

Dependence of C-Reactive Protein & Cholesterol As Prognostic Factors for detective of some Heart Diseases in Duhok

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Abstract

This study had been designed to illustrate the relationship between immunological and biochemical abnormalities in patients with coronary heart disease (CHD) in Dohuk.

These patients were referred to the general Azadi hospital in Dohuk for investigation.

The present study include 80 patients with coronary heart disease (CHD) which consisting of 44 males (55%) and 36 females (45%). The Patients were divided into two groups, the first group included 42 (52.5%) patients with angina pectoris (AP), and the second group included 38 (47.5 %) patients with myocardial infarction (MI).In addition, 10 healthy volunteers were participate as control group. The age distributions of these groups were 56.4 ± 1.43 for CHD patients, 52.66 ± 1.204 for AP patients, 60.52 ± 2.35 for MI patients and 54.9 ± 2.167 for controls (healthy).

Two parameters level in serum of patients and healthy groups we study such as detection of C-reactive protein (CRP) using agglutination test and measurement of total cholesterol (C). The results revealed by statistical analysis the following data:-

- 1- It has been found that CRP was elevated in 66 (82.5%) patients with CHD which include 36 (85.71%) patients with AP & 30 (73.684%) patients with MI in comparison of healthy individuals 1 (10%) ($p < 0.05$).
- 2- The level of total cholesterol was high in patients with CHD $5.75 \text{ mmol/L} \pm 0.14$ which was $6.02 \text{ mmol/L} \pm 0.25$ in patients with AP & $5.44 \text{ mmol/L} \pm 0.09$ in patients with MI as compared with control (healthy) group $4.071 \text{ mmol/L} \pm 0.086$ and the difference highly significant ($P < 0.05$).

This result reflects the importance of using all parameters mentioned in this study to achieve good prognosis especially c-reactive protein in diagnosis of cardiac disease.

Introduction

Coronary heart disease occupies higher ratio look like angina pectoris (AP) and myocardial infarction (MI) which to be established when atheroma and coronary artery thrombosis occur.

Heart diseases are more remarkable reasons which lead to disability and death in different developing countries (Higgins, 1989).

Coronary Heart Disease increases during 50 years notably and usually accompanied with many variables like the imbalance in the level of fat, proteins and enzymes in the blood (Murray, 1997). There are many testes

which could through detection about levels of those variable, which reflects the range of damages which affects the heart and plays an important role in the diagnosis as reflect some of them dangerous level and damage range occurs to the heart and this lead to accurate description for remedy and wending the patient, but the need remains for research about many pointers which have sibylline value contributes in the avoidance of these conditions, which may prophesies with happening potential like these disease. High total cholesterol level and low density lipoprotein (LDL): high density lipoprotein (HDL) level are the major cause of atherosclerosis (Tuzcu, 2001), Recently scientist concentrate on the level of C- reactive protein as an indicator for several cardiovascular disease such as coronary heart disease (CHD) (Sakkinen *et al.*,2002).

In accordance with all mention above, this present study designed and comprised the following:

- 1- Measurement the levels of total serum cholesterol.
- 2- Measurement the levels of C-reactive protein in serum.
- 3- Explain the importance of C - reactive protein in the diagnostic and sibylline sides.

Materials and methods

Materials

1- Study samples:

This study include 80 patients (which consisting of 44 males 55% and 36 females 45%) with a history of coronary heart disease (angina pectoris &myocardial infarction) were enrolled in the study (age ranged 28 – 80 years). These patients were referred to the general Azadi hospital in Dohuk for investigations, in the period between July to October 2008. All patients were interviewed, and a detailed questionnaire was completed for each individual studied.

2- Blood samples collection:

Approximately 5 ml of peripheral venues blood sample was collected in a plain tube. The blood was allowed to coagulate at room temperature for 10-15 minute and then centrifuged at 3000 r. p. m. for 10 minute the resulting sera were collected and either used immediately or stored at -20C° when not used.

Methods

1- Laboratory investigations:

The following investigations were done for all patients included in the study:-

1-1 C- reactive protein detection:

1-1-1 Reagent composition:

R1: Latex Reagent (Blue dropper):

An aqueous suspension of blue latex particles coated with Anti-Human CRP antibody (1 drop 50µl).

R2: Diluent: Isotonic saline

Positive Control: A stabilized liquid containing CRP at a concentration of 30 ± 6 mg/l (1drop 50µl) was used.

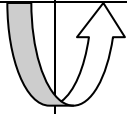
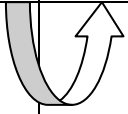
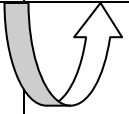
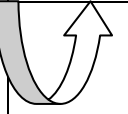
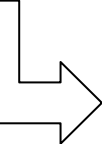
Negative Control: A stabilized liquid containing CRP at a concentration below 6 mg/l (1drop 50µl) was used.

1-1-2- Principle and procedures steps of test:

The CRP reagent contains latex particles coated with Anti- Human CRP antibody, when the reagent is mixed with serum containing CRP at a level greater than 6mg/l the particles will agglutinate; this is interpreted as being a positive sample.

Latex reagent	Sample	Negative control	Positive control
R1 50µl	50µl	50µl	50µl

The reagent may also be used for the semi-quantification of CRP. For this purpose the sample is diluted over a range of dilutions and each is tested qualitatively. The CRP level can be estimated from the last dilution with visible agglutination (Claus, 1976).

Section	1	2	3	4	5	6
isotonic saline	-	50µl	50µl	50µl	50µl	50µl
Serum	50µl	50µl				
Mix and transfer						
		50µl	50µl	50µl	50µl	50µl
Dilution	1:1	1:2	1:4	1:8	1:16	1:32
mg/L	6	12	24	48	96	192

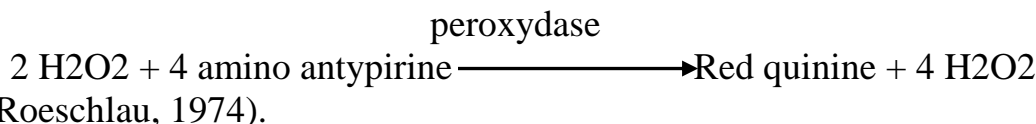
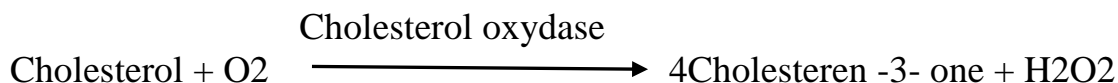
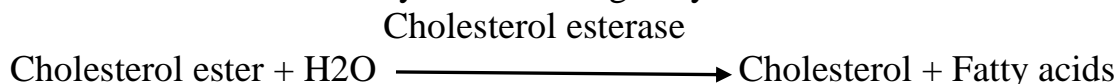
1-2- Measurement of total Cholesterol:

1-2-1- Reagent composition:

- R1: 4-Aminophenoazone 0.7 mmol/L
- Peroxydase 1250 UL/ml
- Cholesterol esterase 300 UL/ml
- Cholesterol oxydase 300 UL/ml
- R2: Phosphate buffer 80 mmol/L
- Phenol 16 mmol/L
- R3: Standard 200 mg/dl
- R1+R2: Working solution

1-2-2- Principle and procedures steps of test:

Cholesterol is measured by the following enzymatic reaction:-



Mix 100µl of serum with 1ml of working solution, and also mixed 100µl of standard with 1 ml of working solution. Incubate both tubes in 37C° for 10 minutes. Transfer 10 ml of each mixture to specific cup of spectrophotometer and measure the optical density at wave length 500 nm.

3- Statistical analysis:

The data was analyzed using the statistical package for social science, SPSS for obtained all the above analysis probability value P<0.05 or less was considered to indicate statistical significance.

Result and discussion

1- Testing for C- reactive protein CRP:

The result for this study proved the positively of specific agglutination of CRP in investigated groups of (CHD 66 (82%), AP. 36(85.71%) & MI 30 (78.94%), while the result of CRP investigation records very low positively in cases of control (healthy) group. The results of statistical analysis show that there is a significant deference (P< 0.05) in the comparison between control and CHD, while there is no significant deference between groups of patients (AP & MI) as shows in table (1).

Table (1): The results of agglutination test of CRP in control and patient with CHD (AP & MI).

Groups	CRP		Total No.
	-	+	
Control	9 90%	1 10%	10
CHD	14 17.5%	66 * 82.50%	80
AP	6 14.285 %	36 * 85.71 %	42
MI	8 21.052 %	30 * 78.94 %	38

* Indicate the presence of significant difference at the level P< 0.05

This study depends on the estimation of the semi quantitative method notify the increasing in mean level of CRP in patients with CHD 27.54 \pm 2.80, AP 28.54 mg/dl \pm 2.75 and MI 26.48 mg/dl \pm 3.34, has been found high disparities between mean levels of patient under studying and normal range (less than 6 mg/dl as in kit used) as show in table (2) that is considered as diagnostic prediction for differentiation between disease and healthy cases. In spite of there is no significant deference between its mean in both cases AP & MI. at P< 0.05 (table 2).

Table (2): The result of CRP (mean \pm S.E.M.) in patient with CHD (AP & MI)

Groups	No.	CRP (mg/dl)	
		Mean	\pm S.E.M.
CHD	66	27.54	\pm 2.80
AP	36	28.54	\pm 2.75
MI	30	26.48	\pm 3.34

P< 0.05

It has been also notified that the increasing of CRP level in male patients (28.11 mg/dl \pm 3.67) when comparing with female patients (27.09 mg/dl \pm 4.02), However, there was no significant deference between them, (Table 3).

Table (3): The result of CRP (mean \pm S.E.M.) in patient with AP & MI according to the gender.

Groups	No.	CRP (mg/dl)		
		Mean	\pm S.E.M	
Male	35	28.11	\pm 3.67	*
Female	31	27.09	\pm 4.02	*

* Indicate the presence of significant difference at the level P< 0.05

The mean level of CRP in patient's age group (less than 50 years was (24 mg/dl \pm 4.89), but obviously increased in 50_60 years to reach (29.76 \pm 2.89), whereas notify increase in patients larger than 60 years 24.92 (\pm 3.79), but there was no significant difference observed as shown in (Table 4).

Table (4): The result of CRP (mean \pm S.E.M.) in patient with AP & MI according to the age distribution.

Groups	No.	CRP (mg/dl)	
		Mean	S.E.M
Less than 50 years	13	24	4.89
50-60 years	35	29.76	2.88
More than 60	18	24.92	3.79

These results are corresponding with the results of many research (Christoph *et al.*, 2002; Tray *et al.*, 1997). were they refer to elevation in c-

reactive protein in patients with CHD, so it is corresponding with the results of researchers (Mendall *et al.*, 1997; Jame *et al.*, 2002) were they refer to the association between increasing in level of c- reactive protein and MI. Other research refer to mighty relationship between increasing in the c-reactive protein and cardiac events specially both stable and unstable angina. Raised plasma concentrations of CRP are associated with atherosclerosis of carotid, coronary, or lower limb peripheral arteries and with progression of atherosclerotic disease (Biasucci, 1999).

The inflammation may stimulate the atherosclerosis, and the high level of CRP and fibrinogen which associated with inflammation, incorporate in the increasing the risks of CHD events and it is reflect intension of inflammation that causes the atherosclerosis in coronary arteries, so it is play a role in the formation and the rupture of plaque. The peak CRP concentration retaliated with value of infarction as refer in (Anzai, 1997) therefore the measurement of CRP can use as a prediction to the value of infarction (Ridker, 2003).

2- Testing for total cholesterol measurement:

In this study, the total cholesterol were measured in serum of 80 patients with coronary heart disease CHD, 42 (52.5%) of them are affected with angina pectoris AP and 38 (47.5%) are with myocardial infarction MI. The result shown that the total of serum cholesterol have been increased in CHD patients with average $5.75 \text{ mmol/L} \pm 0.14$, whereas the mean of total serum cholesterol healthy control was $4.07 \text{ mmol/L} \pm 0.08$. So it was observed elevation in the mean of total cholesterol in patients with AP reach to $6.02 \text{ mmol/L} \pm 0.25$. The same finding can observe this elevation in the mean of total cholesterol in those patients with MI to reach $5.44 \text{ mmol/L} \pm 0.09$. The results of statistical analysis show that the elevation in total cholesterol in patients with CHD had high significant differences ($P < 0.05$) in comparison with the control subject table(5), while the total serum cholesterol in patient with AP and patient with MI, indicates show no significant difference between them.

Table (5): The total cholesterol (mean \pm S.E.M.) in serum of control and patient with CHD (AP & MI).

Group	No. of patient	Level of total cholesterol mmol/L
		Mean \pm S.E.M.
Control	10	4.07 \pm 0.08
CHD	80	5.75 \pm 0.14 *
AP	42	6.02 \pm 0.25*
MI	38	5.44 \pm 0.09 *

* Indicate the presence of significant difference at the level $P < 0.05$

It has been notified that the increasing in the level of total cholesterol in male patient with CHD (5.13 ± 0.14) when compared with female patient (5.27 ± 0.26), but there was no significant difference between them as show in (Table 6).

Table (6): The comparison in the result of total cholesterol (mean \pm S.E.M.) between males and females patients with CHD.

Groups	No.	Level of total cholesterol mmol/L
		Mean \pm S.E.M.
Male	47	5.13 \pm 0.14 N.S
Female	33	5.27 \pm 0.26 N.S

N.S.No significant difference was observed between male and female at the level $P < 0.05$.

In the comparison of the mean of total cholesterol patients with AP in the groups of age less than 50 year-old ($5.154 \text{ mmol/L} \pm 0.029$), 50-60 year-old ($5.918 \text{ mmol/L} \pm 0.17$) & more than 60 year-old ($6.596 \text{ mmol/L} \pm 0.70$), and in patient with MI in groups of age less than 50 year-old ($5.556 \text{ mmol/L} \pm 0.32$), 50-60 year-old ($5.511 \text{ mmol/L} \pm 0.13$) & more than 60 year-old ($5.332 \text{ mmol/L} \pm 0.14$). Statistical analysis of the results reveled that there was no significant difference between cholesterol levels versus age groups ($p < 0.05$), (table 7).

Table (7): The level of total cholesterol (Mean \pm S.E.M.) according to the age distribution

Age Group	Level of total cholesterol mmol/L (Mean \pm S.E.M.)					
	Patients					
	No.	AP =42	S.E.M	No.	MI=38	S.E.M
Less than 50 years	6	5.1541	± 0.0295	10	5.556	± 0.328
60-50 years	22	5.918	± 0.176	18	5.511	± 0.134
More than 60	14	6.596	± 0.78	10	5.332	± 0.147

These results are corresponding with the results of many research, (Gorden, 1981; Law, 1994; Wilson, 1998 and Henry *et al.*, 2002), these researchers are referring that the increase in total cholesterol is associate with risk of coronary heart diseases. High cholesterol (hypercholesterolemia) can cause the formation and accumulation of plaque deposits in the arteries. Plaque is composed of cholesterol, other fatty substances, fibrous tissue and calcium. When it builds up in the arteries, it results in atherosclerosis or CHD. Atherosclerosis can lead to plaque ruptures and blockages in the arteries, which increase the risk for heart attack, stroke, circulation problems, and dead. So the elevations of total cholesterol incorporate in the hypercoagulability, platelets reactivity and blood viscosity (Wald, 1995).

Cholesterol has a relationship with the increase of inflammation sensitive plasma protein, that increases the rate of cardiovascular disease event that associated with cholesterol (Engstom *et al.*, 2002), as in other studied and researchers indicated that the cholesterol constitute as risk factor of the atherosclerosis (Lukas *et al.*, 2002).

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اعتماد البروتين الفعال C والكوليسترول كعوامل استكشافية لبعض أمراض القلب

ملیكة قاسم نجیب البر واری أحمد سعید مرزا البر واری

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الخلاصة

صممت هذه الدراسة لتوضیح العلاقة بین الشذوذ المناعي و الكيموحيوي عند المرضى المصابين بمرض شرايين القلب الإكليلية في مدينة دهوك.

شملت الدراسة الحالية ۸۰ مريض مصاب بأمراض شرايين القلب الإكليلية و تضمنت ۴۴ (۵۵%) ذكور و ۳۶ (۴۵%) إناث ، و قسمت مجموعة المرضى إلى مجموعتين، الأولى تمثل المصابين بالذبحة الصدرية AP (Angina pectoris) وعددهم ۴۲ (۵۲,۵%) و الثانية المصابين بأحتشاء العضلة القلبية (Myocardial infarction) وعددهم ۳۸ (۴۷,۵%) ، بالإضافة إلى ۱۰ متطوعين من الأصحاء يمثلون مجموعة السيطرة في البحث.

كان توزيع الفئات العمرية لمجاميع البحث ۵۶,۴ ± ۱,۳۴ للمصابين بأمراض شرايين القلب الإكليلية و ۵۲,۶۶ ± ۱,۲۰ للمرضى المصابين بالذبحة الصدرية AP و ۶۰,۵۲ ± ۲,۳۵ للمرضى المصابين بأحتشاء العضلة القلبية MI و ۵۴,۹ ± ۲,۱۶ لمجموعة السيطرة (الأصحاء).

تم دراسة مؤشرين في مصل الدم لمجاميع المرضى و السيطرة و هي البروتين الفعال C (CRP) و الكوليستيرول الكلي C. و قد أوضحت النتائج بواسطة التحليل الإحصائي المعطيات التالية:-

۱- ارتفاع مستوى البروتين الفعال C لدى ۶۶ (۸۲,۵%) من المصابين بأمراض شرايين القلب الإكليلية، و الذي يتضمن ۳۶ (۸۵,۷۱%) مصابين بالذبحة الصدرية و ۳۰ (۷۸,۹۴%) مصابين بأحتشاء العضلة القلبية بالمقارنة مع مجموعة السيطرة (الأصحاء) ۱ (۱۰%). وكانت الفروقات معنوية عند احتمالية $P < 0.05$.

۲- ارتفاع مستوى الكوليستيرول الكلي عند المرضى المصابين بأمراض شرايين القلب الإكليلية ۵,۷۵ ملي مول / لتر ± ۰,۱۴ و التي تضمنت ۶,۰۲ ملي مول/ لتر ± ۰,۲۵ لدى المرضى المصابين بالذبحة الصدرية و ۵,۴۴ ملي مول/لتر ± ۰,۰۹ عند المرضى المصابين بأحتشاء العضلة القلبية مقارنة مع مجموعة السيطرة (الأصحاء) والتي كانت ۴,۰۷ ملي مول/لتر ± ۰,۰۸. وكانت الفروقات معنوية جداً عند احتمالية $P < 0.05$.

تعكس هذه النتائج أهمية استخدام المؤشرات التي ذكرت في هذا البحث لبلوغ مستوى جيد في الجانب التكهني و التشخيصي للمرض خاصة البروتين الفعال C .