High sensitivity C-Reactive Protein is a Significant Predictor for Hypertension and Obesity in Iraqi Postmenopausal Women

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Summary:

Background: several new inflammatory markers have emerged as strong predictors of cardiovascular disease in healthy and non-healthy subjects, one of these markers is hs-CRP, which has been proposed as independent risk factor for cardiovascular disease, and it is positively associated with body weight. Little is known, however, about the utility of hs-CRP and other biomarkers in obese hypertensive Iraqi post menopausal women.The aim of study is to examine the hypothesis that there is a relation between obesity, hypertension and a chronic low-grade inflammatory status (represented by high hs-CRP).

Patients and Methods: A total number of 99 postmenopausal women classified into obese hypertensive group (case) and non obese non hypertensive group(control). For these groups, measurement of zinc, calcium, phosphorus, lipid profile, High sensitivity C–Reactive Protein, body mass index and waist circumference were done.

Results: The (Mean ± SD) of hs-CRP in the cases and controls were (5.74 ± 2.1) mg/land (2.1 ± 0.8) mg/l respectively, (P < 0.001).Calcium, phosphorus, zinc and triglycerides showed no statistical significance between the groups (Pvalue >0,05) while body mass index , waist circumference ,HDL ,LDL ,and total were statistically significant (Pvalue <0,05).

Conclusion: There is an elevated serum level of hs-CRP in hypertensive and obese subjects in comparison with low levels in control group (non obese non hypertensive) which suggest a role of hs-CRP in developing hypertension and obesity.

Keywords: high sensitivity C-Reactive protein, hypertension, obesity, postmenopause.

Introduction:

CRP consists of five identical, noncovalently associated ~23-kDa protomers arranged symmetrically around a central pore. Each protomer has a recognition face with a phosphocholine binding site consisting of two coordinated calcium ions adjacent to a hydrophobic pocket. The opposite face of the pentamer is the effector face, where complement C1q binds and Fcγ receptors are presumed to bind[1]. [Steven 2004][1]Although about two-thirds of the American population has plasma CRP levels under 3 µg/ml, circulating CRP levels under 10 µg/ml have historically been regarded as clinically insignificant. In recent years, a plethora of studies have demonstrated an association between slightly elevated CRP plasma levels, between 3 and 10 µg/ml, and the risk of developing cardiovascular disease, metabolic syndrome, and colon cancer. It is felt that many of these conditions involve a low level of underlying chronic inflammation that could be reflected by these minor

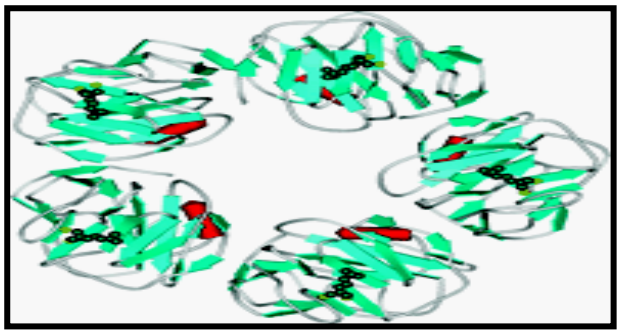

Figure 1 crystal structure of C-reactive protein complex with phosphocholine [1] , calcium iones are yellow & phosphocholine is green

increases. Experimental and clinical evidence accumulated since 1990 have established inflammatory processes as important contributors to atherogenesis as well as to the vulnerability of an atherosclerotic lesion to rupture or erosion .Based upon this evidence, protein markers of inflammation have been studied as noninvasive indicators of underlying atherosclerosis in apparently healthy individuals. The most extensively studied biomarker of inflammation in cardiovascular diseases is serum C-reactive protein (CRP), for which standardized high-sensitivity assays (hs-CRP) are widely available [2,3].CRP is an acute phase protein that is

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produced predominantly by hepatocytes under the influence of cytokines such as interleukin (IL)-6 and tumor necrosis factor-alpha [4].

Subjects & Study Design:

Sample subjects were randomly selected form the patients' relatives attending the external lab department at Al-Zahra' teaching hospital. Subjects recruited to full fill the criteria of being postmenopausal (at least 6-months history of amenorrhea not due to pregnancy and age range 47-66 years).

