The Correlation Between Serum Amyloid A and Reactive Oxygen Metabolites in a Young Mongolian Population

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Objective: Chronic inflammation and oxidative stress are associated with lifestyle-related diseases. Research into the pathophysiology of lifestyle-related diseases is important for Mongolian people. Our study investigated the correlation among the d-ROMs test (a measure of the total oxidant capacity of blood), serum amyloid A (SAA) and high-sensitivity C-reactive protein (hs-CRP) levels in a young Mongolian population.

Methods: The data, including anthropometric and biochemical markers, were collected from 78 Mongolian volunteers (male/female = 27/51, mean age 21 years). The correlation between the SAA and d-ROMs levels was examined, as well as the correlation between the hs-CRP and d-ROMs levels.

Results: The SAA levels were 3.2 μg/mL (median), hs-CRP .04 mg/dL (median) and d-ROMs 309 CARR U, respectively. There was a significant and positive correlation between the SAA and d-ROMs levels ($r=.40$, $P<.01$), in addition to a significant and positive correlation between the hs-CRP and d-ROMs levels ($r=.32$, $P<.01$). These significant correlations remained independent in a multiple linear regression analysis. A subgroup analysis by sex revealed the positive correlation between the SAA and d-ROMs levels to be greater, relative to that between the hs-CRP and d-ROMs levels, particularly in the female group.

Conclusions: The coexistence of chronic inflammation and oxidative stress can be present even in young Mongolian people, suggesting that their coexistence may be a target of early prevention of lifestyle-related diseases. In addition, not only hs-CRP, but also SAA can be used to evaluate the relationship of oxidative stress in this population. Further studies are necessary to confirm the observed relationship. (*Ethn Dis*. 2012;22[3]:329–334)

Key Words: Oxidative Stress, Oxygen Reactive Species, d-ROMs Test, Inflammation, CRP, Lifestyle-Related Disease

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Introduction

Mongolia is a landlocked country in central Asia. The World Health Organization statistical data reported that the average life span was 62 years for Mongolian males and 69 years for females.[1] The data show that Mongolian people have an approximately 17-year shorter life expectancy than the Japanese people, although both ethnic groups are thought to share similar genetic backgrounds.[2] Lifestyle-related diseases, such as cardiovascular disease, are listed as the primary causes of death in Mongolia.[3] The Mongolian people may have a greater atherogenic burden than the Japanese people.[4–6] The long-term regulation of lifestyle-related diseases, including cardiovascular disease, will require a thorough study of preventive strategies in the younger generation.

Subclinically low-grade chronic inflammation and oxidative stress, as well as their coexistence, have attracted great attention as non-traditional pathological conditions associated with lifestyle-related diseases, since they have been proven to be a crucial concept in human health and disease.[7–11] Although C-reactive protein (CRP) is often applied for evaluating chronic inflammation, serum amyloid A (SAA) is also one of the major markers for inflammation.[12] The SAA has not yet been investigated in the same depth as CRP in this area. Furthermore, oxidative stress-related markers that can be used to easily evaluate the oxidative stress status remain under consideration.[13] The d-ROMs test was relatively recently introduced, and this test can quantify the oxidative stress status by measuring the levels of hydroperoxides of global organic compounds (eg, lipids, proteins, nucleic acids).[14–16] Therefore, the measurement of d-ROMs is currently used as an easy clinical marker for evaluating oxidative stress.[17–21]

A few studies have reported that the Mongolian people may show a significantly higher level of CRP in comparison to the Japanese people.[5,6] One study has also reported a significantly higher level of d-ROMs in the Mongolian participants than in the Japanese participants, even in younger ages (although a multivariate-adjusted analysis was not conducted in that study).[22] There have been some studies showing a significantly positive relationship between the high-sensitivity CRP (hs-CRP) and d-ROMs levels in Japanese patients at high risk for coronary artery disease[17] and in general Japanese populations.[18–20] However, the relationship between the CRP and d-ROMs levels has not yet been examined in the Mongolian people and in a restricted population of the younger generation in particular. In addition, the relationship between the SAA and d-ROMs levels has not been examined, and there have been few reports showing a positive correlation between the SAA and 8-isoprostane-prostaglandin F_2 (as an oxidative stress-related marker) in coronary artery disease patients[23] and a positive correlation between the SAA and oxidation-reduction potential maxima (as an oxidative stress-related marker) in multi-trauma patients.[24] Thus, the aim of our study was to investigate

