A Biochemical Study to Evaluate Lipid Profiles and Sex Hormone In Sera of Females Patients Suffering CHD

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الخلاصة

تهدف هذه الدراسة لايجاد علاقة بين صورة الدهون والهرمونات الجنسية (تستوستيرون و الايستراديول) عند النساء المصابات بامراض القلب التاجية والمشخصات بواسطة القسطرة. بالاضافة الى تقدير حالات الحديد والانسولين وتاثير الكتلة. اجريت الدراسه على (٢٠) من النساء المصابات بامراض القلب التاجية تتراوح أعمارهن بين (٥٠-٧٠) سنة كما اشتركت(١٥) امرأه سليمة كمجموعة سيطرة. تم قياس تركيز الكوليستيرول الكلى والدهون عالية الكثافة(HDL) و الحديد وسعة الارتباط بالحديد الكلية (TIBC) باستخدام الطرق الطيفية اللونية بينما تم حساب كل من الدوال الآتية رياضيا: سعة الارتباط بالحديد غير المشبع (UIBC)، مخزون الحديد الكلي المخمن (ETIBS)، نسبة تشبع الترانسفرين (TS%)، وتركيز الترانسفرين والدهون واطنة الكثافة(LDL). تم قياس تراكيز الهرمونات الجنسية(تستوستيرون و الايستراديول) و الفرتين والانسولين في المصل بتقنية الايلايزا. أظهرت النتائج ارتفاعا معنويا ($p<0.05$) في صورة الدهون و مؤشرات الحديد لدى المرضى مقارنة بمجموعة السيطرة عدا مستوى الحديد ونسبة تشبع الترانسفرين (TS%)والدهون عالية الكثافة(HDL) والتي أظهرت انخفاضا معنويا ($p<0.05$). كما اظهرت نتائج الدراسة عدم وجود اي تغير معنوي في الهرمونات الجنسية (التستوستيرون و الايستراديول) لدى المرضى مقارنة بمجموعة السيطرة ($p>0.05$).اظهرت الدراسة ان هنالك علاقة ايجابية بين خزين الحديد ،الدهون الثلاثية ،الدهون واطنة الكثافة والكوليستيرول مع مقدار الانسداد. وعلاقة عكسية بين الايستراديول مع مقدار الانسداد. وبينت الدراسة عدم وجود علاقة بين تركيز الحديد، التستوستيرون، الانسولين وتركيز السكر مع مقدار الانسداد.يمكن الاستنتاج من هذه الدراسة أن النساء المصابات بامراض القلب التاجية لديهن احتمال عالي للتعرض إلى زيادة الدهون وتعقيداتها المختلفة في أجسامهن.

Abstract

The present work was designed to estimate the correlation between lipids profiles and sex hormones (Testosterone & Estradiol) in women with coronary heart disease undergoing catheterization in addition to the measurement of iron status ,insulin, insulin resistance & body mass index . Twenty female patients with coronary heart disease were participated in the present study, with age range 50-70 years. Fifteen apparently healthy females were selected as control group. Serum levels of total Cholesterol ,HDL ,iron and TIBC were measured spectrophotometrically while UIBC, ETIBS, TS% , transferring concentration and LDL were calculated mathematically. Serum ferritin, Testosterone , Estradiol & insulin were measured using ELISA technique. The results showed significant increase ($p<0.05$) in Lipid Profile & iron indices of CHD patients in comparing with healthy control group except iron , HDL,TS, which decreased significantly ($p<0.05$) in those patients in comparing with control group . Serum Testosterone , estradiol and insulin no significantly ($p<0.05$) in those patients in comparing with control group. The results revealed a positive correlation between iron store , TG ,LDL, Cholesterol with CHD , reversible correlation between estradiol with CHD and There is no statistically significant correlation noticed among iron , HDL, testosterone ,insulin, and blood sugar with CHD. It can be concluded that female patients with CHD undergo Catheterization are at high risk for hyperlipidemia .

