Journal of Clinical Laboratory Analysis 30: 672–676 (2016)

Association of Serum hs-CRP Levels With the Presence of Obesity, Diabetes Mellitus, and Other Cardiovascular Risk Factors

Mahmoud Ebrahimi,[1,†] Ali Reza Heidari-Bakavoli,[1,†] Sara Shoeibi,[2] Seyed Reza Mirhafez,[3] Mohsen Moohebati,[1,4] Habibollah Esmaily,[4,5] Hamed Ghazavi,[2] Maryam Saberi Karimian,[3] Seyed Mohammad Reza Parizadeh,[4] Maryam Mohammadi,[4] Hossein Mohaddes Ardabili,[4] Gordon A. A. Ferns,[5] and Majid Ghayour-Mobarhan[1,4*]

[1]*Cardiovascular Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[2]*Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[3]*Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran*

[4]*Department of Biostatistics and Epidemiology, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran*

[5]*Division of Medical Education, Brighton & Sussex Medical School, Brighton, Sussex, UK*

Background: Diabetes mellitus remains one of the major health problems of the 21st century and is associated with comorbidities including obesity and metabolic abnormalities. The study was conducted to evaluate serum high-sensitivity C-reactive protein (hs-CRP) levels, as a marker of inflammation, in a large sample of Iranian population without a history of cardiovascular or inflammatory disease and cancer, and to relate this to fasting blood glucose (FBG) and the presence of diabetes mellitus. *Methods:* The study consisted of 7,762 subjects divided into four groups—nonobese/nondiabetic, obese/nondiabetic, nonobese/diabetic and obese/diabetic—based on the BMI classification and their FBG. Anthropometric characteristics were measured and blood was collected for the evaluation of fasted lipid profile, FBG and serum hs-CRP levels. *Results:* Several clinical and biochemical characteristics were significantly different among the four groups: FBG, $P < 0.001$; total cholesterol (TC), $P < 0.001$; and triglyceride (TG), $P < 0.001$. The subjects with a serum hs-CRP >3 mg/dl had higher TC ($P < 0.001$), low-density lipoprotein cholesterol (LDL-C, $P < 0.001$), TG ($P < 0.001$), fat percentage ($P < 0.001$), and systolic and diastolic blood pressure ($P < 0.001$) compared with subjects with a serum hs-CRP <3 mg/dl. Multivariate analysis showed FBG, LDL-C, and waist circumference (WC) associated with increased serum hs-CRP levels ($P < 0.001$). *Conclusions:* FBG, LDL-C, WC and gender are independently associated with serum hs-CRP concentrations. J. Clin. Lab. Anal. **30**:672–676, 2016. © 2016 Wiley Periodicals, Inc.

Key words: high-sensitivity C-reactive protein (hs-CRP); obesity; diabetes; anthropometric characteristics

Recent predictions estimate that the prevalence of diabetes will increase to 438 million by 2030 (1). The

[†]These authors contributed equally to this work.

Grant sponsor: Mashhad University of Medical Sciences.

*Correspondence to: Majid Ghayour-Mobarhan, Biochemistry and Nutrition Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad 99199-91766, Iran. E-mail: ghayourm@mums.ac.ir

Received 9 September 2015; Accepted 16 November 2015
DOI 10.1002/jcla.21920
Published online in Wiley Online Library (wileyonlinelibrary.com).

prevalence of type 2 diabetes is estimated to be 14.6% in Asia, 40% in the Middle East, and 14.5 % in Iran.

Type 2 diabetes mellitus is associated with a number of other metabolic disorders including elevated triglycerides (TGs), low high-density lipoprotein cholesterol (HDL-C) and central obesity (2, 3). It is also associated with disorders related with protein, carbohydrate, and fat metabolism. Reduced glucose uptake by muscle and adipose tissue can be seen in people with diabetes, which is a consequence of chronic hyperglycemia and eventually tissue damage and chronic vascular problems (4). The absolute number of people with diabetes is increasing due to population growth, ageing of the population, urban settlement, and factors such as obesity (5) and lack of physical activity (6).