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METHODS

A total of 78 Mongolian volunteers (27 males and 51 females), aged 18–25 years, were enrolled into this study. The inclusion criteria were asymptomatic, non-smokers, non-alcoholics and participants not taking any medications (including contraceptive pill) or supplements. The exclusion criteria were participants who had acute infectious diseases, such as a common cold, or who had a history of cardiovascular, cerebrovascular, thyroid, collagen, severe kidney or liver diseases. This study was approved by the Institutional Ethics Committee, and all volunteers provided their informed consent.

All data from each participant were collected during an overnight fasting period. The body mass index (BMI) was calculated based on the weight and height measured while participants wore light clothing without shoes. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the participant's right arm with a mercury sphygmomanometer while the person was in a seated position. The serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and fasting plasma glucose (FPG) levels were measured by standard enzymatic methods. The SAA levels were measured by a latex agglutination immunoassay (Eiken Chemical Co. Ltd., Tokyo, Japan). The serum hs-CRP levels were measured by an enzyme-linked immunosorbent assay (Assaypro Co. Ltd., St. Charles, MO, USA). The d-ROMs level was determined by means of the d-ROMs test, a photometric assay that measures the total oxidant capacity (mainly dependent on total amount of hydroperoxides, a class of reactive oxygen metabolites) of a serum sample against N,N-diethylparaphenylendiamine, used as chromogenic substrate, as previously described.[14] Briefly, serum samples were mixed with an acidic buffered solution and incubated at 37°C with the chromogenic substrate for three minutes, and after that they underwent photometric reading at 505 nm, according to the kinetic mode, in two minutes, at 37°C, by using a dedicated photometer (F.R.E.E system, Diacron International s.r.l., Grosseto, Italy). In these conditions, the d-ROMs test showed acceptable analytical performances as detected by its low intra- and inter-assay coefficients of variation (2.1% and 3.1%, respectively).[15,16] The results were expressed as CARR U, where 1 CARR U is equivalent to the oxidant capacity against N,N-diethylparaphenilendiamine of a .08 mg/dL H_2O_2 solution. The normal range of the test is between 250 and 300 CARR U.[15,16]

The data are expressed as the means ± standard deviation (SD) or the medians plus the interquartile range. Data between sexes were compared using the unpaired *t* test. A simple correlation test (Pearson's test) and a multiple linear regression analysis, adjusted for other variables (eg, age, sex, BMI, SBP, TC, TG, HDL-C and FPG), were used to observe the correlation between the SAA and d-ROMs levels, as well as between the hs-CRP and d-ROMs levels. Only the SBP, but not the DBP, was entered into the multiple linear regression analysis model, because of their collinearities in the adjustments. The TG, SAA and hs-CRP values were log-transformed in these analyses because of their skewed distributions. Statistical significance was defined as a $P<.05$.

RESULTS

The clinical participants of the subjects are listed in Table 1. Males had a significantly higher SBP level than females, while females had significantly higher levels of HDL-C and d-ROMs than males. The other variables, including SAA and hs-CRP, did not show any relative difference.

The results of the correlation between SAA and d-ROMs, as well as between hs-CRP and d-ROMs, among all participants are listed in Table 2. A simple correlation analysis for SAA showed that the d-ROMs level was significantly and positively correlated with the SAA level. A simple correlation analysis for hs-CRP showed that the d-ROMs level was significantly and positively correlated with the hs-CRP level, while there was a significant and positive correlation between the participant age and d-ROMs as well as between the BMI and d-ROMs. In addition, there was a significant and positive correlation between the SAA and hs-CRP levels ($r=.51$, $P<.01$) in a simple correlation analysis.

Subsequently, a multiple linear regression analysis for SAA revealed that the d-ROMs level remained to be significantly and positively correlated with the SAA level, along with female sex, independent of the other variables (Table 2). Similarly, a multiple linear regression analysis for hs-CRP revealed that the d-ROMs level remained to be significantly and positively correlated with the hs-CRP level, along with female sex, independent of the other variables (Table 2).

