〈Original Article〉

Prediction of the presence of atherosclerosis using high-sensitivity C-reactive protein and serotonin in apparently healthy subjects

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Summary Aim: High-sensitivity C-reactive protein (hsCRP) is a marker of inflammation. Serotonin can be released from platelets activated by atherosclerosis. We assessed the efficiency of combined hsCRP and serotonin levels in blood for detection of atherosclerosis.

Methods: Peripheral venous blood samples were taken from 70 office clerks (19 men and 51 women, aged 21 - 68 years) for measurements of serotonin and hsCRP. The Pearson product moment correlation was used to examine the correlations among quantitative variables. ABI/PWV test results were examined for participants who consented to further examination.

Results: The mean serotonin value was 146.7±57.1 ng/mL; 9 participants yielded values above the normal range (>200 ng/mL). The mean hsCRP value was 0.052±0.81 mg/dL; one-fourth of the participants yielded values above the normal range (>0.04 mg/dL). A significant linear correlation between serotonin and hsCRP was found (P<0.05). By using the ABI/PWV test, we could identify atherosclerosis in 4 of the 5 participants in the high serotonin and high hsCRP group.

Discussion: hsCRP is a marker of inflammation, and serotonin, of platelet activation. Both phenomena occur in atherosclerosis, so the combination of hsCRP and serotonin could identify atherosclerosis, particularly in the early stages of the disease.

Conclusion: Combination of serotonin and hsCRP is useful in identifying atherosclerosis.

Key words: Serotonin, High-sensitivity C-reactive protein (hsCRP), Atherosclerosis, Screening

1. Introduction

Clinical and laboratory studies have shown that inflammation plays a major role in the initiation, progression, and destabilization of atheroma. C-reactive protein (CRP) is an acute-phase reactant that is important in nonspecific host defense against inflammation and is synthesized in the liver. A more sensitive CRP assay allows determination of lower levels of CRP. This is known as high-sensitivity CRP

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(hsCRP) and has been established as an inflammatory marker in a wide range of medical conditions including atherosclerosis[1-3].

Atherosclerosis activates platelets. Once activated, these platelets release serotonin, and therefore, evaluation of serotonin levels in blood could be a valuable biomarker for future risk of cardiovascular events. Serotonin is a monoamine neurotransmitter, released from intestinal chromaffin cells and taken up by platelets. When the platelets bind to form a clot, they release serotonin, which then serves as a vasoconstrictor and helps to regulate hemostasis and clotting. Serotonin is also released at sites where platelets are activated, such as at atherosclerotic vascular lesions. Serotonin levels, particularly in platelet-poor plasma, were reported to be a useful marker of atherosclerotic disease[4-7].

Atherosclerosis is a specific form of a chronic inflammatory process resulting from interactions between plasma lipoprotein, cellular components, and the extracellular matrix of the arterial wall[8]. Lately, the ankle-brachial pressure index/pulse wave velocity (ABI/PWV) test, as a parameter of aortic stiffness, has been used to evaluate progression of atherosclerosis[9, 10]. Tanaka et al reported that a significant positive relation exists between brachial-ankle pulse wave velocity (baPWV) and carotid-femoral and brachial-ankle wave velocity (cfPWV), the most recognized index of arterial stiffness[11].

In this study, we assessed the possibility that hsCRP and serotonin are useful markers of atherosclerosis in apparently healthy men and women.

2. Methods

2.1. Research participants

We recruited 70 healthy volunteers (19 men and 51 women, aged 21 - 68 years) who had no physical signs of disease and were not taking any medication. The participants were employees of the Tsukuba University Hospital. At entry, all participants provided written informed consent to participate in the study. The study protocol was approved by the committee of the Tsukuba University Hospital (H24-77).

2.2. Serotonin and hsCRP measurements

Peripheral venous blood samples were collected into tubes containing EDTA or serum separator gel. Serotonin was determined in EDTA whole blood, and hsCRP, in sera. hsCRP levels were measured using the latex-enhanced immunoturbidimetric method on a nephelometric analyzer. Serotonin levels were measured by HPLC using a fluorescence detector.

The reference values in our laboratory were <0.04 mg/dL for hsCRP and <200 ng/mL for serotonin.

2.3. Measurement of atherosclerosis[9, 10]

The ABI/PWV test was conducted in 14 participants who consented to further examination. The subjects reclined on the examination table before the examination to obtain hemodynamic stability.