Key Words: *Coronary Heart Disease, Testosterone , Estradiol & Lipids Profiles.*

Introduction

Coronary heart disease (CHD) is the narrowing or blockage of the coronary arteries, usually caused by atherosclerosis. Atherosclerosis is the buildup of cholesterol and fatty deposits (called plaques) on the inner walls of the arteries. These plaques can restrict blood flow to the heart muscle by physically clogging the artery or by causing abnormal artery tone and function. Without an adequate blood supply, the heart becomes starved of oxygen and the vital nutrients it needs to work properly. This can cause chest pain called angina. If blood supply to a portion of the heart muscle is cut off entirely, or if the energy demands of the heart become much greater than its blood supply, a heart attack (injury to the heart muscle) may occur. It is most commonly equated with atherosclerotic coronary artery disease, but coronary disease can be due to other causes, such as coronary vasospasm (1), where the stenosis to be caused by spasm of the blood vessels of the heart it is then usually called Prinzmetal's angina.(2)

Coronary artery disease has a number of well determined risk factors. The most common risk factors include smoking, family history, hypertension, obesity, diabetes, high alcohol consumption, lack of exercise, stress, and hyperlipidemia. Smoking appears to be the cause for about 54% of cases and obesity 20%. Lack of exercise has been linked to 7-12% of cases(3).

CHD develops 7 to 10 years later in women than in men and is still the major cause of death in women over the age of 65 years(4). The risk of heart disease in women is often underestimated due to the misperception that females are ‘protected’ against cardiovascular disease(4). Recent data from the National Health and Nutrition Examination Surveys (NHANES) have shown that over the past two decades the prevalence of myocardial infarctions has increased in midlife (35 to 54 years) women, while declining in similarly aged men(4). In a report from the European Heart Survey on stable angina pectoris it was found that women are less likely to be referred for functional testing for ischaemia and that a lower rate of diagnostic angiograms and interventional procedures are performed compared with men(5). The under-recognition of heart disease and differences in clinical presentation in women lead to less aggressive treatment strategies and a lower representation of women in clinical trials. Furthermore, self-awareness in women and identification of their cardiovascular risk factors needs more attention which should result in a better prevention of

cardiovascular events. In this review we summaries the major issues that are important in the diagnosis and treatment of coronary heart disease (CHD) in women .It is assumed that exposure to endogenous oestrogens during the fertile period of life delays the manifestation of atherosclerotic disease in women. Before menopause the CHD event rate in women is low and predominantly attributed to smoking(6). Women with an early menopause (<40 years) have a two-year lower life expectancy compared with women with a normal or late menopause(7) Data from the Framingham Heart Study suggest that a harmful cardiovascular risk profile may be more cause than consequence of age at menopause. In the Women's Ischemia Syndrome Evaluation (WISE) study it was shown that young women with endogenous oestrogen deficiency have a more than sevenfold increase in coronary artery risk[8]. Oestrogens have a regulating effect on several metabolic factors, such as lipids, inflammatory markers and the coagulant system. They also promote a direct vasodilatory effect through the α and β receptors in the vessel wall. Furthermore, signs of subclinical atherosclerosis, as visualised by intima-media thickness measurements, can already be found in women before menopause, especially when several CHD risk factors are present. Flow-mediated vasoreactivity by brachial artery measurements declines with the time elapsed since menopause. After menopause atherosclerotic plaque composition changes into more vulnerable lesions with inflammatory factors involved.

Kivimäki et al suggested Job stress appear to play a minor role accounting for about 3% of cases(9). In one study, women who were free of stress from work life saw an increase in the diameter of their blood vessels, leading to atherosclerosis(10).

Contrastingly, women who had high levels of work-related stress experienced a decrease in the diameter of their blood vessels. Also, having a type A behavior pattern, a group of personality characteristics including time urgency, competitiveness, hostility, and impatience (11) is linked to an increased risk of coronary disease(12).

