Journal of Clinical Laboratory Analysis 27: 207–210 (2013)

Serum Levels of High Sensitive C Reactive Protein in Healthy Adults From Southern Brazil

Francieli Delongui,[1] Ana Paula Kallaur,[1] Sayonara Rangel Oliveira,[1] Ana Maria Bonametti,[2] Cintia Magalhães Carvalho Grion,[2] Helena Kaminami Morimoto,[3] Andrea Name Colado Simão,[3] Giuliana Gisele Magalhães,[4] and Edna Maria Vissoci Reiche[3]*

[1]Postgraduate Program, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil
[2]Department of Medical Clinic, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil
[3]Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil
[4]Pharmacy Graduate, Health Sciences Center, State University of Londrina, Londrina, Paraná, Brazil

Background: With the emergence of more sensitive assay techniques, it has been shown that C reactive protein (CRP) is present at low levels in the serum of all the clinically healthy individuals. *Objective:* To determine the interval values of high-sensitivity CRP (hs-CRP) in healthy adults. *Methods:* Serum hs-CRP level was evaluated in 176 healthy blood donors. *Results:* The serum hs-CRP level ranged from $<$0.175 to 48.7 mg/l (median 1.2 mg/l); 127 (72.2%) individuals exhibited values $\geq$0.175 and $<$3.0 mg/l and 31 (17.6%) showed values $>$3.0 and $\leq$10.0 mg. Higher hs-CRP level was observed among the female than male ($P = 0.0001$), and among the older than the younger individuals ($P = 0.0180$). Individuals with body mass index $\geq$25.0 kg/m^2 exhibited higher hs-CRP level than those with normal weight (18.5–24.9 kg/m^2; $P < 0.0005$). When the participants were stratified into gender and low ($\leq$24.9 kg/m^2) and high ($\geq$24.9 kg/m^2) body mass index (BMI) groups, the gender difference in hs-CRP levels remained (female with low BMI vs. male with low BMI, $P = 0.0221$; female with high BMI vs. male with high BMI, $P = 0.0001$). *Conclusion:* Gender, age, and BMI influence serum hs-CRP level in healthy individuals and these variables should be considered for the interpretation of hs-CRP values. The results reinforce the importance in evaluating whether these differences in hs-CRP levels could contribute to alter the cardiovascular risk criteria and clinical outcomes, and whether hs-CRP thresholds for cardiovascular risk assessment should be adjusted for different gender and body mass index groups. J. Clin. Lab. Anal. 27:207–210, 2013.

Key words: C reactive protein; reference values; body mass index; acute-phase proteins

INTRODUCTION

In the presence of inflammation or tissue necrosis, tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and mainly interleukin-6 (IL-6), predominantly produced by macrophages as well as adipocytes, stimulate the liver to synthesize C reactive protein (CRP) and other positive acute-phase proteins of greatest clinical interest (1). In healthy subjects, CRP is a trace plasma protein with a geometric mean of 0.89 mg/l (2) and is usually lower than 10.0 mg/l (1). The levels begin to rise rapidly within 4–6 hr of the onset of signs of infection or tissue injury, duplicating each 8 hr, and normally peaks within

Grant sponsor: Coordination for the Improvement of Higher Level or Education Personnel (CAPES) of Brazilian Ministry of Education; Grant sponsor: Institutional Program for Scientific Initiation Scholarship (PIBIC) of the National Council for Scientific and Technological Development (CNPq); Grant sponsor: State University of Londrina (PROPPG); Grant sponsors: Araucária Foundation.

*Correspondence to: Edna Maria Vissoci Reiche, Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, State University of Londrina, Avenida Robert Koch, 60, CEP 86.038-440, Londrina, Paraná, Brazil. E-mail: reiche@sercomtel.com.br

Received 18 June 2012; Accepted 11 January 2013
DOI 10.1002/jcla.21585
Published online in Wiley Online Library (wileyonlinelibrary.com).

24–48 hr later. Specially, CRP rises after surgery, bacterial infection, acute myocardial infarction, rheumatic diseases, burns, trauma, and high levels may persist indefinitely in chronic inflammatory states (1). In addition, high levels were also observed in patients with depressive disorder (3).

With the emergence of more sensitive and reliable immunoassays, it has been shown that CRP is present at low levels in the serum of all the clinically healthy individuals (2). When CRP concentration was measured using an enzyme-linked immunosorbent assay (ELISA) in sera from healthy blood donors, the values ranged from 0.05 to 57.6 mg/l (median 1.8 mg/l) (4), and a non-Gaussian distribution of CRP was demonstrated, with the 2.5th, 50.0th, and 97.5th percentile values of 0.08, 0.64, and 3.11 mg/l, respectively (5).

Currently, the CRP levels can be measured using high-sensitivity nephelometry (high-sensitivity CRP [hs-CRP]) that improved the quantification with an analytical sensitivity of 0.175 mg/l. Despite the important role of hs-CRP as a marker of different disease processes, to date, few studies have presented data regarding the distribution of serum hs-CRP levels in Brazilian healthy adults (2). The present study was undertaken to determine the hs-CRP levels in serum samples from healthy blood donors from southern Brazil, and to evaluate whether the serum hs-CRP levels are associated with demographic and anthropometric characteristics of the individuals.

The protocol was approved by the institutional Research Ethics Committees of the Londrina State University. A voluntary written consent was obtained from the 176 blood donors enrolled. None of them exhibited either clinical symptoms of disease or epidemiological, clinical, and laboratory criteria for blood donation exclusion according to the government standardization (6). Age, gender, ethnicity, weight, and blood pressure were obtained from the blood bank database. The self-reported ethnicity was stratified as Caucasian and non-Caucasian (Asiatic, black, and Mullato). Body mass index (BMI) was calculated as kg/m^2 and categorized (7) as follows: <18.5 kg/m^2 (underweight), 18.5–24.9 kg/m^2 (normal weight), 25.0–29.9 kg/m^2 (overweight), 30.0–34.9 kg/m^2 (obesity class I), 35.0–39.9 kg/m^2 (obesity class II), and ≥40.0 kg/m^2 (obesity class III). The individuals who presented at least one altered clinical and/or metabolic biomarker value that is considered criteria for metabolic syndrome as defined following the Adult Treatment Panel III criteria (8), and individuals who were in treatment with anti-inflammatory drugs or antioxidants were excluded from the study.

Peripheral blood samples were obtained at 7:00 am to 10:00 am and the hs-CRP was determined using the high-sensitive nephelometric method (Nephelometer II^{TM}, Dade Behring, Siemens Healthcare Diagnostics, Inc., Deerfield, IL), with an analytical sensitivity of 0.175 mg/l and linearity up to 1,100 mg/l. Samples with result below the assay sensitivity threshold, which was registered as < 0.175 mg/l, were converted to 0.17 mg/l for statistical analysis. The qualitative variables were analyzed by chi-square test or Fisher's exact test, when appropriate. The quantitative variables were reported as minimum (<0.175 mg/l), maximum, mean, standard deviation, median, interquartile range (IQR, 25.0th and 75.0th percentile values) and were analyzed using the Mann–Whitney test, Kruskal-Wallis, and Dunn posttest. The level of significance was set at $P<0.05$.

TABLE 1. Demographic Characteristics and Serum High Sensitive C-reactive Protein (hs-CRP) Levels Obtained on Healthy Blood Donors from Londrina, Southern Brazil

		hs-CRP serum levels (mg/l)		
Characteristic	*n* (%)	Range	Median	IQR
Age (years)[a]				
18 – 25	33 (18.8)	<0.175 – 48.70	0.56	0.29–1.69
26 – 30	30 (17.0)	<0.30– 11.50	1.71	1.06–3.13
31 – 40	56 (31.8)	<0.175– 24.0	1.36	0.54–2.78
41 – 50	38 (21.6)	<0.175 – 16.3	1.62	0.82–3.07
≥ 51	19 (10.8)	0.24 – 5.13	1.17	0.80–2.04
Gender[b]				
Female	139 (79.0)	<0.175 – 48.70	1.60	0.76–3.26
Male	37 (21.0)	<0.175 – 5.16	0.76	0.44–1.17
Ethnicity[c]				
Caucasian	142 (80.7)	<0.175 – 48.7	1.17	0.59–2.70
Non-Caucasian	34 (19.3)	<0.175 – 24.00	1.35	0.74–2.75
BMI (kg/m^2)[d]				
< 18.5	5 (2.9)	<0.175–4.23	0.46	<0.175–3.66
18.5–24.9	97 (56.4)	<0.175–48.70	1.07	0.48–2.02
25–29.9	48 (27.9)	<0.175–12.30	1.39	0.74–3.02
30.0–34.9	18 (10.5)	0.33–24.0	2.65	1.46–5.22
35–39.9	3 (1.7)	2.60–10.10	3.37	2.76–8.45
≥ 40	1 (0.6)	2.60	2.60	2.60–2.60

hs-CRP, high-sensitive C reactive protein was evaluated using nephelometric method; IQR, interquartile range.

[a]Kruskal–Wallis test, $P = 0.0180$. The categories of 51–60 and ≥61 years were evaluated together ($n = 19$). Dunn's test, PCR values from individuals with 18–25 years differed from those individuals with 26–30 and with 41–50 years old.

[b]Mann–Whitney test, $P < 0.0001$.

[c]Non-Caucasian, Asiatic ($n = 5$, 2.8%), black ($n = 9$, 5.2%) and Mullato ($n = 20$, 11.3%); Mann–Whitney test, $P = 0.5689$.

[d]BMI, body mass index was evaluated in 172 individuals; Kruskal–Wallis test, $P = 0.0005$; Dunn's test, the group with BMI 18.5–24.9 kg/m^2 differed from those with BMI 30.0–34.9 kg/m^2.

Table 1 summarizes the results. The serum hs-CRP levels were detected in almost all the samples analyzed, ranging from < 0.175 to 48.7 mg/l (median 1.2 mg/l, IQR 0.6–2.7). Only nine (5.1%) individuals showed levels below the detection limit of 0.175 mg/l. Most of the serum samples ($n = 127$, 72.2%) presented hs-CRP concentration <3.0 mg/l. Thirty-one (17.6%) individuals presented hs-CRP concentration between 3.1 and 10.0 mg/l and nine

(5.1%) exhibited levels greater than the clinically relevant threshold of 10.0 mg/l.

The range of hs-CRP levels was very wide in each age group and showed lower levels among the younger individuals (18–25 years old) when compared with those with 26–30 and with 41–50 years old ($P = 0.0180$). The serum hs-CRP values did not differ among the individuals when the ethnicity was considered ($P = 0.5689$). The gender of individuals showed an association with the serum hs-CRP levels. Women exhibited substantially higher median hs-CRP levels compared with men ($P < 0.0001$).

The BMI of the individuals ranged from 16.5 to 40.2 kg/m^2 (median 23.9, IQR: 21.6–26.9 kg/m^2). The BMI also presented a strong direct association with serum hs-CRP levels. Individuals that were included in the obese category of BMI ($\geq$30.0 kg/m^2) exhibited higher serum hs-CRP levels than those with both normal weight (18.5–24.9 kg/m^2) and overweight (25.0–29.9 kg/m^2) categories ($P < 0.0005$). The individuals with BMI ranging from 18.5 to 24.9 kg/m^2 differed from those with BMI ranging from 30.0 to 34.9 kg/m^2; and the individuals with BMI $<$18.8 kg/m^2 did not differ from any other group (Dunn test, $P > 0.05$). When the participants were stratified into gender and low ($\leq$24.9 kg/m^2) and high ($\geq$24.9 kg/m^2) BMI groups, the gender difference in hs-CRP levels remained (female with low BMI vs. male with low BMI, $P = 0.0221$; female with high BMI vs. male with high BMI, $P = 0.0001$).

All the individuals enrolled in the study exhibited normal levels of total cholesterol, HDL, LDL, tryglicerides, glucose, and did not present the criteria for metabolic syndrome (data not shown).

The wide range in the serum hs-CRP levels obtained in the present study is consistent with previous studies (4, 9–11), and may be resulted from the different undetectable stimulus and the genetic heterogeneity of the inflammatory response among the individuals (11, 12). In the present study, middle age, female gender, and high BMI were associated with high serum hs-CRP levels. These results are also consistent with previous studies in which some host factors, such as age, gender, race/ethnicity, waist circumference, BMI, leptin, impaired glucose tolerance, smoking, and physical activity, were significantly associated with variations in circulating hs-CRP levels (11, 13–17).

The results underscore that BMI strongly influences hs-CRP levels and that high BMI tended to exert strong inflammatory effects in the individuals, finding consistent with the notion that excess adipose tissue contributes to a chronic inflammatory state through a range of metabolic pathways (2, 11, 18). In the present study, the serum hs-CRP levels were higher in women compared with men despite accounting for BMI and other common confounding variables. This gender difference was maintained across the BMI groups and all the ethnic subgroups. These results suggest that evaluation of gender-specific hs-CRP cut points to determine cardiovascular risk should be considered (19).

In the present study, no association was observed between race/ethnicity of the individuals and the serum hs-CRP levels, differing from previous study (15) in which black subjects exhibited higher hs-CRP levels than white subjects (median 3.0 vs. 2.3 mg/l; $P < 0.001$) and women exhibited higher hs-CRP levels than men (median 3.3 vs. 1.8 mg/l; $P < 0.001$). Other study also showed that black individuals exhibited the highest CRP values and the Chinese participants presented the lowest CRP values (17).

In addition to environmental factors that trigger cytokine secretion, individuals may demonstrate consistent differences in the inflammatory response probably genetically predetermined (12). The *CRP* gene is located on the first chromosome (1q21-q23) and it was demonstrated that 2.9% of the total variation in circulating CRP levels seems to be explained by haplotypes two among the eight single nucleotide polymorphisms (SNPs) genotyped of the *CRP* gene, conferring different secretion of this acute phase protein (16).

The present study was restricted to healthy people according to the blood donation criteria (6), and those who were treated with anti-inflammatory drugs or antioxidants were excluded; however, in addition with the weakness of the cross-sectional design of this study, data about smoking, physical activity, and hormonal therapy were not obtained and these factors could exert some influence in the results obtained.

Taken all together, this study underscored that age, gender, and BMI differences also exert effect on the distribution of serum hs-CRP levels among the healthy adult individuals from southern Brazilian population. The cardiovascular diseases stand out as the main cause of death (28.8% among males and 36.9% among females) in the whole Brazilian population and the southern region reports the highest rates and accounts for 40.0% of deaths among women (20). Considering the high risk of cardiovascular disease in females from southern Brazilian population, the clinical implications of gender differences in hs-CRP levels require more investigation and long-term outcome studies in this population are needed to determine whether these differences in serum hs-CRP levels could contribute to alter the cardiovascular risk and clinical outcomes, and whether hs-CRP thresholds for cardiovascular risk assessment should be adjusted for different gender and BMI groups on this population.

ACKNOWLEDGMENTS

The study was supported by grants from the Coordination for the Improvement of Higher Level or Education

Personnel (CAPES) of Brazilian Ministry of Education; Institutional Program for Scientific Initiation Scholarship (PIBIC) of the National Council for Scientific and Technological Development (CNPq), State University of Londrina (PROPPG), and Araucária Foundation, Paraná, Brazil.

CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

REFERENCES

1. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–454.
2. Nazmi A, Oliveira IO, Victora CG. Correlates of C-reactive protein levels in young adults: A population-based cohort study of 3827 subjects in Brazil. Braz J Med Biol Res 2008;41:357–367.
3. Reiche EM, Morimoto HK, Nunes SOV. Stress and depression-induced immune dysfunction: Implications for the development and progression of cancer. Int Rev Psychiatry 2005;17:515–527.
4. Ribeiro MA. Levels of C-reactive protein in serum samples from healthy children and adults in São Paulo, Brazil. Braz J Med Biol Res 1997;30:1055–1059.
5. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: Implications for reference intervals and epidemiological applications. Clin Chem 1997;43:52–58.
6. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Diretoria Colegiada. Resolução RDC nº. 153, de 14 de junho de 2004. Brasília: Ministério da Saúde, publicada no Diário Oficial da União em 24/06/2004.
7. WHO. Physical status: The use and interpretation of anthropometry. Report of a WHO expert committee. World Health Organ Tech Rep Ser 1995;854:1–452.
8. Jacobs DR, Jr. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and high blood cholesterol in adults (Adults Treatment Panel III). JAMA 2001;285:2486–2497.
9. Povoa P, Almeida E, Moreira P, et al. C-reactive protein as an indicator of sepsis. Intensive Care Med 1998;24:1052–1056.
10. Yan M, Zhao L, Zheng F, Sun X, Zhang Y, Wang C. The relationship between gene polymorphism and CRP level in a Chinese Han population. Biochem Genet 2007;45:1–9.
11. Danik JS, Ridker PM. Genetic determinants of C-reactive protein. Curr Atheroscler Rep 2007;9:195–203.
12. Waterer GW, Wunderink RG. Science review: Genetic variability in the systemic inflammatory response. Critical Care 2003;7:308–314.
13. Harris TB, Ferruci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 1999;106:506–512.
14. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. Am J Cardiol 2020;89:1117–1119.
15. Khera A, McGuire DK, Murphy SA, et al. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464–469.
16. Martínez-Calatrava MJ, González-Sánchez JL, Martínez-Larrad MT, Pérez-Barba M, Serrano-Ríos M. Common haplotypes of the C-reactive protein gene and circulating leptin levels influence the interindividual variability in serum C-reactive protein levels. The Segovia study. Thromb Haemost 2007;98:1088–1095.
17. Majka DS, Chang RW, Vu T-H T, et al. Physical activity and high-sensitivity C-reactive protein. The multi-ethnic study of atherosclerosis. A J Prev Med 2009;36:56–62.
18. Hashimoto K, Kasayama S, Yamamoto H, Kurebayashi S, Kawase I, Koga M. Strong association of C-reactive protein with body mass index and 2-h post challenge glucose in non-diabetic, non-smoker subjects without hypertension. Diabetic Med 2004;21:581–585.
19. Lakoski SG, Cushman M, Criqui M, et al. Gender and C-reactive protein: Data from the Multiethnic Study of Atherosclerosis (MESA) cohort. Am Heart J 2006;152:593–598.
20. Brazilian Institute of Geography and Statistics (IBGE). Summary of Social Indicators 2002. Available from: http://www.ibge.gov.br/home/presidencia/noticias/12062003indic2002.shtm, accessed on 12 June 2003.