

## The Correlation between C-Reactive Protein and Regulation of Glycemia in Type-2 Diabetic Patients

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### Article info

Received: 28/02/2017  
Accepted: 05/06/2017

### Keywords:

C-reactive protein  
inflammation  
Type 2 diabetes

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**Abstract:** Inflammation plays a significant role in the development of Type 2 diabetes mellitus (T2D). Studies have indicated that C-reactive protein (CRP) as inflammatory marker is an important risk factor for insulin resistance (IR) and T2D. The purpose of this study was to determine concentrations of fasting C-reactive protein, glucose, and hemoglobin A1c (HbA1c) in a total of 40 adults with Type 2 diabetes (40-60 years of the age) and 40 healthy subjects as control group (the same ages). We found that C-reactive protein concentrations in diabetic subjects were higher than those in control group. Also, our results have shown the significant association between CRP and hemoglobin A1c levels ( $p < 0.05$ ) and positive association with glucose concentrations ( $p > 0.05$ ) in T2 diabetics. A negative, but not significant correlation of CRP with glucose and hemoglobin A1c levels was demonstrated in controls. Therefore, our findings suggest an association between glycemic control and systematic inflammation in people with diagnosed diabetes.

## INTRODUCTION

The prevalence of Type 2 diabetes mellitus (T2D) as a metabolic disease with inappropriate hyperglycemia either due to deficiency of insulin secretion or reduction in the biologic effectiveness of insulin, is rapidly raising worldwide. Elevated inflammatory markers and altered adipokine concentrations have been observed in obese T2 diabetic patients. The main physiological abnormalities in Type 2 diabetic are insulin resistance (IR) and impaired insulin secretion but specific underlying mechanisms for the disease remain uncertain yet (Pradhan et al., 2001; Sattar et al., 2003; Luft et al., 2013). Some studies suggest that inflammation has a crucial intermediary role in pathogenesis of T2D, thereby linking diabetes with a number of commonly coexisting conditions thought to originate through inflammatory mechanisms. Chronic systemic inflammation can induce IR and is a key

mechanism linking obesity and diabetes. As a nonspecific marker of systemic inflammation, commonly elevated in human insulin resistant states, C-reactive protein (CRP) is an acute-phase reactant synthesized in the liver in response to cytokine, especially interleukin-6 (IL-6). As such it has been associated with hyperglycemia, IR and overt type 2 Diabetes and its complications (Qi et al., 2009; Bandyopadhyay et al., 2013; Sah et al., 2015).

C-reactive protein belongs to the pentraxin family of calcium dependent ligand-binding plasma proteins. The human CRP molecule is composed of five identical non-glycosylated polypeptide subunits each containing 206 amino acid residues. Most functions of CRP are easily understood in the context of the body's defenses to pathogen. Despite structural differences with immunoglobulin molecule, CRP shows similar functional properties with the immunoglobulins. Importantly, acute-phase CRP values show no relationship to fasting state or

diurnal patterns and have a long half-life. Analysis of serum concentration of CRP, may be performed using different instrumental methods of quantitative chemical analysis. ELISA (enzyme-linked immunosorbent assay), immunoturbidimetry, nephelometry, rapid immunodiffusion, and visual agglutination are methods used to measure of concentration of C-reactive protein. However, mostly used method nowadays is the method of immunoanalysis i.e. turbidimetry or nephelometry.

CRP, a robust clinical marker is easily measured and standardized in high-sensitivity immunoassay (detecting of CRP concentration <5mg/L), therefore providing similar results in fresh, stored, or frozen serum/plasma. Serum levels of CRP are independent of age and ethnicity. All of these factors make it a relatively stable serum protein compared with many other markers which can be used in screening for organic diseases; in monitoring the response to the treatment of inflammation and infection and detection of intercurrent infection in immune-compromised individuals, and in the few specific diseases characterized by modest or absent acute-phase responses (King *et al.*, 2003; Bandyopadhyay *et al.*, 2013; Mohammed *et al.*, 2015).

Therefore, the objective of this study was to determine the concentration of C-reactive protein in Type 2 diabetes and examine the relationship between CRP with glucose and hemoglobin A1c levels.

## EXPERIMENTAL

### *Subjects*

A total of 80 participants (40 control and 40 diabetic) have been screened for serum C-reactive protein (CRP), glucose, and hemoglobin A1c (HbA1c) after obtaining informed consent. Participants involved in this study were free of evidence of hepatitis B or C viral infection or active liver and kidney damage and were selected on the basis of presence of history of diabetes for more than five years.

Initial diagnosis of T2D was established by a specialist of internal medicine who used World Health Organization (WHO) criteria for diagnosis of the disease. All research involving human subjects and material derived from human subjects in this study was done in accordance with ethical principles outlined in World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000). Nondiabetic controls were of approximately same age (40-60 years old), with normal glucose tolerance (fasting plasma glucose less than 6.2 mmol/l and two hours postprandial glycemia less than 7.8 mmol/l).

### *Sample Analysis*

Blood samples from all of the participants in the study were collected into siliconized tubes (BD Vacutainer Systems, Plymouth, UK).

Blood samples were withdrawn by using sterile syringe from the 12 to 14 hours of overnight fasting diabetic and control patients in the morning. All samples, after collection in sterile tubes were centrifuged at 3000 rpm for 10 minutes and serum was stored at 4°C. Fasting blood glucose concentration was measured by an enzymatic glucose hexokinase method while ion-exchange high-performance liquid chromatography was used for measurement of hemoglobin A1c (HbA1c). C-reactive protein (CRP) was measured by Sandwich Enzyme Linked Immunosorbent Assay Method (immunoturbidimetric method) applied on BT PLUS 2000-Biotechnic Instruments Bioanalyzer (Rome, Italy).

### *Immunoturbidimetric assay for CRP*

Briefly, the test samples were treated with a specific antibody to human CRP in a suitable buffer. The turbidity induced by the formation of immune complexes was measured at 546 nm, and the values were then calculated automatically from a known standard. All the assay steps were performed automatically by the instrument. A commercial control serum was used to verify the assay performance.

### *Statistical analysis*

All statistical analyses were done by SPSS (version 17.0 for Windows, SPSS Inc; Chicago, IL, USA). *P* values smaller than 0.05 were accepted as significant.

Within the programme, nonparametric Mann-Whitney U-test was used in order to estimate differences in glucose, hemoglobin A1c, insulin, and CRP concentration between groups. Spearman's correlation coefficient was calculated in order to analyze the relationships between the study variables.

## RESULTS AND DISCUSSION

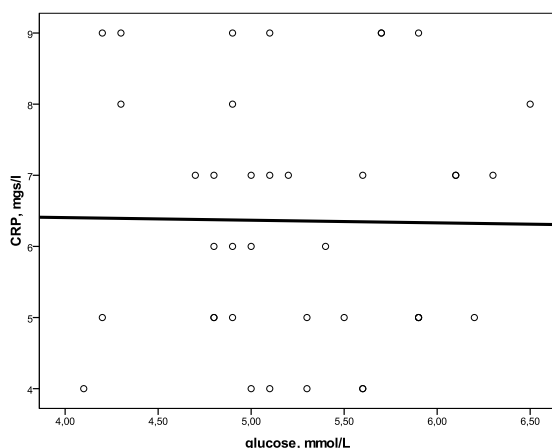
The study was conducted on 80 participants of both genders and of similar age. In our study, C-reactive protein concentrations, in Type 2 diabetes patients were slightly higher when compared to control subjects (6.33mg/L and 5.55 mg/L, respectively). Fasting serum concentrations of glucose and hemoglobin A1c (HbA1c), as expected, were significantly higher in T2D group of patients compared to controls. Results for tested clinical parameters in study participants are presented in Table I.

**Table 1.** Concentrations of testing parameters in studied participants.

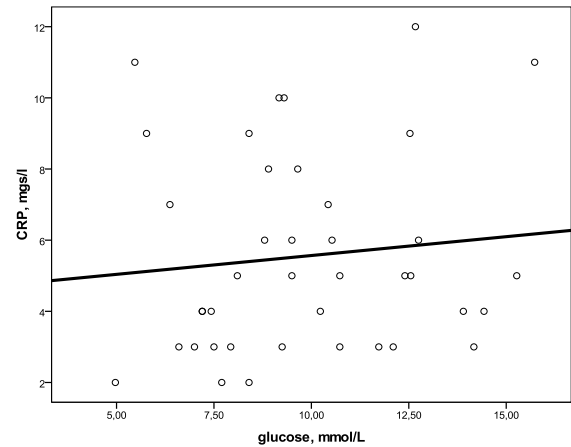
Parameter	Controls	Type 2 Diabetics	P values	Normal range
<b>Fasting glucose, mmol/L</b>	5.3	10.3	$p < 0.001$	3.9-7.2 mmol/L
<b>C-reactive protein, mg/L</b>	5.55	6.33	$p < 0.05$	Low risk $\leq 2$ mg/L Moderate risk 2-6mg/L High risk $\geq 6$ mg/L
<b>Hemoglobin A1c, %</b>	4.5	6.9	$p < 0.001$	6.0%

C-reactive protein a marker of systemic inflammation is emerging as an independent risk factor for insulin resistance, and cardiovascular disease. Elevated CRP levels have also been linked to an increased risk of later development of diabetes. Furthermore, CRP levels are higher in people with diabetes compared with those without diabetes. These findings are in line with our results (King et al., 2003; Sattar et al., 2003; Luft et al., 2013).

One of the goals of the study was to investigate the relation between CRP and hemoglobin A1c (HbA1c) in adults with diabetes. Recent research evidence supports a link between hyperglycemia and inflammation. Such evidence is consistent with the findings in the current study, which further documented the association between hyperglycemia and inflammation in adults with diabetes (Figures 1 and 2).

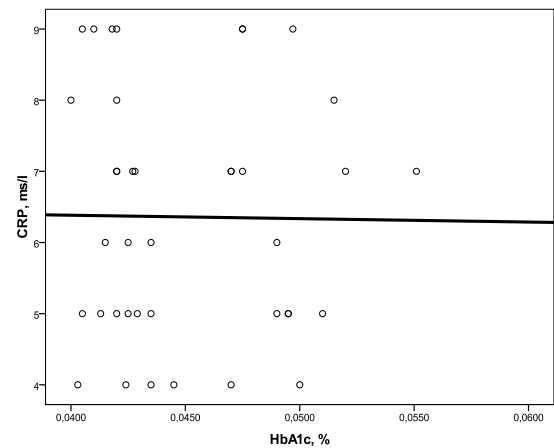


**Figure 1a**

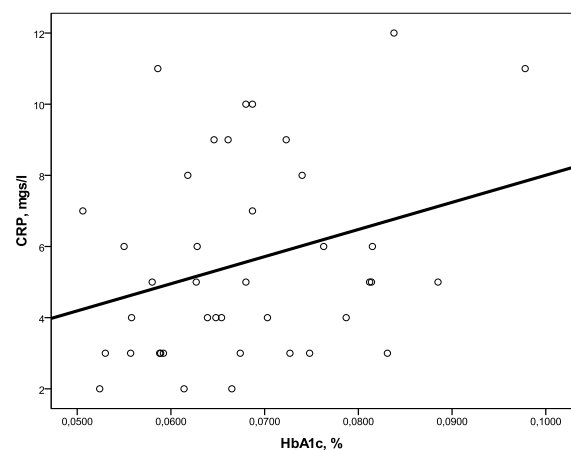


**Figure 1b**

**Figure 1.** Spearman’s correlation coefficient between glucose and CRP levels in studied patients (1a controls:  $r = - 0.007, p > 0.05$ ; 1b diabetics:  $r = 0.179, p > 0.05$ )



**Figure 2a**



**Figure 2b**

**Figure 2.** Spearman’s correlation coefficient between hemoglobin A1c and CRP levels in studied patients (1a controls:  $r = - 0.073, p > 0.05$ ; 1b diabetics:  $r = 0.371, p < 0.05$ )

CRP is known to be higher in people with impaired glucose tolerance and frank diabetes. Furthermore, increased CRP has been found to be a risk factor for later development of diabetes. De Luca *et al.*, 2008; Ehiaghe *et al.*, 2013 and Rajkovic *et al.*, 2014, found links between CRP and insulin resistance. Other studies have related hyperglycemia to inflammation by demonstrating simultaneous inflammation, endothelial dysfunction, and insulin resistance at the physiologic level. In this study, the likelihood of elevated CRP levels increased with increase in hemoglobin A1c levels.

## CONCLUSION

This is one of the first studies addressing state of hyperglycemia and its correlation to C-reactive protein levels. The study indicated that concentrations of the CRP, as a pro-inflammatory cytokine, were higher in diabetic patients compared to controls. In addition, we observed that CRP concentration significantly correlated with glycemic control i.e. hemoglobin A1c in these patients. However, due to possible influence other factors on inflammation, further studies should be observed this problem more clearly and include of high number of patients.

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## Summary/Sažetak

Inflamacija ima značajnu ulogu u razvoju Tip 2 diabetes mellitusa (T2D). Istraživanja su pokazala da je C-reaktivni protein (CRP) kao inflamatorni marker važan faktor rizika za insulinsku rezistenciju (IR) i T2D. Cilj ovog rada bio je odrediti koncentraciju natašte C-reaktivnog proteina, glukoze i hemoglobina A1c (HbA1c) kod ukupno 40 odraslih osoba s Tip 2 dijabetesom (40-60 godina starosti) i 40 zdravih ispitanika kao kontrolne grupe (iste starosne dobi). Nađeno je da je koncentracija C-reaktivnog proteina kod dijabetičara veća od izmjerene koncentracije u kontrolnoj grupi. Takođe, naši su rezultati pokazali i statistički značajnu korelaciju između CRP i vrijednosti hemoglobina A1c ( $p < 0,05$ ) i pozitivnu asociranost s koncentracijama glukoze ( $p > 0,05$ ) kod T2 dijabetičara. Pokazana je negativna korelacija ali ne i statistički značajna između CRP i vrijednosti glukoze i hemoglobina A1c u kontrolama. Dakle, naši rezultati ukazuju na povezanost regulacije glikemije i sistemske inflamacije kod osoba s dijagnosticiranim dijabetesom.