SERUM C-REACTIVE PROTEIN CONCENTRATION AND MEASURES OF ADIPOSITY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract

We investigated serum concentration of C-reactive protein (CRP) and measures of adiposity in 30 patients with type 2 diabetes mellitus (15 male, 15 female) and 30 age and sex-matched apparently healthy subjects.

CRP concentration was determined by laser nephelometry (BN II Analyzer) and CardioPhase high-sensitivity CRP (DADE BEHRING) was used as reagent which consists of polystyrene particles coated with mouse monoclonal antibodies to CRP.

Results have shown that serum CRP concentration in patients with type 2 diabetes mellitus was statistically significantly higher compared to control group of healthy subjects ($p<0,05$). Body mass index (BMI) correlated significantly with serum concentration of CRP in patients with type 2 diabetes mellitus ($r=0,614$; $p<0,001$). Statistically significant positive correlation was also found between waist to hip ratio and serum CRP concentration in patients with type 2 diabetes mellitus ($r=0,426$; $p<0,05$).

Elevated serum CRP concentration in patients with type 2 diabetes mellitus is probably caused by the presence of chronic low-grade inflammation in these patients. It is possible that determined increase of CRP concentration reflects activation of innate immune system components in patients with type 2 diabetes mellitus. Implications of established association between measures of adiposity and serum CRP level in type 2 diabetes mellitus remain unclear.

KEY WORDS: serum C-reactive protein, adiposity, type 2 diabetes mellitus

Introduction

C-reactive protein (CRP) is an acute phase protein and it represents extremely sensitive systemic marker of inflammation and tissue damage. CRP is primarily synthesized in hepatocytes and interleukin-6 (IL-6) is considered to be one of the major stimuli for CRP synthesis. Interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), corticosteroids and other hormones can also stimulate CRP production. Alongside with IL-6 and TNF-α which are mainly synthesized in adipose tissue, increased production of CRP may be the result of other adipocytokines action that is cytokines which originate from adipose tissue. Even though liver is primary site of CRP synthesis, its generation is possible in kidneys, neurons and bowels (1). Ability to activate complement system is one of the leading mechanisms through which CRP mediates in the process of phagocythosis. Because of these properties CRP is considered to be an important component of innate immune system. In vivo *studies* have shown that CRP has an important role in the elimination of pathogens and it also influences defensive functions of the host (2). Recent studies indicate that CRP might be activator of nonspecific immunity and modulator of specific immunity. Du Clos et al. (3) postulate that CRP serves as a link between innate and acquired immunity and that it may directly participate in amplification of immune response. Serum CRP concentration reflects the level of inflammatory processes in the body. Inflammation develops as a response to tissue damage, lipid peroxidation or infection and it manifest itself through binding of monocytes, macrophages as well as activated T-lymphocytes to the endothelium of blood vessels. Even though role of CRP in inflammation has been extensively investigated it still remains controversial. *In vivo* and *in vitro* studies have established that CRP has both pro-inflammatory and anti-inflammatory features. Many authors believe that CRP is not only a marker but an important mediator of inflammatory processes (4).

Numerous findings point to significant role of inflammation in all phases of atherosclerosis development and progression. It seems that an earliest event in atherogenesis is the dysfunction of endothelial cells. Endothelial dysfunction may be caused by different cardiovascular risk factors, metabolic disorders and systemic or local inflammation. Hypertension, diabetes mellitus, smoking, dyslipidemia, hyperhomocysteinemia as well as other factors have a significant role in the development of endothelial cells dysfunction. Endothelial dysfunction primarily manifests itself as nitric oxide (NO) deficiency. NO, generated in endothelium by endothelial nitric oxide synthase (eNOS), leads to vasodilatation and in the same time confronts the actions of powerful vasoconstrictors such as endothelin-1 and angiotensin II. In vitro studies on endothelial cell cultures incubated with human recombinant CRP have demonstrated that CRP causes decrease in eNOS expression and by that it also causes decreased production and bioactivity of NO. It is believed that through this manner CRP indirectly inhibits angiogenesis (5). Novel findings are suggesting that activation of innate immune system as well as low-grade inflammation may have an important role in the pathogenesis of type 2 diabetes mellitus (6). It has been demonstrated that systemic low-grade inflammation is associated with an increased risk for the development of type 2 diabetes mellitus. Inflammation is considered to be a link between known risk factors for diabetes mellitus (obesity, smoking and hypertension) and the development of type 2 diabetes mellitus. Thorand et al. (7) have shown that CRP is a significant predictor for type 2 diabetes mellitus development in middle aged men independent of classical risk factors such as triglycerides level, body mass index, fasting glucose or smoking. Several lines of evidence point to possible role of adipocytokines in the pathogenesis of diabetes mellitus. Main source of adipocytokines are visceral and subcutaneous adipose tissue. Adipocytokines, as cytokines, represent biologically active proteins with low molecular weight and with certain endocrine and metabolic functions by which they mediate in inflammation and insulin resistance. Studies have shown that resistin and TNF-α are associated with diminished insulin sensitivity, whereas two other adipocytokines, adiponectin and leptin are associated with increased insulin sensitivity (8). Ford et al. (9) demonstrated correlation between body mass index and serum CRP concentration which was independent of age, gender, ethnical background, level of education and presence of diabetes mellitus. It is still not established whether relationship between measures of adiposity and CRP is a consequence of increased production of pro-inflammatory cytokines from adipose tissue which in turn stimulate CRP production or decreased insulin sensitivity in conditions of adiposity leads to increased hepatic production of CRP. The aim of this study was to determine whether serum CRP concentration is increased in patients with type 2 diabetes mellitus as well as to test whether there is an association between CRP and measures of adiposity in these patients.

Materials and Methods

Two groups of subjects were enrolled in the present study: Control group of subjects consisted of 30 community dwelling age-matched persons both gender (15 male, 15 female) that were according to subjective and objective parameters of general health condition absent from pathophysiological changes. Group of subjects with type 2 diabetes mellitus consisted of 30 persons (15 male, 15 female) that were selected from the group of registrated patients with type 2 diabetes mellitus in Family Medicine Outpatient Clinic "Višnjik" in Sarajevo. Inclusion criteria were clinically diagnosed type 2 diabetes mellitus, with the duration of 5-10 years. Exclusion criteria were presence of coronary heart disease, peripheral blood vessel abnormalities and infection. Subjects that were taking medicaments such as statins and hormonal contraceptives which can influence CRP values were not included in the study. Informed consent was obtained from all subjects before the investigation and the Research Ethics Committee within the Faculty of Medicine at the University of Sarajevo approved the study protocol. Subjects underwent history, clinical examination and laboratory investigations. Non-fasting blood samples were drawn from antecubital vein into siliconized tubes. Samples were centrifuged at 4000 r.p.m. for 10 minutes to separate serum and were immediately used for the measurement of serum CRP concentration. Height was measured with stadiometer and weight was measured with a Toledo self-zeroing weight scale. From these two measurements, Body Mass Index (BMI) for each subject was calculated (weight in kilograms divided by height in meters squared). To determine waist to hip ratio (WHR), the standardized clinician`s tape measure was placed around the widest part of the hips and then placed around the narrowest part of the waist above the belly button. The ratio was determined by dividing the waist measurement by the hip measurement. Serum CRP concentration was determined by means of particle enhanced immunonephelometry with the use of BN II analyzer at the Institute of Clinical Chemistry and Biochemistry, University of Sarajevo Clinics Centre. CardioPhase high-sensitivity CRP (DADE BEHRING) was used as a diagnostic reagent. CardioPhase hsCRP consists of a suspension of polystyrene particles coated with mouse monoclonal antibodies to CRP. Reference interval for CRP with the use of this method is from 0 to 5 mg/l. Data are reported as mean ± SEM or median and interquartile range (IQR). Since CRP is highly skewed and the study sample is small, data were analyzed with Mann-Whitney U Test which is the nonparametric alternative for the unpaired t test. Associations between continuous variables were tested with Spearman's rank correlation analysis. Two-tailed p values <0,05 were considered statistically significant. Statistical analyses were performed using SPSS 12.0 statistical software system.

Results

The baseline characteristics of the two groups enrolled in the study are reported in Table 1. Number of subjects, age, body mass index, waist to hip ratio as well as the duration of diabetes in patients with type 2 diabetes mellitus are presented.

Variables	Control group (n=30)	DM type 2 group (n=30)
Age (years)	57,80±2,96	58,83±3,55
BMI (kg/m^2)	25,86±3,49	28,75±2,94
WHR	0,89±0,02	0,92±0,04
Duration of diabetes (years)	—	7,10±1,73

TABLE 1. Baseline characteristics of control subjects and patients with type 2 diabetes mellitus.

Data are presented as mean±SEM.
BMI: body mass index; WHR: waist-hip ratio; Diabetes Mellitus (DM) type 2 group

Figure 1. shows the median and inter-quartile range of serum CRP concentration in the control group and patients with type 2 diabetes mellitus. Serum CRP concentration in patients with type 2 diabetes mellitus was statistically significantly higher compared to the control group (p<0,05).

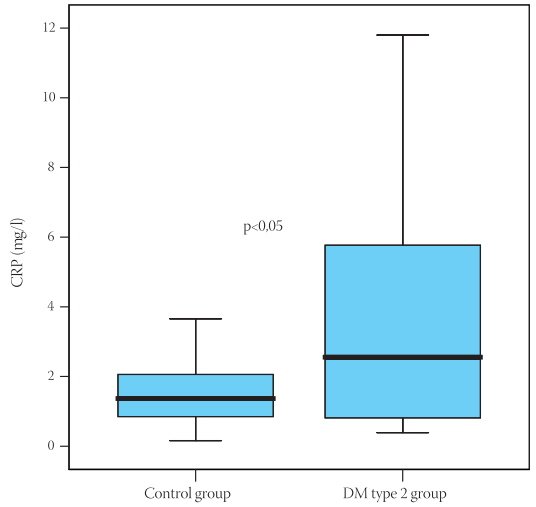

FIGURE 1. Serum C-reactive protein concentration in the control and Diabetes Mellitus (DM) type 2 group. Each bar shows upper and lower quartile, while the square and its central bar indicate inter-quartile range and median.

Correlation between body mass index (BMI) and serum CRP concentration in patients with type 2 diabetes mellitus is shown in Figure 2. There was a statistically significant positive correlation between BMI and serum CRP concentration in patients with type 2 diabetes mellitus (r= 0,614; p<0,001).

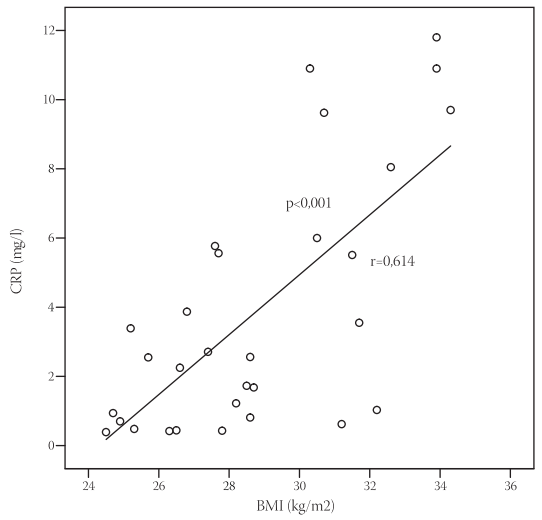

FIGURE 2. Correlation between body mass index (BMI) and serum C-reactive protein concentration in patients with type 2 Diabetes Mellitus.

As presented in Figure 3, there was a statistically significant positive correlation between waist to hip ratio (WHR) and serum CRP concentration in patients with type 2 diabetes mellitus (r= 0,426; p< 0,05).

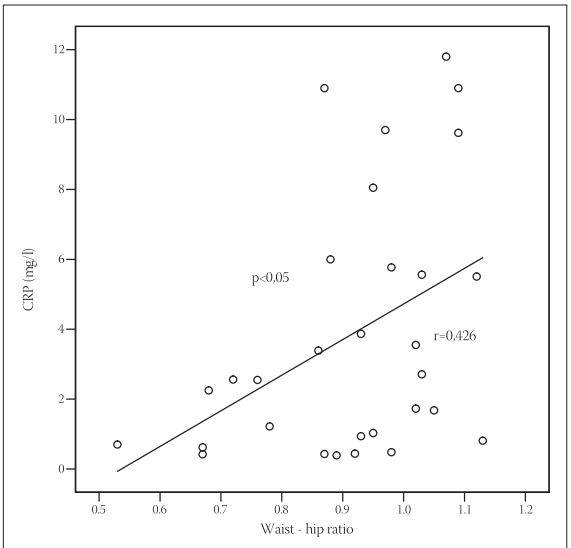

FIGURE 3. Correlation between waist-hip ratio and serum C-reactive protein concentration in patients with type 2 Diabetes Mellitus.

Discussion

Role of C-reactive protein, extremely sensitive but not specific marker of inflammation, in the pathogenesis of type 2 diabetes mellitus is a subject of extensive investigations.

Studies conducted with the purpose to elucidate role of inflammatory processes in the development of type 2 diabetes mellitus and consequent complications have given discordant results. This discordance of results is a consequence of the fact that it is still not established whether in persons predisposed for development of diabetes mellitus first develops chronic vascular inflammation which by numerous chain reactions leads to hyperglycemia or hyperglycemia causes chronic low-grade inflammation. Recent studies suggest that activation of innate immune system has an important role in the development of atherosclerosis and type 2 diabetes mellitus. Genetic factors, age, obesity, physical inactivity, smoking and stress are thought to be possible activators of innate immune system (6). Activation of innate immune system is followed with increased production of different pro-inflammatory cytokines (IL-6 and TNF-α) which in liver stimulate generation of acute phase reactants. Also, it is known that hyperglycemia stimulates release of inflammatory cytokines from different types of cells and leads to induction and secretion of acute phase reactants such as CRP. It has been demonstrated that determination of serum CRP levels may predict possibility for development of type 2 diabetes. Tan et al. (10) have shown that men with CRP concentration higher then 3 mg/l have 2,7 larger risk for development of type 2 diabetes compared to men whose CRP value was below 1 mg/l. As CRP is considered to be marker of low-grade inflammation, authors concluded that inflammation might be one of the mechanisms through which known risk factors such as hypertension, obesity and smoking lead to development of type 2 diabetes mellitus. In future, determination of serum CRP concentration should be used as additional measure in identification of persons that are in highest risk for development of type 2 diabetes mellitus and in whom preventive measures might give good results. Numerous investigations have proven that serum CRP concentration in patients with type 2 diabetes is increased. There is the discordance of opinions on mechanisms that lead to increase of CRP values in these patients. Most of the authors consider that increased serum CRP concentration might be the reflection of present low-grade inflammation which precedes the development of type 2 diabetes mellitus (11). However, results of Choi et al. (12) do not support the hypothesis that systemic inflammation caused by pro-inflammatory cytokines represents early metabolic dis-

order that precedes the development of type 2 diabetes. Authors suppose that elevated serum CRP concentration is probably reflection of insulin resistance that leads to increased production of acute phase reactants. Stehouwer et al. (13) have shown that increased albumin excretion in urine, endothelial dysfunction and chronic inflammation represent interrelated processes. These processes develop in parallel, progress in time and are strongly and independently related to death of patients with type 2 diabetes mellitus in whom conventional cardiovascular risk factors are present. Results of our study determined statistically significant increase of serum CRP concentration in patients with type 2 diabetes mellitus compared to control group of subjects ($p<0,05$). Obtained results are in the accordance with results of other authors that have also established elevated serum CRP concentration in patients with type 2 diabetes mellitus (14). Conversely, our results are not in the accordance with results of Pereira et al. (15) who did not find difference in values of CRP, LDL-cholesterol, total cholesterol and HDL-cholesterol between patients with type 2 diabetes mellitus and control group of subjects. Serum CRP concentration reflects the extent of its synthesis in hepatocytes. We believe that increased serum CRP concentration in patients with type 2 diabetes mellitus may partly be explained with its increased synthesis in the liver under the actions of adipocytokines from adipose tissue. Also, it is possible that elevated serum CRP concentration in patients with type 2 diabetes mellitus might be the reflection of existing atherosclerosis or decreased insulin sensitivity in these patients. It has been established that different adipocytokines lead to increase of CRP production in liver. CRP stimulates formation of pro-inflammatory mediators in endothelial cells, decreases bioactivity of vascular NO and activates smooth muscle cells of blood vessels. In the conditions of adiposity, adipocytes generate and secrete wide spectrum of inflammatory molecules such as IL-6 and TNF-α, which are main stimuli for hepatic production of CRP. Adipose tissue of obese persons is infiltrated with macrophages, which may be important source of local production of pro-inflammatory cytokines. Aldhahi et al. (16) consider that adiposity and diabetes are related through condition of chronic low-grade inflammation of adipose tissue that develops as a result of chronic activation of innate immune system.

Numerous authors investigated association between CRP and measures of adiposity. It is believed that visceral adiposity has a role in inflammatory processes as well as in metabolic abnormalities that characterize insulin resistance (17). Our results have shown statistically significant positive correlation between body mass index and serum C-reactive protein concentration in patients with type 2 diabetes mellitus ($r=0,614$; $p<0,001$). Also, statistically significant positive correlation was determined between waist-hip ration and serum C-reactive protein concentration in patients with type 2 diabetes mellitus ($r=0,426$; $p<0,05$). Reasons for the existence of association between CRP and measures of adiposity are still not fully understood but it seams probable that different and complex mechanisms may be in the background of this relationship. Because of the established ability of adipocytes to generate, in the conditions of adiposity, certain pro-inflammatory cytokines that have metabolic effects adiposity is now regarded as an inflammatory condition that has an important role in the pathogenesis of insulin resistance. This can at least partly explain association between on one side CRP, as a marker of inflammation and on the other side body mass index and waist-hip ration as clinical measures of adiposity in patients with type 2 diabetes mellitus. However, as it has been shown that obese persons have increased risk for the development of different chronic conditions, certain number of authors explains the correlation between CRP and measures of adiposity in patients with type 2 diabetes with the existence of different chronic conditions in these patients that are followed with elevated CRP values (9). It is possible that adipocytokines, probably through CRP, indirectly participate in the process of atherogenesis and atherosclerosis contributing to the development of endothelial dysfunction and insulin resistance. Further physiological, molecular and clinical studies are needed that will better explain role of adipocytokines and CRP in the etiology of type 2 diabetes mellitus. We believe that focus of future research should be targeted on explanation of interrelations between innate immune system activation, adipocytokines secretion, low-grade inflammation, hyperglycemia, endothelial dysfunction and atherosclerosis in patients with type 2 diabetes mellitus.

Conclusion

CRP as an acute phase reactant represents one of the components of nonspecific immunity but also the link between innate and acquired immune system. According to newest hypothesis, activation of innate immune system has an important role in the pathogenesis of type 2 diabetes mellitus. Increased serum CRP concentration determined in our study might be the reflection of activation of innate immune system components in patients with type 2 diabetes mellitus. Further studies are needed that will explain implications of established association between CRP and measures of adiposity in type 2 diabetic patients.

List of Abbreviations

CRP	-	C-reactive protein
IL-6	-	Intreleukin-6
IL-1	-	Intreleukin-1
TNF-α	-	Tumor Necrosis Factor-alpha
NO	-	Nitric Oxide
eNOS	-	endothelial nitric oxide synthase
BMI	-	Body Mass Index
WHR	-	Waist to hip ratio

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