Iron is essential to life, because of its unique ability to serve as both an electron donor and acceptor. Iron can also be potentially toxic. Its ability to donate and accept electrons means that if iron is free within the cell, it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to a wide variety of cellular structures, and ultimately kill the cell. To prevent that kind of damage, all life forms that use iron bind the

iron atoms to proteins. That allows the cells to use the benefits of iron, but also limit its ability to do harm (13).

The general mechanism that Fe(II) and certain Fe(II) chelates react with lipid hydroperoxides (ROOH), as they do with hydrogen peroxide, splitting the O–O bond. This gives RO•, an alkoxyl radical, which can also abstract H• from polyunsaturated fatty acids and from hydroperoxides (Figure 1). The resulting peroxyl radicals ROO• can continue propagation of lipid peroxidation. Oxidative stress also leads to considerable DNA damage (14) and to the polymerisation and denaturation of proteins (15) and proteolipids that can together form insoluble structures typically known as lipofucsin (16).

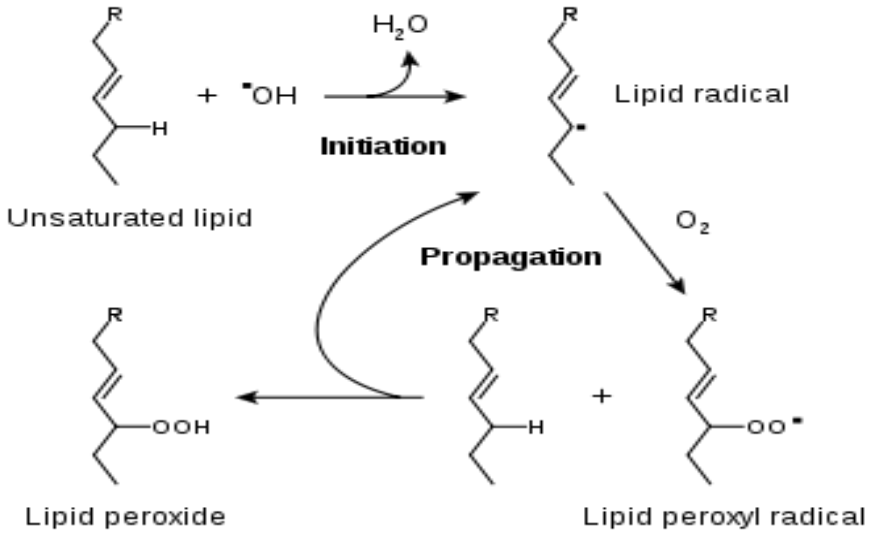

Fig. 1: Lipid Peroxidation Process (16).

The mechanism by which iron may stimulate atherogenesis . It is suggested that the catalytic role of iron in lipid peroxidation may be an important factor in the formation of atherosclerotic lesions. Normal native LDL-C can cross the arterial wall without causing damage to the vessel wall. Iron-catalyzed free radical reactions cause oxidation of LDL-C, which occurs in endothelial cells, smooth muscle cells, lymphocytes, or macrophages(17). Unlike native LDL-C, oxidized LDL-C is recognized by so-called scavenger receptors on tissue macrophages, followed by accumulation of lipids in these cells and the formation of foam cells, the characteristic cells of the fatty-streak lesions of early atherosclerosis(18).

Subjects and Methods

Subjects: twenty female patients with CHD undergoing Catheterization were participated in the present study. The age range was (50-70) years old. These patients were registered as CHD patients in " Catheterization Unit" at "AL-Sader Teaching Hospital" in Najaf city. The diagnosis was established by clinical symptoms, angiographical analysis . The present study excluded the patients with apparent diabetes mellitus **.** fifteen apparently healthy females were selected as a control group. Their age ranges were comparable to that of patients.