The ABI and PWV were measured using an automated device (BP-203RPE III form PWV/ABI; Omron-Colin, Kyoto, Japan). The average ABI/PWV measurements were used for the analysis.

2.4. Statistical analysis

The Pearson product moment correlation was used to determine correlations among the quantitative variables.

3. Results

3.1. hsCRP and serotonin distribution

As shown in Figure 1A, the distribution of hsCRP was highly skewed to the right: 74% of the participants yielded hsCRP levels below 0.04 mg/dL. The mean hsCRP value was 0.052±0.081 mg/dL, and the median value was 0.022 mg/dL; 18 participants (26%) yielded levels higher than 0.04 mg/dL, findings consistent with those of an earlier report[2, 3].

As shown in Figure 1B, the distribution of serotonin showed 2 peaks, one large (distributed from 80 to 200 ng/mL) and one small (250 to 280 ng/mL), a finding consistent with those of other reports[5, 6]. The mean serotonin value was 146.7±57.1 ng/mL, and the median value was 136.0 ng/mL. The second small peak was around 250 ng/mL, and 9 participants (13%) yielded levels higher than 200 ng/mL, suggesting presence of platelet activation.

Fig. 1A

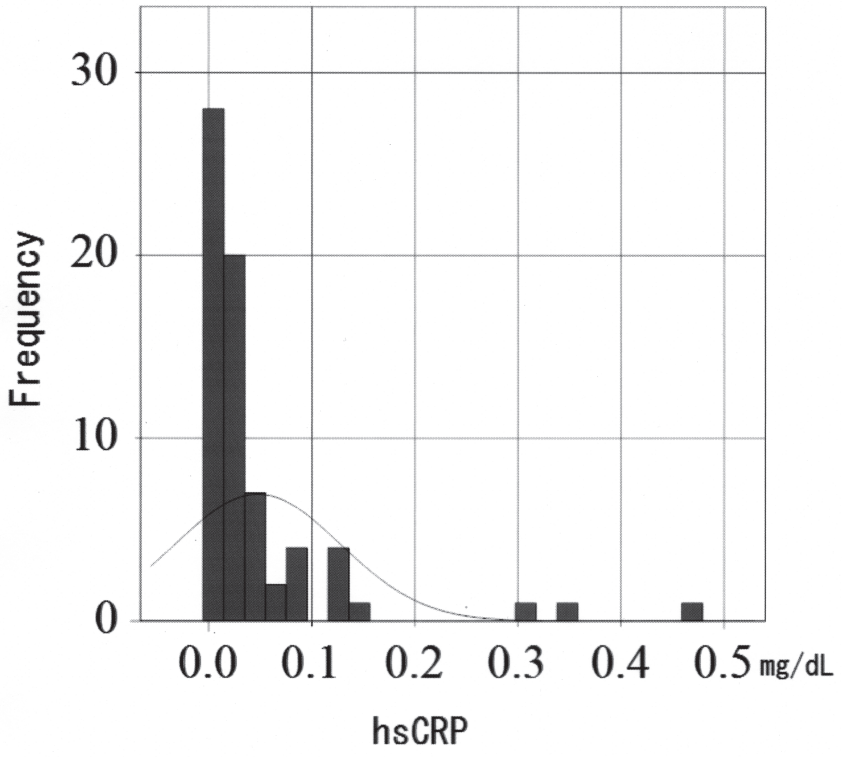

Fig. 1B

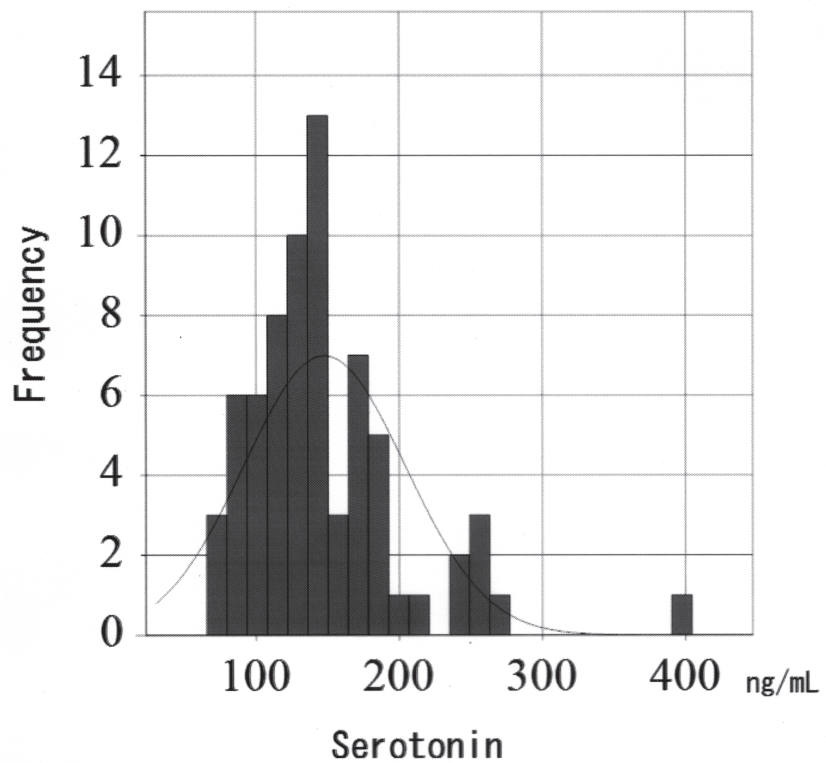

Fig. 1 hsCRP and serotonin levels in healthy participants
The distribution of hsCRP was highly skewed to the right: 26% of the participants yielded hsCRP levels higher than 0.04 mg/dL. The distribution of serotonin showed 2 peaks, one large and one small. Thirteen percent of the participants yielded levels higher than 200 ng/mL.

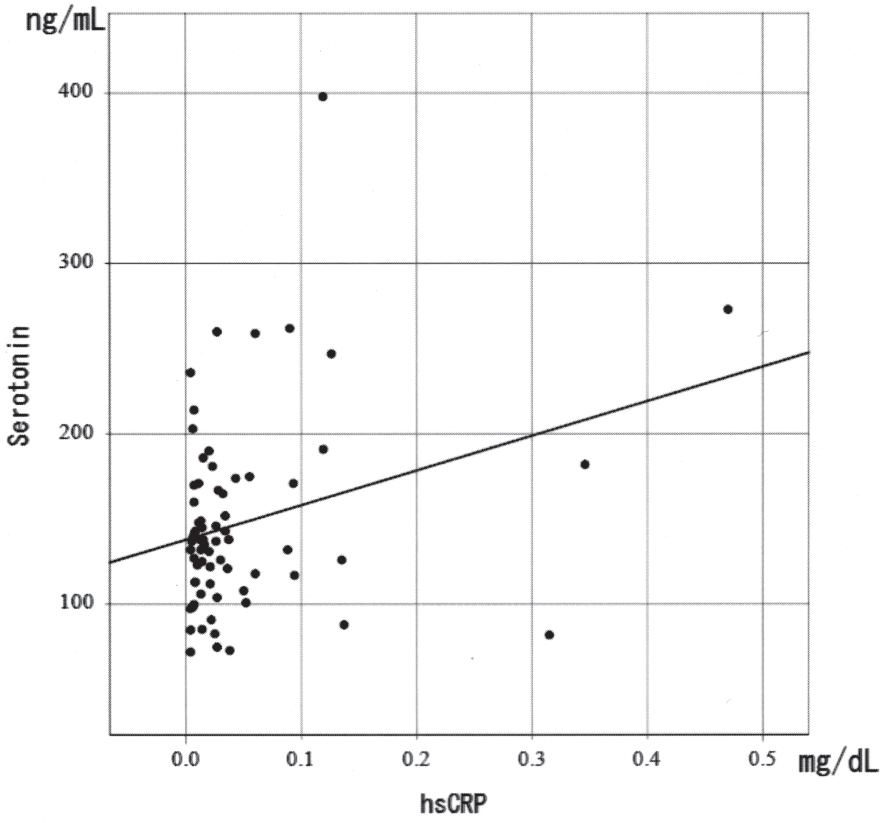

Fig. 2 Correlation between hsCRP and serotonin levels in healthy participants
A significant linear correlation between the hsCRP and serotonin levels was found (r=0.271, P<0.05).

3.2. Correlation between hsCRP and serotonin

As shown in Figure 2, a significant linear correlation between the hsCRP and serotonin levels was found (r=0.271, P<0.05). We could classify the high-serotonin group (>200 ng/mL) into 2 subgroups, one with accompanying high hsCRP (>0.04 mg/dL) and the other without it (<0.04 mg/dL).

