risk factor for developing cardiovascular disease. High glucose levels can damage the artery walls and make the buildup of fatty deposits(atheroma) more likely. If these fatty deposits occur in the coronary arteries, they can lead to possible coronary heart disease and heart attack [27].

1.5.3 Smoking

Cigarettes are the major form of smoked tobacco.] Risks to health from tobacco use result not only from direct consumption of tobacco, but also from exposure to second-hand smoke .Approximately 10% of cardiovascular disease is attributed to smoking ,however, people who quit smoking by age 30 have almost as low a risk of death as never smokers[28].

1.5.4 Alcohol

The cardiovascular system is affected by alcohol. At the time of drinking, alcohol can cause a temporary increase in heart rate and blood pressure. In the long-term,

drinking above the guidelines can lead to on-going increased heart rate, high blood pressure, weakened heart muscle and irregular heartbeat .All of which can increase the risk of alcohol-caused heart attack and stroke[29].

1.5.5 Cholesterol

High levels of low-density lipoprotein (LDL) cholesterol – also known as “bad cholesterol” are linked to a range of cardiovascular diseases. Cholesterol is a fatty substance that is carried around the body by proteins. If too much LDL cholesterol is present, it can cause fatty substances to build up in the artery walls and lead to complications and CVD[30].

1.5.6 Obesity

Cardiovascular disease (CVD) mortality and morbidity has been shown to be elevated in individuals who are overweight, particularly with central deposition of adipose tissues. Abdominal obesity has been shown to be a risk factor for CVD worldwide.Obesity may be associated with hypertension, dyslipidemia, diabetes, or insulin resistance, and elevated levels of fibrinogen and C-reactive protein, all of which increase the risk of CVD events [31].

In addition to CVD, obesity has been shown to increase the risk of high blood pressure.[32] Persistent hypertension is one of the risk factors for stroke, myocardial infarction (MI), heart failure, and arterial aneurysm, and is a leading cause of chronic

kidney failure. Moderate elevation of arterial blood pressure leads to shortened life expectancy, which also increases the risk of heart diseases [31].

1.5.7 Family history

Family history of CVD could be used as a tool to identify which hypertensive individuals are at particularly high risk. A positive family history of CVD is an independent predictor of both myocardial infarction[32] and stroke.[33] Prognostic models for stroke that include both hypertension and family history have predictive value greater than models that include hypertension or family history alone. Among individuals with hypertension, family history of CVD has been shown to be independently associated with mortality by ischemic heart disease [34] and family history of CVD mortality with incidence ofoverall CVD .Therefore, a positive family history of CVD could reflect an underlying genetic predisposition related to CVD [35] .

1.6Aim of Research

The present study was designed to achieve the following aims:

1-To determine the role of phosphate in occurrence of cardiovascular disease in Babylon province

CHAPTER TWO

2.1.Subjects Groups and Study Design

This study was performed at the laboratory of Chemistry and Biochemistry Department, College of pharmacy, University of Babylon. The subjects in this prospective case control study, included a total of 44 subjects, 22 of these subjects suffering from cardiovascular disease and 22 volunteer apparently healthy control subjects .All samples were collected from Marjan and Imam Al-Sadiq teaching Hospitals.

Methods

Samples Collection

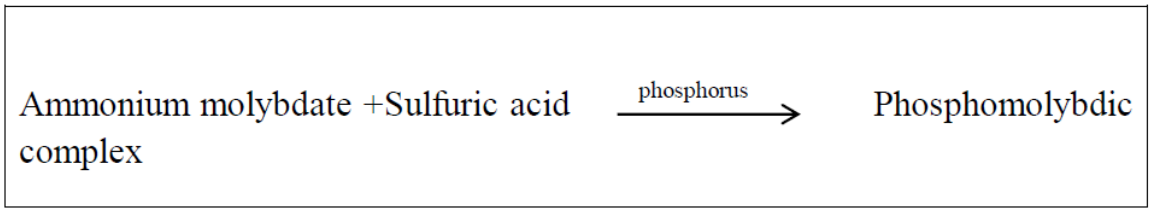
The blood sample was collected from participants vein by needle puncture about (3ml) in disposable plane tubes .whereas the blood sample in the plane tube were kept for about 5 minutes at room temperature to be clot and then centrifuged for 5 minutes at 3000×g subsequently

2.2.Determination of Phosphorous by Spectrophotometric

1. Test principle:

The basic principle is that each compound absorbs or transmitted its light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance. Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications.

Phosphate ion react with ammonium molybdate in acidic solution yielding yellow complex , which by the action of an alkaline buffer is reduced to blue molybedenum that is colorimetric measured as shown in the following equation .



2. Reagents preparation to measuring phosphate ion :

A. Catalystor- Store at 15- 25 °C

Reagent label bears expiration date. Polyvinylpirrolidone and hydroxylamine chloride (2.88 mol/L). This reagent may have a slight yellow color to orange.

B. Reagent Molybdate - Store at 15- 25°C

Reagent label bears expiration date. Ammonium molybdate (41 mmol/L) and sulfuric acid(900 mmol/L)

C. Buffer -Store at 15 - 25°C

Reagent label bears expiration date. Sodium carbonate (50 mmol/L) and sodium hydroxide (10mol/L)

D. Standard 5.0mg/dl - Store at 15- 25°C

Reagent label bears expiration date. In order to avoid evaporation of the Standard, keep the bottle tightly closed at 2- 8 °C

3. Procedure:

Every reagents were placed at room temperature until use

	Blank	Stander	Sample
Reagent	1000 µl	1000 µl	1000 µl
Stander	-	20 µl	-
Sample	-	-	20 µl

All of the previous ingredient were mixed and incubate for 5 minutes and measured the absorbance of standard, sample agents and the reagent blank.

Calculation of Phosphorous Ion concentration:

From the following formula the concentration of phosphate calculated

$$Phosphorous\ Con.\ mg/dl = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times 5$$

CHPTER THREE RESULTS AND DISCUSION

3.1 Demographic characteristics of Studied groups:

The results of the current comparative study of the patient and control groups were calculated statistically using the *t*-test to determine the difference in mean between the control and cardiovascular groups as well as the correlation between the different parameters of all patients. The total number of study groups was 44 adults (male and female) divided into equal two groups .The results of demographic data are shown in Table (3.1).

variabele	***group***	**Mean ±SE**	**P-Value**
Age	***c***	***30.7735±0.4882***	***0.58***
	p	**33.983±*0.7008***	

P-Value : Significant level less than 0.05

SE : Stander Error

3.1.1 Age

The results were expressed as mean and standard error (SE). There is **no** significant **(p> 0.05)** changes in age (as mean) between control and CVD patients, mean±SE for the case **)**and for control as shown in Table(3-1). This age matching helps to eliminate differences in parameters result that may originate due to the big variation in age. [36].

3.2. Estimation of Phosphate Ions in Serum:

The normal value of phosphate may vary from laboratory to another but it's normally similar to calcium range , usually from 3.4 to 4.5 mg/dl ().Also Phosphate concentration is characterized by a high physiologic variation, depending on age, gender, , the spectroscopic measurement of phosphate in controls and patients showed the following results .

3.3. Mean Differences of Phosphate Level According to Studied Groups:

As shown in table (3.2) the significant difference (**p<0.05**) in phosphate (as mean) between control and cardiovascular patients, mean ±SE for case and for control

Parameter	***group***	**Mean ±SE**	**P-Value**
Phosphate (mg/dl)	***c***	**2.141±0.102**	***0.03***
	p	**5.631±0.340**	

phosphate accumulation produces detrimental effects on cardiovascular system resulting in poor patient outcomes. The accumulation of phosphate occurs long before the rise in serum phosphate above the normal range. Increased concentrate extracellular phosphate is toxic to endothelial cells and induces vascular smooth muscle cell to osteogenic phenotype[37]**.**

previous publication have also shown a relation between serum phosphate and the risk for death and cardiovascular events in patients with prior Myocardial Infraction(MI). Similarly, higher phosphate concentrations are related to increased

CVD risk in individuals with no CKD [38] Most recent data suggest that serum phosphate is associated with greater left ventricular mass cross-sectionally and with increased risk for heart failure prospectively in a large community based sample of individuals without prior MI or CKD [39].

In the present study, phosphate level show significant elevation from baseline in CVD patients without CKD that supported by above observations. Many studies agreement With current results such as Yamamto, *et al*[40]. and ,Kwak SM *et al* [169].found relationship between phosphate and CVD

3.4.Conclusion

We noticed in previous research's suggest that not high phosphate levels, might be markedly asso- ciated with all-cause and cardiovascular mortality .

In our research we concluded that high phosphate product levels exhibit complex associations with traditional cardiovascular risk factors and outcomes.

3.5. Recommendation:

1.Study the effect of long-term particulate matters (PM) exposure and it's relationship to CVD, especially in men and the elder age.

2.Study the role calcium of cardiovascular disease that correlated with other chronic disease

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