Blood samples*:* Blood samples were collected from individuals in the morning in plain tubes and the serum separated by centrifugation after clotting. Serum levels of iron were estimated using Ferrozine colorimetric method (19) , total Iron Binding Capacity (TIBC) were estimated colorimetrically by the following procedure (20).An excess of iron is added to the serum to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation, the iron in the supernatant was determined.

Unsaturated iron-binding capacity (UIBC), the amount of protein (apotransferrin) still available to bind iron, can be estimated from the formula:

UIBC=TIBC – Serum iron.

The ferritin quantitative kit based on a solid phase enzyme-linked immunosorbent assay (ELISA) was supplied by Monobind® Inc. USA. The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme horseradish peroxidase (HRP) conjugate solution.

Estimated Total Iron Body Stores (ETIBS) were calculated using the following formula (21):

ETIBS (in µmol) = (serum ferritin in µg/L) * 143

Transferrrin saturation percentage (TS%) was calculated from the following equation

TS%=(Serum Iron/TIBC)*100%

Transferrin concentration was calculated using the following formula (Morgan,2002):

Transferrin Conc. (g/L) =S.Iron (µmol/L)/ (TS%*3.98)

The formula is based on the maximal binding of 2 mol Fe^{3+}/mol of transferrin and a molecular weight of 79,570gm/mol for transferrin (22).

Serum hormones Serum ferritin, Testosterone , Estradiol & insulin

were measured using ready for use ELISA kits supplied by Monobind ,Total cholesterol: Enzymatic cholesterol kit were obtained from Biomareux® Company-France.Total cholesterol was determined by enzymatic methods using Roche-Diagnostics standards and kits (23).

Low Density Lipoprotein were estimated using the following formula:

LDL-c = TC (mmol/l)- VLDL-c(mmol/l)- HDL-c(mmol/l)

Serum High Density Lipoprotein cholesterol:

Principle (Biolabo /France):This reagent is only for treatment of specimens before determination of HDL-Cholesterol with a reagent for total cholesterol. Low density lipoproteins (LDL) ,very low density (VLDL) and chylomicrons from specimens are precipitated by phosphotungstic acid (PTA) and Magnesium chloride. HDL-C obtained in supernatant after centrifugation is then measured with Total Cholesterol reagent (i. e. : Choleterol kit) (24).

Biostatistical Analysis: The results were expressed as mean ± standard deviation. Pooled t-test was used for the comparison between the patients and control groups in the measured parameters were calculated using Microsoft Excell 2007 program.

Results and Discussion

The result in table[1] shows no significant change in the iron level of patient with CHD comparison to control group **.** in the other hand the result in same table shows a significant increase Ferritin levels , TIBC , UIBC , ETIBS , Transferrin in patient with CHD comparison to control group **.**These Results may explain as a difference in number of cases (Patient), environment of study in addition several of these cases in current study are have a chemical therapy which is lowering the level of iron in CHD.

Table 1: The Level Of Iron Indices In Patient With CHD As Compared Of Cholesterol With Their Control Group

Parameter	Patient Female=20	Control Female=15	P value
IRON. µg/dl	104.73±57.48	96.64±22.71	0.571742
FERR.ng/ml	71.35±32.43	50±28.35	0.049049 *
TIBC. µg/dl	445.7±159.8	352±76	0.029122 *
UIBC µg/dl	340.9±104.9	255.4±60.68	0.004862 *
TS%	21.58±6.15	28.49±7.91	0.009439 *
TRANSF.	1.12±0.4	0.88±0.19	0.029122 *
ETIBS ng/ml	10203±4639	7188±4055	0.049049 *

Early epidemiologic investigations [25,26 and 28] related body iron stores to CAD were criticized because of the use of nonspecific markers, such as serum iron and/or transferrin. Previous studies that used serum ferritin have been considered more informative because ferritin is strongly correlated with body iron stores in healthy individuals in addition the result of high level of ferritin and another iron status with normal level of iron this study is agreed with the previous study as well as study produced by WHO/UNICEF/UNU. That showed body ferritin levels, in contrast to haemoglobin, are not affected by residential elevation above sera level or smoking behavior. However, ferritin is a positive acute phase response protein whereby concentrations increase during inflammation and thereby no longer reflect the size of the iron store . This makes the interpretation of normal or high serum ferritin values difficult in areas of widespread infection or inflammation In the absence of inflammation or liver disease, high serum ferritin concentrations indicate iron overload (28).

Table 2: The Cholesterol , TG , LDL , VLDL , TC/HDL & TG/HDL Levels In Sera amples

Parameter	Patient Female=20	Control Female=15	P Value
CHOLES.	188±37	120±44	0.000049 *
TG	205±91	100±46	0.000114 *
HDL	39±**5**	49±19	0.068109
LDL	108±30	51±42	0.000159 *
VLDL	41±18	20±9	0.000114 *
TC/HDL	4.84±1.14	3.01±2.77	0.027281 *
TG/HDL	5.25±2.41	2.43±1.68	0.000273 *
LDL/HDL	2.79±0.94	1.53±2.61	0.091408

Table 2 shows significant increase in level of TG, Cholesterol , LDL_C & $VLDL_C$ in patients with CHD as compared with their control group in other hand the results appearance no significant change in HDL_C in CHD patients as paralleled with their control group. this consequence leads to the deposition of fat on the walls of the coronary arteries lead to difficulty pumping blood, shortness of breath and high blood pressure . Abnormal cholesterol levels (hypercholesterolemia) that is, higher concentrations of LDL-C and lower concentrations of functional HDL-C are strongly associated with hypertension ,cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke, and peripheral vascular disease. Since higher blood LDL-C, especially higher LDL particle concentrations and smaller LDL particle size, contribute to this process more than the cholesterol content of the LDL particles, LDL particles are often termed "bad cholesterol" because they have been linked to atheroma formation (29).

The results in **Table 3** refers no significant change in level of testosterone hormone and estradiol in patients in comparing with their control group. this result is agreement with study *(Cauley 1994*) reported that appears no relationship between estrone levels and degree of CAD in postmenopausal women(30).

Table 3: Levels Of Sex Hormone , Insulin And Blood Sugar In Sera Samples

Parameter	Patient Female=20	Control Female=15	P Value
Testosterone	0.61±0.97	0.32±0.22	0.213465
E2	9.59±11.1	10.7±16.6	0.821613
BMI	25.14±2.73	24.3±3.1	0.446439
INSU.	6.8±3.7	7.74±5.45	0.594089
Blood S.	95.35±10.75	92.7±11.8	0.506164

The result in **Table 4** shows a significant increase correlation between obstruction with ferritin , ETIBS , cholesterol , TG , LDL , VLDL & TC/HDL in patients with (CHD) . In the same table that shows a significant decrease correlation between obstruction with E_2 in patients with (CHD) .In the other hand there is no correlation between obstruction with Iron , TIBC , UIBC , TS% , Transferrin , HDL , TG/HDL , LDL/HDL , Testosterone , BMI& Insulin in patients with (CHD)**.**

Table 4: Shows The Correlation Between Obstruction Of Coronary With Iron Status & Lipid Profiles In Patients With Coronary Heart Disease

Parameter	R	p Value
IRON	0.139	0.559
FERR.ng/ml	0.941	0.018*
TIBC. µg/dl	0.125	0.600
UIBC µg/dl	0.114	0.633
TS%	0.728	0.083
TRANSF.	0.125	0.600
ETIBS ng/ml	0.018	0.941
CHOLES.	0.639	0.002 *
TG	0.437	0.054*
HDL	0.019	0.938
LDL	0.517	0.020*
VLDL	0.437	0.054 *
TC/HDL	0.524	0.018 *
TG/HDL	0.412	0.071
LDL/HDL	0.424	0.062
TESTO.	-0.154	0.516
E2	-0.722	0.000 **
BMI	0.166	0.483
INSU.	0.089	0.709
Blood S.	0.309	0.184