Table 1. Clinical characteristics of the study participants

	All	Male (*n*=27)	Female (*n*=51)	*P*
Age, years	21 ± 2 (18–25)	20 ± 2 (18–25)	21 ± 2 (18–25)	.30
Body mass index, kg/m^2	21.7 ± 3.0 (16.9–33.5)	22.2 ± 3.5 (16.9–32.8)	21.4 ± 2.7 (17.6–33.5)	.30
Systolic blood pressure, mm Hg	115 ± 9 (101–145)	120 ± 11 (103–145)	113 ± 8 (101–134)	<.01[a]
Diastolic blood pressure, mm Hg	70 ± 7 (56–85)	70 ± 8 (56–82)	70 ± 7 (58–85)	.80
Total cholesterol, mmol/L	4.00 ± .60 (2.78–5.75)	3.99 ± .58 (2.96–4.91)	4.00 ± .61 (2.78–5.75)	.98
HDL cholesterol, mmol/L	1.28 ± .27 (.80–2.00)	1.11 ± .20 (.80–1.54)	1.38 ± .27 (.94–2.00)	<.01[a]
Triglyceride, mmol/L	.58 [.44–.89]	.66 [.44–1.29]	.55 [.44–.77]	.06
Plasma glucose, mmol/L	4.26 ± .51 (3.22–5.89)	4.35 ± .53 (3.44–5.89)	4.22 ± .50 (3.22–5.61)	.28
SAA, μg/mL	3.2 [2.1–7.2]	3.4 [1.7–6.6]	3.2 [2.1–7.4]	.83
hs-CRP, mg/dL	.04 [.02–.08]	.06 [.02–.09]	.03 [.01–.08]	.23
d-ROMs, CARR U	309 ± 50 (222–431)	289 ± 35 (232–350)	320 ± 54 (222–431)	<.01[a]

[a] $P<.05$.

Data are expressed as means ± standard deviations (range) or medians [interquartile ranges].

HDL, high-density lipoprotein; SAA, serum amyloid A; hs-CRP, high-sensitivity C-reactive protein.

The difference between sexes was examined by the unpaired *t* test. The triglyceride, SAA and hs-CRP values were log-transformed because of their skewed distributions.

The results of the correlation between SAA and d-ROMs, as well as between hs-CRP and d-ROMs, by sex, are listed in Table 3, which displays only the results of a multiple linear regression analysis for SAA or hs-CRP. A simple correlation analysis for SAA showed that the d-ROMs level was significantly and positively correlated with the SAA level in the male group (r=.40, P=.04) and that there were no significant correlations between the other variables and the d-ROMs levels (data not shown). A correlation analysis, adjusted for age and BMI (as a basic confounder), for SAA showed that the d-ROMs level was insignificantly, but moderately positively correlated with the SAA level (r'=.35, P=.08) (data not shown). A subsequent multiple linear regression analysis for SAA revealed that the correlation between the SAA and d-ROMs levels remained to be mildly positive, but thereafter decreased to a non-significant level (Table 3). On the other hand, a simple correlation analysis for hs-CRP showed that the d-ROMs level was significantly and positively correlated with the hs-CRP level in the male group (r=.41, P=.03) and that there were no significant correlations between the other variables and the d-ROMs levels (data not shown). A correlation analysis, adjusted for age and BMI, for hs-CRP showed that the d-ROMs level was insignificantly, but moderately positively correlated with the hs-CRP level (r'=.35, P=.08). A subsequent multiple linear regression analysis for hs-CRP revealed that the correlation between the hs-CRP and d-ROMs levels remained mildly positive, but thereafter decreased to a non-significant level (Table 3).

In the female group, a simple correlation analysis for SAA showed that the d-ROMs level was significantly and positively correlated with the SAA level (r=.46, P<.01) and no significant correlations were observed between the other variables and the d-ROMs levels (data not shown). A correlation analysis, adjusted for age and BMI, for SAA showed that the d-ROMs level was significantly and positively correlated with the SAA level (r'=.44, P<.01) (data not shown). A subsequent multiple linear regression analysis for SAA revealed that the correlation between the SAA and d-ROMs levels remained significant, independent of the other variables (Table 3). On the other hand, a simple correlation analysis for hs-CRP

Table 2. The correlation between the d-ROMs (CARR U; dependent variable) and SAA levels, as well as between the d-ROMs (dependent variable) and hs-CRP levels, in all participants

	SAA		hs-CRP	
	r (*P*)	β (*P*)	*r* (*P*)	β (*P*)
Age, years	.06 (.59)	.18 (.09)	.24 (.03)[a]	.12 (.27)
Sex, female	.02 (.83)	.29 (.01)[a]	.14 (.23)	.36 (<.01)[a]
Body mass index, kg/m^2	.21 (.07)	.16 (.16)	.28 (.01)[a]	.18 (.13)
Systolic blood pressure, mm Hg	.04 (.70)	−.15 (.18)	−.06 (.61)	−.12 (.32)
Diastolic blood pressure, mm Hg	−.05 (.69)	–	−.14 (.22)	–
Total cholesterol, mmol/L	.13 (.24)	.03 (.82)	−.09 (.42)	.15 (.21)
HDL cholesterol, mmol/L	−.04 (.75)	.02 (.91)	−.07 (.57)	−.05 (.69)
Triglyceride, mmol/L	−.02 (.87)	.21 (.07)	.03 (.80)	.14 (.23)
Plasma glucose, mmol/L	−.14 (.22)	.03 (.76)	.06 (.60)	−.06 (.58)
SAA, μg/mL	.40 (<.01)[a]	.37 (<.01)[a]	–	–
hs-CRP, mg/dL	–	–	.32 (<.01)[a]	.29 (<.01)[a]

[a] $P<.05$.

The triglyceride, SAA and hs-CRP values were log-transformed because of their skewed distributions.

SAA, serum amyloid A; hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; *r*, coefficient of simple correlation test (Pearson's test); β, coefficient of a multiple linear regression analysis, adjusted for the measured variables (–:, not entered into the adjusted model).

Table 3. The correlation between the d-ROMs (CARR U; dependent variable) and SAA levels, as well as between the d-ROMs (dependent variable) and hs-CRP levels, by sex

	Male		Female	
	For SAA	For hs-CRP	For SAA	For hs-CRP
Age, years	−.10 (.66)	−.09 (.69)	.20 (.13)	.12 (.44)
Body mass index, kg/m^2	.32 (.17)	.33 (.16)	.19 (.15)	.22 (.13)
Systolic blood pressure, mm Hg	−.27 (.30)	−.24 (.38)	−.05 (.68)	−.07 (.58)
Total cholesterol, mmol/L	−.27 (.25)	−.21 (.36)	.15 (.28)	.29 (.05)
HDL cholesterol, mmol/L	−.35 (.22)	−.38 (.16)	.01 (.92)	−.04 (.80)
Triglyceride, mmol/L	.16 (.55)	.12 (.66)	.23 (.11)	.13 (.38)
Plasma glucose, mmol/L	−.15 (.50)	−.27 (.20)	−.03 (.83)	−.07 (.61)
SAA, μg/mL	.29 (.17)	–	.43 (<.01)[a]	–
hs-CRP, mg/dL	–	.30 (.14)	–	.29 (.05)

[a] $P<.05$.

The data are β-coefficients of a multiple linear regression analysis, adjusted for the measured variables (–: not entered into the adjusted model).

The triglyceride, SAA and hs-CRP values were log-transformed because of their skewed distributions.

SAA, serum amyloid A; hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein.

showed that the d-ROMs level was significantly and positively correlated with the hs-CRP level in the female group ($r=.39$, $P<.01$) and that there was also a significant and positive correlation between the participant age and d-ROMs levels ($r=.38$, $P<.01$) as well as between the BMI and d-ROMs levels ($r=.30$, $P=.03$) (data not shown). A correlation analysis, adjusted for age and BMI, for hs-CRP showed that the d-ROMs level was positively but non-significantly correlated with the hs-CRP level ($r'=.25$, $P=.09$) (data not shown). A subsequent multiple linear regression analysis for hs-CRP revealed that the correlation between the hs-CRP and d-ROMs levels showed a borderline significance level (Table 3).

We found an independent, significant and positive correlation between the SAA and d-ROMs levels in a young Mongolian population.