Previous research has proposed that inflammation resulting from β-cell dysfunction plays an important role in the pathogenesis of diabetes mellitus and links diabetes with the inflammatory responses. C-reactive protein (CRP) is a classic acute-phase protein that is produced in the liver under the stimulation of cytokines such as tumor necrosis factor, interleukin-1 (IL-1), and IL-6 (2,4,7). IL-6 is expressed in adipose tissue in inflammatory conditions. It regulates the expression of CRP in hepatocytes at the transcriptional level (8). CRP is a pentameric and nonimmunoglobulin protein having five identical subunits that have been introduced as the most important marker of inflammation. Serum levels of high-sensitivity CRP (hs-CRP) can be measured at very low levels using highly sensitive assays and may indicate increased inflammatory activity in the vessel wall. Thus, chronic systemic inflammation has been identified as an associated factor in the metabolic syndrome and diabetes mellitus (2, 9). An understanding of the pathogenesis of type 2 diabetes mellitus, including its interaction with inflammatory responses, may provide a better insight into preventing its complications. We decided to study the factors that are associated with serum hs-CRP in a population without cardiovascular (CVD) and inflammatory disease or cancer and its association with diabetes and adiposity.

This study was a hospital-based cross-sectional study conducted at Ghaem Nutritional Clinic, Mashhad, Iran and included a total of 7,762 subjects between the ages of 18 and 65. Study participants were recruited using a random sampling method based on the selection criteria and individuals were categorized into four groups based on the body mass index (BMI) and presence or absence of diabetes.

Group I ($n = 4{,}953$): This group consisted of people with normal weight (BMI = 18–25 kg/m^2) without diabetes (fasting blood glucose (FBG) ≤ 100 mg/dl). They were free from any problems that might affect the parameters under study. This group was recruited from a normal population.

Group II ($n = 2{,}116$): This group consisted of patients who were obese (BMI ≥ 30 kg/m^2) but without diabetes (FBG ≤ 100 mg/dl).

Group III ($n = 448$): This group consisted of patients with type 2 diabetes (FBG ≥ 126 mg/dl) but were nonobese (BMI = 18–25 kg/m^2).

Group IV ($n = 245$): This group consisted of obese individuals (BMI ≥ 30 kg/m^2) with diabetes (FBG ≥ 126 mg/dl).

Individuals with known CVD, inflammatory disease, stroke, cancer and systemic diseases such as lupus and kidney failure were excluded from this study. Pregnant and lactating women and individuals who were taking dietary supplements were also excluded.

The purpose and risks of the study were explained carefully to all eligible subjects. Informed written consent was obtained from all subjects. The study was approved by the ethical review board in Mashhad University of Medical sciences.

A questionnaire was designed to obtain personal information according to the selection criteria of the study. The questions and anthropometric measurements focused on baseline information and background characteristics of diabetes including height, weight, waist, hip, waist–hip ratio, and fat percentage during a standard interview.

Blood samples (20 ml) were collected after 12-h fasting, for the measurement of lipid profile and FBG serum or plasma, and separated and stored at –80°C temperature till analysis was done. Serum was used for the assay of lipid profile and hs-CRP. Blood glucose, serum cholesterol, TGs, low-density lipoprotein (LDL) and HDL were determined by enzymatic colorimetric assays using standard kits (Pars Azmun, Karaj, Iran). Serum hs-CRP level was measured following the method of immunoturbidity measurement (Kit: Quantitative determination of hsCRP in human blood by latex turbidimetry assay). Previous studies have demonstrated that a serum CRP >3 mg/l may be associated with future risk of CVD (10) and this was used as the cutoff for determining a high hs-CRP level.

The collected data were analyzed statistically to determine the significance of different parameters by the SPSS program (v. 14.0). Normal distribution of variables was assessed by Kolmogorov–Smirnov test. The values among groups are compared using one-way ANOVA and chi-square tests. Nonparametric Kruskal–Wallis and Mann–Whitney tests were used for quantity variables that were not normally distributed. A P-value <0.05 was considered as statistically significant. Regression analysis was used to study association among parameters.

Groups differed significantly for age (but not for the normal weight versus the obese/diabetic with nobobese/diabetic group), gender ($P < 0.001$), history of smoking ($P = 0.037$), FBG ($P < 0.001$), serum TG