A total number of 99 postmenopausal women classified into obese hypertensive group (case) and non obese non hypertensive group(control). (table 1).

Obese hypertensive (BMI ≥ 30 kg/m^2 and blood pressure ≥ 140/90 mmHg ± history of taking anti hypertensive medications), [n = 65, Age = 56.02 ± 4.37, BMI = 35.68 ± 4.78; mean ± SD].

Non-obese normotensive (BMI < 30 kg/m^2- blood pressure < 140/90 mmHg), [n=34, Age = 56.443.17, BMI = 26.02 ± 3.05; mean ± SD].

To compare the significance of the difference in the mean values in comparison groups, student t-test was applied; $p < 0.05$ was considered statistically significant.

After an overnight fasting, venous blood samples were aspirated at 8:00-10:00 am, blood samples were collected into tubes and centrifuged after 30 minutes of collection, serum was removed and stored at -18 c until the time of assay.

High sensitivity C - reactive protein (Hs-CRP)

High sensitivity enzyme immunoassay for the quantitative determination of C - reactive protein concentration in human serum was used. **DRG International, Inc., USA.** This is done by ELISA test

Normal values (adult serum):***0.068-8.2mg/l***

Cutoff values (YEH2003):

Hs-CRP<1mg/l Low risk

From (1-3) mg/l Moderate risk

Hs-CRP>3mg/l High risk

Lipid profile tests: include total cholesterol(TC) ,triglycerides(TG) ,high density lipoprotein(HDL) and low density lipoprotein(LDL).These are measured by using enzymatic methods.

Calcium and phosphorus: are measured spectrophotometrically. Reference Values: Adults 8.62-10.23mg/dl, Adults 3-4.5mg/dl ,respectively.

Zinc: Measurement of zinc was done by using flame atomic absorption.

Normal level 80-120 µg/dl.

Results:

Obese (BMI>=30 kg/m^2) Hypertensive subjects (Cases) , number = 65,

Non-obese (BMI<30 kg/m^2) normotensive (Controls) , number = 34.

There is no significant difference in mean age, phosphorous, calcium, zinc and triglycerides between case and control groups (p value > 0.05).

Triglycerides is elevated in case group compared to control group but the difference is not statistically significant (p = 0.48) (table 1).Mean BMI is highly elevated in case group compared to control group (P < 0.001) (table 1). Mean waist circumference is higher in case group than in control group (P < 0.001) (table 1). Mean CRP is significantly elevated in case group compared to control group (P < 0.001) (table 1). Total cholesterol is significantly higher in case group compared to control group (P < 0.05) (table 1). Mean HDL is slightly lower in case group compared to control group (p < 0.05) (table 1). Mean LDL is significantly higher in case group than control group (p < 0.05) (table 1).

Table 1 mean ± SD values of age, phosphorous, calcium, zinc, triglycerides, BMI, Waist Circumference, CRP, cholesterol, HDL, and LDL in case (n=65) and control (n=34) subjects.

Characteristics	*Case group*	*Control group*	*P value*
Age (year)	56.02 ± 4.379	56.44 ± 3.17	0.628
Phosphorous (mg/dl)	3.411 ± 0.73	3.356 ± 0.52	0.707
Ca++ (mg/dl)	9.04 ± 0.89	9.35 ± 0.58	0.074
Zinc (mg/dl)	108.46 ± 20.7	103.13 ± 13.3	0.188
TG (mg/dl)	134.75 ± 52.33	126.88 ± 49.705	0.48
BMI (kg/m^2)	35.68 ± 4.78	26.06 ± 3.05	< 0.001
Waist Circumference	112.06 ± 8.66	87.43 ± 13.72	< 0.001
CRP (mg/l)	5.74 ± 2.15	2.14 ± 0.85	< 0.001
Cholesterol (mg/dl)	215.17 ± 33.3	197.81 ± 27.3	< 0.05
HDL (mg/dl)	51.31 ± 7.45	54.63 ± 5.091	< 0.05
LDL (mg/dl)	136.91 ± 32.87	117.81 ± 24.73	< 0.005

Discussion:

When hypertensive patients are compared to normals, one of the major differences is an increased prevalence of obesity [5]. Furthermore, weight gain appears to be a main determinant of the rise in blood pressure (BP) that is commonly seen with aging [6]. In addition to the risk of hypertension, obesity further enhances total cardiovascular risk by increasing LDL-cholesterol levels, reducing HDL-cholesterol levels, diminishing glucose tolerance, and predisposing to the development of left ventricular hypertrophy (independent of the systemic BP) [7,8]. The importance of these associations and their associations with inflammation are presented in the present study. It appears that the search for the link between hypertension and inflammation represents a new, stimulating field of research. However, some recent epidemiological studies showed that the presence of a chronic low grade inflammatory status can anticipate the future development of hypertension. This novel observation suggests that the increase in plasma levels of

inflammatory mediators observed among hypertensive patients cannot be solely attributed to the vascular damage induced by high blood pressure (Paolo 2006)[9]. In our study there is statistically significant relation between hypertension and CRP (MEAN±SD, 5.62±2.23 vs 3.02±1.92 in hypertensive and normotensive subjects respectively) and this is concordant with the results obtained in an analysis from the Women's Health Study of over 20,000 female health professionals in the United States with a baseline blood pressure <140/90 mmHg and no history of hypertension [10]. Serum CRP was measured at baseline and the women followed for a median of 7.8 years; hypertension developed in 11.5 percent. There was a progressive increase in the rate of developing hypertension with increasing values of serum CRP.This observation suggests a role for inflammation in the pathogenesis of hypertension. The association could be related in part to an association between serum CRP and the metabolic syndrome [11]. It is also possible that CRP may directly contribute by reducing nitric oxide synthesis in endothelial cells, leading to increased vascular resistance [12]. Since nitric oxide (endothelium-derived relaxing factor) is a vasodilator, its production would have to be reduced for it to have a pathogenetic role in the development of hypertension. Findings compatible with this hypothesis include the following [13]:

Endothelium-dependent vasodilation is impaired in essential hypertension; the mechanism for this may be a reduction in nitric oxide activity in response to shear stress, which is normally the most important stimulus for nitric oxide release [14].

Long-term blockade of nitric oxide (NO) synthesis or knock-out of the endothelial nitric oxide synthase genes leads to the development of hypertension [15].

Mice overexpressing the nitric oxide synthase gene are significantly hypotensive [16].

Our results about the relation between CRP and hypertension are different from that reached by [George2005][17] who found that CRP levels are associated with blood pressure ,pulse pressure and hypertension , but adjustment for life course confounding suggest that CRP levels do not lead to elevated blood pressure .Our results also indicate that CRP is strongly associated with BMI and these results are compatible with previous studies as seen in study done by (A.Elisabeth et al 1999) [18].Potential mechanisms relating the degree of obesity and circulating CRP levels have not clearly elucidated .It has been suggested that CRP level reflect the amount and the activity of pro inflammatory cytokines such as tumor necrosis factor –α ,IL-1, and IL-6 , which are implicated in the process of atherosclerotic plaque formation and acute coronary syndrome (Andre 2002et al)[19]. In this regard, IL-6, which is induced by both TNF-α and, IL-1, has been proposed to play a central role in the relationship between CRP and cardiovascular disease (Andre 2002)[19].IL-6 is secreted in several sites including activated macrophages and lymphocytes but also in adipose tissues. The contribution of adipose tissues in IL-6 secretion has been proposed to be the link between plasma CRP and adiposity, as CRP synthesis in the liver is largely under the control of IL-6.Thus, it is possible that this mechanism explains the higher CRP levels in obese patient.What support this hypothesis is the study done by (Brian L et al 2003)[20] who found that there is lower CRP levels in habitually physically active postmenopausal women and he also observed lower plasma IL-6 levels associated with physical activity, which suggest a lower level of inflammation in this population and this low level of CRP may be due to , at least in part ,to lower IL-6 concentration as result of less body fat in habitually active postmenopause when compared to sedentary women.

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