3.3. ABI/PWV test

As shown in Table 1, we could identify 5 cases (cases 5 to 9) of atherosclerosis using the PWV test results with reference to age and sex. Four (cases 5 to 8) of those 5 atherosclerosis cases belonged to the high serotonin (3 cases had serotonin levels >200 ng/mL and 1 case had a level of 191 ng/mL) and high hsCRP (>0.04 mg/dL) group. The remaining case (case 9) was probably a case of progressive atherosclerosis. In all of 14 cases, the ABI test results were within normal levels. Although case 1 had higher baPWV (1329 cm/s), he was not diagnosed as having atherosclerosis because of his older age (59 years).

4. Discussion

In the present study, we measured hsCRP and serotonin levels in 70 healthy individuals. Even though apparently healthy, one-fourth of the participants showed high levels of hsCRP, indicating the presence of some kind of inflammation. However, the causes of inflammation are so diverse that it is hard to identify atherosclerosis by measurement of the hsCRP level alone.

The meaning of high serotonin levels is also diverse, including personal difference, platelet activation, and, in rare cases, carcinoid tumors. Hara et al reported that platelets are overactivated to release serotonin when renal damage is advanced[4]. We speculated that individuals with high levels of serotonin can be classified into 2 groups: those with accompa-

Table 1 Participants' characteristics

cases	sex	age	Serotonin ng/mL	hsCRP mg/dL	ABI	baPWV cm/s	Systolic Blood Pressure, mmHg	Atherosclerosis
1	M	59	260	0.027	1.27	1329	119	
2	W	38	236	<0.004	1.09	1072	98	
3	W	37	214	0.007	0.96	1182	102	
4	W	34	398	0.119	0.93	1065	100	
5	W	37	273	0.47	1.04	1371	128	present
6	W	46	262	0.09	1.15	1497	125	present
7	W	39	247	0.126	1.11	1249	119	present
8	W	44	191	0.119	1.14	1430	131	present
9	M	61	137	0.145	1.18	1945	159	present
10	W	33	126	0.135	1.11	879	97	
11	W	27	112	0.107	1.15	1005	114	
12	W	50	88	0.137	1.02	1214	119	
13	W	48	50	0.212	1.16	1234	143	
14	W	53	25	0.221	1.06	1154	120	

Presence of atherosclerosis was diagnozed by PWV values, considering age and sex.

nying atherosclerosis and those without.

Hirowatari et al developed a more sensitive assay system for plasma serotonin and reported that the level of serotonin in platelet-poor plasma (PPP) correlated significantly with the level of HDL-C[5]. They also reported that the PPP/WB-serotonin ratio also correlated with Framingham 10-year risk scores (FRS)[6]. Rifai et al reported that LDL-C combined with CRP had good ability to predict future cardiovascular disease[1]. Memon et al also reported that fibrinogen combined with CRP had good ability to verify coronary artery disease[3].

hsCRP is a marker of inflammation and serotonin, a marker of platelet activation. Both phenomena occur in atherosclerosis; therefore, combination of hsCRP and serotonin could identify atherosclerosis, particularly in the early stages of the disease.

Conflicts of Interest

There are no conflicts of interest associated with this manuscript.

Acknowledgements

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References

1. Rifai N, Ridker PM: High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem, 47: 403-411, 2001.
2. Rifai N, Ridker PM: Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. Clin Chem, 49: 666-669, 2003.
3. Memon L, Spasojevic-Kalimanovska V, Bogavac-Stanojevic N, Kalimanovska-Ostric D, Jelic-Ivanovic Z, Spasic S, Topic A: Association of C-reactive protein with the presence and extent of angiographically verified coronary artery disease. Tohoku J Exp Med, 209: 197-206, 2006.
4. Hara K, Hirowatari Y, Shimura Y, Takahashi H: Serotonin levels in platelet-poor plasma and whole blood in people with type 2 diabetes with chronic kidney disease. Diabetes Res Clin Pract, 94: 167-171, 2011.

5. Hirowatari Y, Hara K, Kamihara H, Iwasaka T, Takahashi H: High-performance liquid chromatographic method with column-switching and post-column reaction for determination of serotonin levels in platelet-poor plasma. Clin Biochem, 37: 191-197, 2004.
6. Hirowatari Y, Hara K, Shimura Y, Takahashi H: Serotonin levels in platelet-poor plasma and whole blood from healthy subjects: relationship with lipid markers and coronary heart disease risk score. J Athero Thromb, 18: 874-882, 2011.
7. Ohkawa R, