Reference Value for C-Reactive Protein and Its Distribution Pattern in Thai Adults

Navapun Charuruks, MD; Bunpayao Laohajinda, MSc*; Suthee Rujwanitgun, MD**; Mathuros Chaiworaporn, MT, MPPM†

Background To set a reference value for high-sensitivity C-reactive protein (hs-CRP) in a healthy Thai population and study the effect of time, gender and age on that value.

Methods and Results Three hundred and sixty-four subjects, aged between 18 and 74 years, comprising 185 men and 279 women, were studied. Another 10 healthy subjects, aged between 18 and 54 years, were recruited for the study of circadian variation in hs-CRP over the days of the week and the months of a year. The reference value for the Thai adults in the present study was 1.8 mg/L, range 0.2–7.9 mg/L. There was no significant difference in the hs-CRP concentration because of region, time, gender or age (p>0.05), nor was the value affected by time.

Conclusion The determination of hs-CRP can be performed at any time and the hs-CRP value determined by this study can be used as the reference for Thai adults. (Circ J 2005; 69: 339–344)

Key Words: hs-CRP; Reference value; Thai adults

C-reactive protein (CRP) is an acute-phase constituent that has been measured for more than 70 years in the diagnosis and monitoring of active infection and inflammation because it is one of the most fundamental and earliest host responses to inflammatory injury. It is mainly regulated by the CRP gene, located on the long arm of chromosome 1, induced at the transcriptional level by interleukin-6 (IL-6), and its major synthetic site is the hepatocyte. In fact, it is released into the circulation with equal distribution in the vascular compartment without substantial tissue sequestration at the site of inflammation. However, the clinical importance of CRP has been limited for many decades because of its large intra-individual variation and methods of measurement that were not thoroughly sensitive and accurate. Now, a method of detecting high-sensitive CRP (hs-CRP) is available and many study results demonstrate its greater value, especially as a potential risk marker for cardiac diseases. However, as stated before, there is a large intra-individual variation and thus a need for regional studies of CRP. Several factors influence the concentration of CRP: different ethnicity, different physical factors and lifestyle (eg, diet, exercise, stress, hygiene, and environment). Hence, the reference value and distribution pattern of hs-CRP are needed as the basic background knowledge for further studies in each region of the world. For this reason, the authors designed the present study to determine a reference value of hs-CRP in a healthy Thai population, and to examine the effect of time, gender and age.

Methods

Population This investigation was a multicenter study formed by the collaboration of 4 centers with the aim of covering all the regions (center, north, south, and northeast) of Thailand. The 4 centers were (1) the Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, located in the center of Thailand, (2) the Department of Clinical Chemistry, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, located in the north, (3) the Department of Pathology, Maharaj Nakornsithammarat Hospital, located in the south, and (4) the Department of Clinical Pathology, Maharaj Nakornrajima Hospital, located in the northeast. The study protocol complied with the requirements of the Ethical Committee of the Faculty of Medicine, Chulalongkorn University and was agreed to by all collaborating centers. The volunteers from each center were randomized from private and governmental employees who attended the annual check-up program during July 2003 to June 2004. They were given a physical examination by a doctor and underwent laboratory screening. Any individuals with abnormal vital signs or abnormal blood pressure were excluded from this study, as were those with a history of chest pain or antihypertensive medication. Only subjects with a normal physical examination were recruited and interviewed. Each subject was tested for complete blood count (CBC), blood chemistry (glucose (Glu), blood urea nitrogen (BUN), creatinine (Cr), uric acid, cholesterol (Chol), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and urinalysis (UA). An abnormal level of any of the laboratory parameters meant exclusion from the study. Although 1,000 subjects agreed to join the study, only 464 were recruited.
with an age range between 18 and 74 years, comprising 185 men and 279 women. An additional 10 healthy subjects (5 men, 5 women; age range 18–54 years) were enrolled in the study of temporal variation in the hs-CRP concentration.

