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 mmol/L ± 0.14 which was 6.02 mmol/L ±0.25 in patients with AP & 5.44 mmol/L ±0.09 in patients with MI as compared with control (healthy) group 4.071 mmol/L ± 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 50 μl).
R2: Diluent: Isotonic saline
Positive Control: A stabilized liquid containing CRP at a concentration of 30 ± 6 mg/l (1 drop 50 μl) was used.
Negative Control: A stabilized liquid containing CRP at a concentration below 6 mg/l (1 drop 50 μl) 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 6 mg/l the particles will agglutinate; this is interpreted as being a positive sample.

<table>
<thead>
<tr>
<th>Latex reagent</th>
<th>Sample</th>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>50 μl</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
</tbody>
</table>

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).

1-2- Measurement of total Cholesterol:

1-2-1- Reagent composition:
R1: 4-Aminophenoazone 0.7 mmol/L
Peroxidase 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:-

\[
\begin{align*}
\text{Cholesterol esterase} & : \\
\text{Cholesterol ester} + \text{H}_2\text{O} & \rightarrow \text{Cholesterol} + \text{Fatty acids} \\
\text{Cholesterol oxidase} & : \\
\text{Cholesterol} + \text{O}_2 & \rightarrow \text{4Cholesten -3- one} + \text{H}_2\text{O}_2 \\
\text{Peroxidase} & : \\
2 \text{H}_2\text{O}_2 + 4 \text{amino antypirine} & \rightarrow \text{Red quinine} + 4 \text{H}_2\text{O}_2
\end{align*}
\]

(Roeschlau, 1974).

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 37°C 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>
<thead>
<tr>
<th>Groups</th>
<th>CRP</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>10%</td>
</tr>
<tr>
<td>CHD</td>
<td>14</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>17.5%</td>
<td>82.50%</td>
</tr>
<tr>
<td>AP</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>14.285 %</td>
<td>85.71 %</td>
</tr>
<tr>
<td>MI</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>21.052 %</td>
<td>78.94 %</td>
</tr>
</tbody>
</table>

* 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 ±2.80, AP 28.54 mg/dl ±2.75 and MI 26.48 mg/dl ±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 ± S.E.M.) in patient with CHD (AP & MI)

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>CRP (mg/dl) Mean ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
<td>66</td>
<td>27.54 ±2.80</td>
</tr>
<tr>
<td>AP</td>
<td>36</td>
<td>28.54 ±2.75</td>
</tr>
<tr>
<td>MI</td>
<td>30</td>
<td>26.48 ±3.34</td>
</tr>
</tbody>
</table>

P< 0.05

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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>CRP (mg/dl) Mean ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>35</td>
<td>28.11 ±3.67 *</td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>27.09 ±4.02 *</td>
</tr>
</tbody>
</table>

* 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 ±4.89), but obviously increased in 50_60 years to reach (29.76 ±2.89), whereas notify increase in patients larger than 60 years 24.92 (±3.79), but there was no significant difference observed as shown in (Table 4).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>CRP (mg/dl) Mean</th>
<th>S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 50 years</td>
<td>13</td>
<td>24</td>
<td>4.89</td>
</tr>
<tr>
<td>50-60 years</td>
<td>35</td>
<td>29.76</td>
<td>2.88</td>
</tr>
<tr>
<td>More than 60</td>
<td>18</td>
<td>24.92</td>
<td>3.79</td>
</tr>
</tbody>
</table>

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 mmol/L ± 0.14, whereas the mean of total serum cholesterol healthy control was 4.07mmol/L ± 0.08. So it was observed elevation in the mean of total cholesterol in patients with AP reach to 6.02 mmol/L ± 0.25. The same finding can observe this elevation in the mean of total cholesterol in those patients with MI to reach 5.44 mmol/L ± 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>
<thead>
<tr>
<th>Group</th>
<th>No. of patient</th>
<th>Level of total cholesterol mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± S.E.M.</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>4.07 ±0.08</td>
</tr>
<tr>
<td>CHD</td>
<td>80</td>
<td>5.75 ±0.14 *</td>
</tr>
<tr>
<td>AP</td>
<td>42</td>
<td>6.02 ±0.25*</td>
</tr>
<tr>
<td>MI</td>
<td>38</td>
<td>5.44 ±0.09 *</td>
</tr>
</tbody>
</table>