TABLE 1. Characteristics and Biochemical Data From All Subjects in Each Group

		Normal ($n = 4,953$)	Obese ($n = 2,116$)	Diabetic ($n = 448$)	Obese and diabetic ($n = 245$)	*P*1	*P*2	*P*3	*P*4	*P*5	*P*6
Age (years)		47.7 ± 8.3	48.3 ± 7.9	52.7 ± 7.6	51.9 ± 7.1	0.012	<0.001	<0.001	<0.001	<0.001	0.732
Gender, no. (%)	Male	2,340 (47.2)	488 (23.1)	197 (44.0)	67 (27.3)	<0.001					
	Female	2,613 (52.8)	1,628 (76.9)	251 (56.0)	178 (72.7)						
Former smoker, no. (%)	Male	1,587 (32.0)	619 (29.3)	139 (31.0)	82 (33.5)	0.037					
	Female	3,365 (67.9)	1,497 (70.7)	308 (68.8)	163 (66.5)						
Current smoker, no. (%)	Male	1,129 (22.8)	445 (21.0)	90 (20.1)	52 (21.2)	0.078					
	Female	3,823 (77.2)	1,671 (79.0)	357 (79.7)	193 (78.8)						
Mean FBG (mg/dl)		80.6 ± 12.7	83.8 ± 13.5	200.1 ± 61.5	182.8 ± 53.6	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Mean serum TC (mg/dl)		187.0 ± 37.8	194.6 ± 38.4	208.2 ± 56.7	201.1 ± 47.6	<0.001	<0.001	<0.001	<0.001	0.214	0.411
Median serum TG (mg/dl, IQ range)		111.0 (78.0–161.0)	135.0 (97.2–184.0)	153.0 (104.0–230.7)	170.0 (125.0–242.0)	<0.001	<0.001	<0.001	<0.001	<0.001	0.924
Mean serum LDL-C (mg/dl)		116.5 ± 34.2	119.8 ± 36.4	125.0 ± 44.3	119.5 ± 36.2	0.002	<0.001	0.741	0.109	1.000	0.379
Mean serum HDL-C (mg/dl)		42.0 ± 10.5	41.2 ± 9.0	41.3 ± 10.2	40.8 ± 8.7	0.009	0.554	0.178	1.000	0.967	0.989
Mean SBP (mmHg)		118.2±20.8	124.6 ± 22.1	126.4 ± 19.7	132.9 ± 20.2	<0.001	<0.001	<0.001	0.461	<0.001	<0.001
Mean DBP (mmHg)		77.1 ± 13.5	81.0 ± 13.5	79.7 ± 11.3	84.5 ± 11.7	<0.001	<0.001	<0.001	0.231	<0.001	<0.001
Weight (kg)		66.7 ± 10.2	86.6 ± 139.2	67.4 ± 9.9	83.8 ± 10.6	<0.001	0.998	0.002	<0.001	0.944	0.025
WC		90.4 ± 9.8	105.0 ± 10.1	93.9 ± 9.0	108.6 ± 9.3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Height		1.6 ± 0.09	1.5 ± 0.08	1.6 ± 0.09	1.5 ± 0.07	<0.001	0.024	<0.001	<0.001	0.952	<0.001
BMI (kg/m^2)		25.4 ± 2.9	34.8 ± 60.1	26.0 ± 2.5	33.9 ± 3.4	<0.001	0.977	<0.001	<0.001	0.971	0.009
HC		99.5 ± 6.8	112.2 ± 8.3	99.2 ± 6.3	112.9 ± 8.6	<0.001	0.963	<0.001	<0.001	0.821	<0.001
MUAC		29.2±3.6	33.1 ± 3.5	29.2 ± 3.0	32.8 ± 3.0	<0.001	0.999	<0.001	<0.001	0.766	<0.001
Fat percentage		30.5 ± 11.0	43.9 ± 84.5	32.3 ± 8.4	41.9 ± 8.3	<0.001	0.858	0.001	<0.001	0.913	0.038
Median serum hs-CRP (mg/l)		1.3 (0.8–2.3)	2.2 (1.2–4.4)	2.1 (1.1–4.1)	3.6 (1.8–7.1)	<0.001	<0.001	<0.001	0.579	0.043	0.006

Note Values are presented as mean ± SD, median, and interquartile range for normally and nonnormally distributed variables, respectively. Comparisons were performed by one-way ANOVA and Kruskal–Wallis tests. Also, the posthoc Tukey and Mann–Whitney *U* tests were used for comparison among groups.

*P*1, comparison between normal & normal and obese & normal; *P*2, comparison between normal & normal and normal & diabetes; *P*3, comparison between normal & normal and obese & diabetes; *P*4: comparison between obese & normal and normal & diabetes; *P*5, comparison between obese & normal and obese & diabetes; *P*6, comparison between normal & diabetes and obese & diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure.

($P < 0.001$), serum total cholesterol (TC, $P < 0.001$), systolic and diastolic blood pressure ($P < 0.001$). Also, LDL-C concentrations only showed significant differences between quite normal and obese groups ($P = 0.002$), as well as between quite normal and diabetic groups ($P < 0.001$). The concentrations of hs-CRP, except between groups of obese and diabetic ($P = 0.579$), showed significant differences in the other groups. Among the anthropometric factors, only waist circumference (WC, $P < 0.001$) showed a significant difference among all groups (Table 1).