**Blood Samples**

All samples were taken and stored using the same protocol to ensure comparability. The blood was centrifuged within 2 h of collection at 30,000 G/min and the serum was stored at –20°C until examination. Samples from each center were sent on dry ice by air transportation to the Central Laboratory, King Chulalongkorn Memorial Hospital (KCMH) where all samples were examined. The Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University is responsible for the functioning of the Central Laboratory of KCMH.

For temporal variation study, individual blood samples were collected at 06.00, 12.00, 18.00 and 24.00 h of the same day; daily samples were collected from each participant for a period of 1 week; and monthly samples were collected for 12 consecutive months of a year.

**Reagents**

All samples were analyzed for hs-CRP using a hs-CRP reagent kit (CRPLx, Lot 20764930 322, Roche Diagnostics GmbH D-68298 Mannheim, Germany) supported by the Roche Diagnostics (Thailand) company. The analysis was performed according to the manufacturer’s instructions. All samples were determined for hs-CRP using Integra-700 (Roche Diagnostics, IN, USA). Intra- and inter-assay variability for the kit were 1.5% and 2.7%, respectively, but the intra-assay variability was also assessed in this study by assaying a pool serum 20 times and the inter-assay variability was also determined by assaying a control in every assay cycle.

**Statistical Analysis**

The total concentration of hs-CRP was determined and the range was decided using the National Committee for Clinical Laboratory Standards (NCCLS) recommendation.15 The standard deviation (SD) and the 2.5th and 97.5th percentiles were calculated.14 The frequencies of the hs-CRP concentration were followed to generate the distribution pattern. Furthermore, differences in region, gender, and age were studied using Student’s t-test (unpaired samples, two tailed). A p-value ≤ 0.05 was considered statistically significant. In addition, the concentrations of CRP at different intervals of the day (6-h periods from 06.00h to midnight), on different days of the week, and different months of the year were analyzed using Kendall’s W test.

**Results**

The intra-assay variability was assessed as stated earlier, the mean value was 2.9 mg/L, and the coefficient of variation (CV) was 1.71%. The mean inter-assay variability was 2.8 mg/L and the CV was 2.47%. Although our intra-assay variability was higher than the manufacturer’s, it was still in the acceptable range. The characteristics of the subjects and their CRP concentrations are shown in Table 1. The overall reference value for hs-CRP in healthy Thai adults was 1.8 mg/L (range 0.2–7.9 mg/L). The range was calculated using the 2.5th and 97.5th percentiles to cover 95% reference interval because the hs-CRP data does not have a normal distribution or Gaussian pattern.16 In addition our results for hs-CRP in healthy adults plotted either as the total or by region and sex showed a left skew (Figs 1,2).

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From our study there was no significant difference between the hs-CRP reference values for each region of Thailand (p>0.05). The gender effect in each region is shown in Table 1 and the effect of different ages in Table 2; our results show no significant difference in hs-CRP concentration because of region, gender or age (p>0.05). The CRP concentrations at different intervals of the day, different