The result in **Table 4** shows a positive correlation between the iron store and CHD . The association of high iron stores and CHD was first suggested by *Sullivan1989* (31) .after that several observational and epidemiological studies have identified many new emerging potential risk factors like elevated blood levels of triglycerides and atherogenic lipoproteins fibrinogen. But apart from these there is strong evidence that oxidative free radicals have a role in the development of degenerative disease including coronary heart disease. Thus oxidized low density lipoprotein (LDL) exerts several potentially atherogenic effects (32) . and other study refers to the levels of serum ferritin are increased by two- to threefold from before menopause to after menopause. in

addition to estrogen deficiency, increased iron as a result of menopause could be a risk factor affecting the health of postmenopausal women (33).

The results in **Table 4** refer to reversible correlation between estradiol and abstraction in patients in comparing with their control group. this result is agreement with studies (Gerald B. Phillips et al (1996) were reported that appears to be the first to find a relationship between an endogenous sex hormone level and CHD in women . Have proved in their study a correlation between estradiol and CAD was in the negative direction, an observation consistent with the evidence that estrogen administration to postmenopausal women may prevent CAD (34). And this results disagreement with other studies*(Cauley JA et al 1994)* reported that appears no relationship between estrone levels and degree of CAD in postmenopausal women(35).

HDL-$_C$ is an independent risk factor for CHD. This study favours the view that decrease in estradiol level and associated decrease in HDL-C seen in postmenopausal women may be responsible for the increased risk of coronary heart disease after menopause. Oestrogens have a favourable effect on lipid profile, they lower LDL-C and elevate HDL-C (36) Oestrogens are thought to increase HDL cholesterol by reducing hepatic triglycerides lipase activity that catabolizes HDL (37).

Menopause is an oestrogen deficient state but unlike other hormone deficient states menopause is not a disease, every women who lives long enough becomes postmenopausal. After menopause the incidence of CHD rises to approach that for men of similar age (38). This is most probably due to oestrogen deficiency because in young woman where oestrogen production is high serum lipids are normal (39).But after menopause abnormal lipid levels and increased incidence of coronary heart disease show a possible relationship among oestrogen, normal lipid levels and a relative immunity to CHD (40).

HDL Cholesterol is a ‘good cholesterol’[9] and has an inverse relationship with CHD (41). It has been suggested that transport of cholesterol from peripheral tissues to liver for subsequent catabolism and excretion, is the function of plasma HDL-C. A reduction of plasma HDL-$_C$ may impair the normal clearance of cholesterol from arterial wall and thereby accelerate the

development of atherosclerosis (42).This study was designed to evaluate the effect of oestrogen deficiency due to menopause on serum HDL-total cholesterol level.

Table 5: Correlation Of Estradiol To Lipid Profiles

Parameter	R	p Value
Cholesterol	-0.502	0.024*
TG	-0.435	0.05*
HDL	-0.261	0.266
LDL	-0.312	0.181
VLDL	-0.435	0.05*

This result refers to a negative correlation between serum estradiol and serum total cholesterol in postmenopausal women (C.A.O Usoro 2006). Suggesting that estrogen deficiency increases serum total cholesterol and vice versa. and This result refers to a negative correlation between estradiol and LDL values in postmenopausal women (43). Similarly there is a negative correlation between estrodial and serum triglycerides (44). Hence present study supports the view that the elevated TC,LDL, TG in postmenopausal women greater than 45 years have been attributed to hormone changes and failure of follicular development, where the plasma estradiol levels that reduce the risk of coronary heart disease falls below the levels seen in premenopausal women(45). which predisposes for atherosclerosis.in the other hand no significant correlation between E_2 and HDL-$_C$, Hence present study supports the postmenopausal women greater than 45 years have been suffered from hormone changes and failure of follicular development despite of the normal value of HDL-$_C$ (45).

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