We found that FBG, TC, LDL-C, and TG levels are positively associated with CVD risk and also hs-CRP ≥3 mg/l level. Also, the percentage of those who were diabetic (FBG ≥ 126 mg/dl) in subjects with hs-CRP ≥3.0 mg/l was 14.7% in comparison with 6.8% in subjects with hs-CRP <3 mg/l. We also found that an FBG <100 mg/dl was present in 85.6% subjects with a serum hs-CRP <3 mg/l compared with 73.2% of subjects with a serum hs-CRP ≥3.0 mg/l (data not shown).

Using univariate analysis, we found that there was a positive association between age ($P = 0.035$); FBG, TC, TG, LDL-C, WC, hip circumference (HC, $P < 0.001$); mid-upper arm circumference (MUAC, $P = 0.002$); fat percentage ($P = 0.04$) and serum hs-CRP, and a negative association between height ($P < 0.001$) and serum hs-CRP. We also found that there was an association between gender and hs-CRP ($P < 0.001$). The

TABLE 2. Association of Baseline and Laboratory data and hs-CRP Levels by Linear Regression

Predictors	Univariate β[a]	CI		*P*
Age (years)	0.022	0.002	0.043	0.035
Gender (female)	0.806	0.459	1.154	<0.001
Current smoker	–0.281	–0.689	0.126	0.176
FBG (mg/dl)	0.018	0.014	0.023	<0.001
TC (mg/dl)	0.017	0.013	0.021	<0.001
TG (mg/dl)	0.002	0.000	0.004	0.019
LDL-C (mg/dl)	0.018	0.014	0.023	<0.001
HDL-C (mg/dl)	–0.004	–0.020	0.012	0.649
SBP (mmHg)	0.002	–0.005	0.010	0.527
DBP (mmHg)	–0.001	–0.013	0.012	0.916
Weight (kg)	0.001	–0.001	0.004	0.212
WC	0.062	0.049	0.075	<0.001
Height	–3.381	–4.838	–1.925	<0.001
BMI (kg/m^2)	0.005	0.000	0.010	0.066
HC	0.088	0.070	0.107	<0.001
MUAC	0.064	0.023	0.104	0.002
Fat percentage	0.004	0.000	0.008	0.040
Predictors	Multivariate β	CI		*P*
Age (years)	–0.008	–0.030	0.013	0.435
Gender (female)	0.546	0.188	0.904	0.003
FBG (mg/dl)	0.015	0.011	0.020	<0.001
TG (mg/dl)	<0.001	–0.002	0.002	0.893
LDL-C (mg/dl)	0.015	0.010	0.020	<0.001
WC	0.056	0.041	0.071	<0.001
Fat percentage	0.001	–0.003	0.005	0.580

[a]Regression coefficient.

Correlation between baseline and laboratory data and hs-CRP levels was assessed using Spearman's correlation analysis.

SBP, systolic blood pressure; DBP, diastolic blood pressure; CI, confidence interval.

results of the multivariate analysis showed FBG, LDL-C, and WC were associated with higher hs-CRP levels ($P < 0.001$). Also, there was an association between being female and hs-CRP ($P = 0.003$, Table 2).

As reported, there were significant differences among the groups of subjects defined by adiposity and FBG, for age, gender distribution and history of smoking. A serum hs-CRP ≥3 mg/l was positively related to age. Ferrucci et al. also showed serum hs-CRP concentrations are positively associated with age (11). Multivariate analysis of our data showed that being female compared with being male was associated with increased hs-CRP level. Saltevo et al. have reported women with metabolic syndrome (based on the National Cholesterol Education Program (NCEP) criteria) have higher hs-CRP concentrations compared with men (12). It seems that use of oral contraceptives may be a cause for this significant increase in hs-CRP levels (13). Unfortunately, we have not collected data on oral contraceptives in the current study. We found there was a significant relationship between history of smoking habit and serum hs-CRP concentration. Previous studies have reported that smokers have increased serum CRP levels that could be decreased after cessation (14). All anthropometric indices (including weight, WC, BMI, HP, MUAC and fat percentage) were positively related to serum hs-CRP levels. Several studies have reported the association between obesity BMI (15) and WC (16) as a major predictor of hs-CRP levels. According to the multivariate analysis, WC was the most important factor associated with hs-CRP level.

In the current study, there was a significantly higher FBG in obese, diabetic and obese with diabetes patients than normal subjects. Barzilay et al. showed increased hs-CRP levels can predict the type 2 diabetes mellitus (17) and metabolic syndrome (18), which is consistent with our results. In current study, the results of the univariate analysis did not show any significant independent association between blood pressure and increased hs-CRP level.

Previous studies have reported that there is a relationship between higher levels of circulating CRP and hypertension. It has been suggested that this association may be noncausal. Various disease states, obesity, smoking and adverse socioeconomic circumstances can increase CRP levels. These factors may themselves influence blood pressure levels (19).

We concluded that FBG, LDL-C, and WC are independently associated with serum hs-CRP concentrations. In addition, gender can also have an association with increased levels of hs-CRP.

ACKNOWLEDGMENT

We thank the financial support of Deputy for Research of the Mashhad University of Medical Sciences.

CONFLICT OF INTEREST

The authors confirm no conflict of interest.

REFERENCES

1. World Health Organization. Collaborative framework for care and control of tuberculosis and diabetes. http://www.who.int/diabetes/publications/tb_diabetes2011/en/index.html (accessed Nov 2011).
2. Gohel MG, Chacko AN. Serum GGT activity and hsCRP level in patients with type 2 diabetes mellitus with good and poor glycemic control: An evidence linking oxidative stress, inflammation and glycemic control. J Diabetes Metab Disord 2013;12(56):1–8.
3. Belalcazar LM, Reboussin DM, Haffner SM, et al. A 1-year lifestyle intervention for weight loss in individuals with type 2 diabetes reduces high C-reactive protein levels and identifies metabolic predictors of change. Diabetes Care 2010;33(11):2297–2303.
4. Amanullah S, Jarari A, Govindan M, et al. Association of hs-CRP with diabetic and non-diabetic individuals. Jordan J Biol Sci 2010;3(1):7–12.
5. Dev N, Marcus SR. High sensitive C-reactive protein, an independent and early novel inflammatory marker in healthy obese women. J Biomed Res 2012;23(1):73–77.
6. Wild S, Roglic G, Green A, et al. Global prevalence of diabetes. Diabetes Care 2004;27(5):1047–1053.
7. Ahmed Baig MS, Sarwari KN, Sabeer TM. Study of serum hs-crp in type 2 diabetic patients. Int J Basic Appl Med Sci 2013;3(3):235–240.
8. Black S, Kushner I, Samols D. C-reactive protein. J Biol Chem 2004;279(47):48487–48490.
9. Lin CC, Kardia SL, Li CI, et al. The relationship of high sensitivity C-reactive protein to percent body fat mass, body mass index, waist-to-hip ratio, and waist circumference in a Taiwanese population. BMC Public Health 2010;10:579.
10. Kovacic S, Bakran M. Genetic susceptibility to atherosclerosis. Stroke Res Treat 2012;2012:1–5.
11. Ferrucci L, Corsi A, Lauretani F, et al. The origins of age-related proinflammatory state. Blood 2005;105(6):2294–2299.
12. Saltevo J, Vanhala M, Kautiainen H, Kumpusalo E, Laakso M. Gender differences in C-reactive protein, interleukin-1 receptor antagonist and adiponectin levels in the metabolic syndrome: A population-based study. Diabet Med 2008;25(6):747–750.
13. Cauci S, Di Santolo M, Culhane JF, et al. Effects of third-generation oral contraceptives on high-sensitivity C-reactive protein and homocysteine in young women. Obstet Gynecol 2008;111(4):857–864.
14. Sesmilo G, Biller BM, Llevadot J, et al. Effects of growth hormone administration on inflammatory and other cardiovascular risk markers in men with growth hormone deficiency: A randomized, controlled clinical trial. Ann Intern Med 2000;133(2):111–122.
15. Kang ES, Kim HJ, Ahn CW, et al. Relationship of serum high sensitivity C-reactive protein to metabolic syndrome and microvascular complications in type 2 diabetes. Diabetes Res Clin Pract 2005;69(2):151–159.
16. Rensburg MA, Matsha T, Hoffmann M, Hassan S, Erasmus RT, Rensburg M. Distribution and association of hs-CRP with cardiovascular risk variables of metabolic syndrome in adolescent learners. Lab Med 2012;1(1):1–6.
17. Jacobi J, Maas R, Cardounel AJ, et al. Dimethylarginine dimethylaminohydrolase overexpression ameliorates atherosclerosis in apolipoprotein E-deficient mice by lowering asymmetric dimethylarginine. Am J Pathol 2010;176(5):2559–2570.
18. Hasegawa K, Wakino S, Tatematsu S, et al. Role of asymmetric dimethylarginine in vascular injury in transgenic mice overexpressing dimethylarginie dimethylaminohydrolase 2. Circ Res 2007;101(2):2–10.
19. Davey Smith G, Lawlor DA, Harbord R, et al. Association of C-reactive protein with blood pressure and hypertension: Life course confounding and mendelian randomization tests of causality. Arterioscler Thromb Vasc Biol 2005;25(5):1051–1056.