### Table 1 Characteristics of All Subjects and C-Reactive Protein (CRP) Concentration

<table>
<thead>
<tr>
<th>Study center</th>
<th>n</th>
<th>Age (range) (years)</th>
<th>CRP (range) (mg/L)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>167</td>
<td>40.6 (18–70)</td>
<td>1.9 (0.3–8.4)</td>
<td>0.8132</td>
</tr>
<tr>
<td>Faculty of Medicine, Chulalongkorn University</td>
<td>Total</td>
<td>148</td>
<td>38.3 (20–71)</td>
<td>1.8 (0.2–6.3)</td>
</tr>
<tr>
<td>M</td>
<td>61</td>
<td>40.1 (23–68)</td>
<td>1.8 (0.2–6.5)</td>
<td>0.8132</td>
</tr>
<tr>
<td>F</td>
<td>106</td>
<td>40.9 (18–70)</td>
<td>1.9 (0.4–8.4)</td>
<td>0.8132</td>
</tr>
<tr>
<td>Faculty of Associated Medical Sciences, Chiangmai University</td>
<td>Total</td>
<td>148</td>
<td>38.3 (20–71)</td>
<td>1.8 (0.2–6.3)</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>40.0 (20–71)</td>
<td>2.0 (0.2–6.5)</td>
<td>0.8132</td>
</tr>
<tr>
<td>F</td>
<td>101</td>
<td>37.5 (23–69)</td>
<td>1.7 (0.2–5.4)</td>
<td>0.8132</td>
</tr>
<tr>
<td>Maharaj Nakhon Chaiyaphum Hospital</td>
<td>Total</td>
<td>80</td>
<td>48.9 (28–74)</td>
<td>1.7 (0.2–8.2)</td>
</tr>
<tr>
<td>M</td>
<td>30</td>
<td>51.8 (33–74)</td>
<td>1.0 (0.1–3.5)</td>
<td>0.0270*</td>
</tr>
<tr>
<td>F</td>
<td>50</td>
<td>47.2 (28–73)</td>
<td>2.1 (0.2–12.4)</td>
<td>0.0270*</td>
</tr>
<tr>
<td>Maharaj Nakornrajsima Hospital</td>
<td>Total</td>
<td>69</td>
<td>46.8 (30–59)</td>
<td>1.5 (0.4–6.4)</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>47.0 (30–59)</td>
<td>1.4 (0.4–4.3)</td>
<td>0.4672</td>
</tr>
<tr>
<td>F</td>
<td>22</td>
<td>46.4 (35–59)</td>
<td>1.8 (0.2–6.4)</td>
<td>0.4672</td>
</tr>
<tr>
<td>Total study</td>
<td>Total</td>
<td>464</td>
<td>42.2 (18–74)</td>
<td>1.8 (0.2–7.9)</td>
</tr>
<tr>
<td>M</td>
<td>185</td>
<td>43.9 (20–74)</td>
<td>1.6 (0.2–6.4)</td>
<td>0.3087</td>
</tr>
<tr>
<td>F</td>
<td>279</td>
<td>41.2 (18–73)</td>
<td>1.9 (0.2–8.1)</td>
<td>0.3087</td>
</tr>
</tbody>
</table>

*p≤0.05 considered statistically significant.
Fig 1. Distribution pattern of high-sensitivity C-reactive protein (hs-CRP) in healthy Thai adults shows a leftward skew. Distribution pattern of (A) the total group (n=464 adults), (B) the 167 adults from the Faculty of Medicine, Chulalongkorn University, (C) the 148 adults from the Faculty of Associated Medical Sciences, Chiang Mai University, (D) the 80 adults from Maharaj Nakhonsithammarat Hospital and (E) the 69 adults from Maharaj Nakornrajima Hospital.

Fig 2. Distribution pattern of high-sensitivity C-reactive protein (hs-CRP) in healthy Thai adults plotted according to sex also shows a leftward skew. Distribution pattern of (M) the 185 males and (F) the 279 females.
The mean values and CV of each volunteer calculated for circadian, daily and monthly variation are presented in Table 3. Using Kendall’s W test, we observed that the asymptomatic significance of CRP for the different time intervals of the day, different days of the week, and different months of the year was 0.392, 0.973, and 0.155, respectively, showing no significant difference for any time period.

**Table 2 Reference Value for C-Reactive Protein (CRP) at Different Ages**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No.</th>
<th>Age (range) (years)</th>
<th>CRP (range) (mg/L)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤34</td>
<td>129</td>
<td>28.4 (13–34)</td>
<td>1.8 (0.2–8.1)</td>
<td>0.9825</td>
</tr>
<tr>
<td>35–60</td>
<td>302</td>
<td>45.6 (35–60)</td>
<td>1.7 (0.2–7.6)</td>
<td>0.7586</td>
</tr>
<tr>
<td>&gt;60</td>
<td>33</td>
<td>65.2 (61–74)</td>
<td>2.2 (0.3–6.5)</td>
<td>0.3444</td>
</tr>
<tr>
<td>18–74</td>
<td>464</td>
<td>42.2 (18–74)</td>
<td>1.8 (0.2–7.9)</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 considered statistically significant.

**Discussion**

A previous study determined CRP reference values by the methods recognized by the work of the International Federation of Clinical Chemistry (IFCC) Expert Panel, using citrated plasma samples from 143 healthy individuals (74 males, 69 females)\(^\text{10}\). The median was 0.64 mg/L with a range of 0.08–3.11 mg/L and there were no major gender differences. Another tri-ethnic study of women, 44 African Americans, 45 native Americans and 46 Caucasians, had mean CRP results of 4.3, 2.5, and 2.3 mg/L, respectively\(^\text{17}\) and another recent study of CRP baseline concentrations in 24,455 white, 475 black, 357 Asian, and 254 Hispanic women in the United States had results of 2.02, 2.96, 1.12, and 2.96 mg/L, respectively\(^\text{18}\). The reference value for hs-CRP in the healthy Thai adults of our study was 1.8 mg/L with a range of 0.2–7.9 mg/L. These studies highlight the different concentrations of CRP in healthy populations and
the reasons for the different results relate to study methods and techniques, as well as sample types, ethnic effects and lifestyle. We also calculated the mode of our data as 0.8 mg/L, which is close to the median value of a previous study\(^\text{10}\) which was 0.64 mg/L. In addition, our reference value for 279 healthy women is 1.9 mg/mL (0.2–8.1 mg/L), which is higher than for the 357 healthy Asian women (1.12 mg/L) in the United States study\(^\text{18}\) which included Asian and Pacific Islander women in the 1 group. All these data support the necessity of establishing of regional hs-CRP reference values. Furthermore, studies of the pathophysiology of inflammatory diseases, especially coronary artery disease, should include the differences in the ethnic group, lifestyle, and environment between Western and Eastern countries.

In the present study the distribution pattern of hs-CRP in healthy adults showed a leftward skew rather than a normal distribution or Gaussian pattern. Although the mean or average value of hs-CRP was 1.8 mg/L, the mode was 0.8 mg/L, which suggests that a few healthy adults had higher concentrations of hs-CRP and this should be taken into account when interpreting the hs-CRP result. In addition a few subjects had a result of 0.0 mg/L for hs-CRP, which raises some doubt about the sensitivity of the present hs-CRP method. In fact, it raises the question whether all healthy adults have CRP in their circulation and further study is needed to provide this additional basic knowledge about CRP.

Because of its short half-life of 19 h in circulation, the rate of CRP synthesis may lead to considerable variability in the CRP concentrations. One study investigated the existence of time-of-day baseline CRP values in hourly blood samples drawn from 13 healthy subjects and found that over the course of 24 h, there was no diurnal variation\(^\text{19}\) Furthermore a seasonal variation study done in 16 healthy subjects for 12 consecutive months showed inconsistent evidence of intra-individual and inter-individual seasonal variation of CRP concentration\(^\text{20}\) We also found no significant differences in hs-CRP concentration by region, circadian rhythm, day or month, which confirms the results of those prior studies. The results also suggest that ethnic factors have an impact on the reference values, but not durational effects, although we found a tendency for higher variation during a month than during the day of the week or time of the day, as shown in Table 3. These results could be explained by the effect of several factors such as diet, physical activity, and stress, including the stability of the subject’s health status. These factors are difficult to control. The longer the subjects were followed up the higher the variation in hs-CRP concentration.

In conclusion, our reference value for hs-CRP differs from that determined in previous studies done in other populations. There are variations in those prior studies, which were performed in the same racial groups. Our result
is 3-fold less than the result from one study\textsuperscript{10} but lower than 2 others\textsuperscript{17,18} We found, no significant differences in the hs-CRP concentration because of region, time, gender, or age and therefore the determination of hs-CRP for medical purpose can be performed at any time without concern. Moreover our study was conducted using NCCLS recommendations and our result can be used as the reference value for hs-CRP in healthy Thai adults.

**Acknowledgments**

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**References**