DISCUSSION

We found an independent, significant and positive correlation between the SAA and d-ROMs levels in a young Mongolian population. The significant correlation between the SAA and d-ROMs appeared to be plausible based on earlier studies showing the association of the SAA with oxidative stress,[23,24] although these earlier studies examined diseased populations and the oxidative stress-related markers used were different from our study.[23,24] The independent, significant and positive correlation between the hs-CRP and d-ROMs levels observed in this population was consistent with earlier studies, although these study populations and ethnicities were not always consistent with our study (ie, these earlier studies examined the Japanese diseased populations, or general but not restricted younger populations).[17–20] Our findings are new information since no report has previously investigated the correlation between the levels of d-ROMs and chronic inflammatory markers, such as hs-CRP and SAA, in the Mongolian people. This indicates that not only hs-CRP, but also SAA can be used to evaluate the relationship of oxidative stress in this population. It is important to note that our findings presented the coexistence of chronic inflammation and oxidative stress by using the d-ROMs, an established test to evaluate oxidative stress especially in clinical practice, [17–21] and the representative chronic inflammatory markers, hs-CRP and SAA, even in a young Mongolian population. Early prevention is crucial for lifestyle-related diseases, in which the cooperation of chronic inflammation and oxidative stress is a noticeable concept.[7–11] Thus, our findings implicate that the coexistence of both conditions can be considered as a target to early prevention of lifestyle-related diseases in this population.

Furthermore, we found that the correlation between the SAA and d-ROMs levels could be greater relative to the correlation between the hs-CRP and d-ROMs levels in the female, but not in the male, subpopulation in particular. The clinical significance of the relationship between the d-ROMs and SAA vs hs-CRP, in the association with sex, should be confirmed in further studies, but this may provide unique information, since the association between SAA and oxidative stress has not been thoroughly investigated (indeed, there is no report on the correlation between the SAA and d-ROMs levels) and because the comparison between SAA and CRP is often a focus to the use of inflammatory markers and the pathophysiological roles of both proteins (in that SAA has a wider dynamic range and can reflect different aspects of inflammatory pathologies than CRP).[25–28]

Our study did not address the biological mechanism(s) underlying the correlation between the oxidative stress marker, d-ROMs, and the chronic inflammatory markers, SAA and hs-CRP (which are produced in a relatively common pathway); however, there are several possible explanations. Oxidative stress, which stems from an imbalance in the oxidative/antioxidative system (eg, caused by an unhealthy lifestyle), and chronic inflammation both induce an oxidative stress response and inflammatory

molecules via cell dysfunction in systemic organs, including the adipose tissue, liver and vasculature.[7,8,29–31] Furthermore, the pathways produce a vicious cycle of oxidative stress and chronic inflammation.[32] This oxidative stress-inflammation interplay may be seen even in young patients.

Our study showed that female participants had a significantly higher d-ROMs level than males. This is consistent with earlier studies reporting a high d-ROMs level in females in not only Japanese[21] but also Mongolian patients.[22] The reasons for the sex difference in the d-ROMs level remain unclear. Further research is needed to confirm whether this can affect the possible predominance of female participants (sex difference) in the correlation between the d-ROMs and SAA vs hs-CRP observed our study.

There were some limitations to our study. The sample size was small (in males in particular), and the study population was basically limited to non-diseased participants. Unfortunately, serum antioxidant capacity was not measured in our study, although a balance oxidative/antioxidative system is a key to assess the oxidative stress status. Future studies with a prospective design, larger and more varied populations, as well as with antioxidant measurements, are necessary in order to generalize the findings.

In summary, our study showed that there was an independent, significant and positive correlation between the SAA and d-ROMs levels, in addition to a correlation between the hs-CRP and d-ROMs levels, in the young Mongolian population. The correlation between the SAA and d-ROMs levels might be greater relative to that between the hs-CRP and d-ROMs levels in the female subpopulation in particular. Thus, the coexistence of chronic inflammation and oxidative stress can be present even in young Mongolian people, suggesting that their coexistence may be a target to early prevention of lifestyle-related diseases. In addition, not only hs-CRP, but also SAA can be used to evaluate the relationship of oxidative stress status in this population. Further studies are therefore warranted to clarify the clinical relevance of these results and the biological mechanism(s) underlying the observed correlation.

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