* 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 ± S.E.M.) between males and females patients with CHD.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Level of total cholesterol mmol/L Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>47</td>
<td>5.13 ±0.14 N.S</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>5.27 ±0.26 N.S</td>
</tr>
</tbody>
</table>

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 mmol/L ± 0.029), 50-60 year-old (5.918 mmol/L ± 0.17) & more than 60 year-old (6.596 mmol/L ± 0.70), and in patient with MI in groups of age less than 50 year-old (5.556 mmol/L ±0.32), 50-60 year-old (5.511mmol/L ±0.13) & more than 60 year-old (5.332 mmol/L ± 0.14). Statistical analysis of the results revealed 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 ±S.E.M.) according to the age distribution

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Level of total cholesterol mmol/L (Mean ± S.E.M.) Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Less than 50 years</td>
<td>6</td>
</tr>
<tr>
<td>60-50 years</td>
<td>22</td>
</tr>
<tr>
<td>More than 60</td>
<td>14</td>
</tr>
</tbody>
</table>

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).

References


اعتماد البروتين الفعال C والكولسترول كعوامل استكشافية لبعض أمراض القلب

مليكة قاسم نجيب البر واري
قسم علوم الحياة / كلية العلوم - جامعة دهوك
تاريخ الاستلام: 8/01/2010، تاريخ القبول: 12/01/2011

الخلاصة

صممت هذه الدراسة لتوضيح العلاقة بين الشذوذ المناعي والكيميائي عند المرضى المصابة بمرض شرايين القلب الإكليلية في مدينة دهوك. شملت الدراسة الحالية 80 مريض مصاب بمرض شرايين القلب الإكليلية وتضمنت 44 (55%) ذكور و36 (45%) إناث. وقامت المجموعة المرضى إلى مجموعتين: الأولى تمثل المصابين بالذبحة الصدرية (Angina pectoris) وعددهم 42 (52.5%) و الثانية المصابين بأحتشاء العضلة القلبية (Myocardial infarction) وعددهم 38 (47.5%)، بالإضافة إلى 10 متطوعين من الأصحاء يمثلون مجموعة السيطرة في البحث.

كان توزيع الفئات العمرية لمجامعي الدراسة ونهائيات البحث 56.4±52.6 سنوات للمصابين بأمراض شرايين القلب الإكليلية و53.2±45.9 سنوات للمصابين بالذبحة الصدرية (AP) و51.3±42.6 سنوات للمرضى المصابين بأحتشاء العضلة القلبية (MI) والذكور لجميع المجموعات. تم دراسة مؤشرين في مصل الدم لمجامعي المرضى والسيطرة وهما البروتين الفعال (CRP) و الكولسترول الكلي. وقد أوضحت النتائج بواسطة التحليل الإحصائي المعطيات التالية:

1- ارتفاع مستوى البروتين الفعال C لدى 66 (82.5%) من المصابين بمرض شرايين القلب الإكليلية، والذي يتضمن 36 (85.7%) مصابين بالذبحة الصدرية و30 (78.9%) مصابين بأحتشاء العضلة القلبية بمقارنة مع مجموعة السيطرة (الأصحاء) 1 (10%). وكانت الفروقات معنوية عند احتمالية P<0.05.

2- ارتفاع مستوى الكولسترول الكلي عند المرضى المصابين بمرض شرايين القلب الإكليلية، 5.65 مللي مول/لتر. و التي تضمنت 33.3% مللي مول/لتر، 55% مللي مول/لتر و11.5% مللي مول/لتر عند المرضى المصابين بأحتشاء العضلة القلبية مقابلة مع مجموعة السيطرة (الأصحاء) والتي كانت 4.08 مللي مول/لتر، كانت الفروقات معنوية جداً عند احتمالية P<0.05.

تعكس هذه النتائج أهمية استخدام المؤشرات التي ذكرت في هذا البحث لليدج مستوى جيد في الجانب التكنولوجي والتشخيصي لمرض خاصة البروتين الفعال C.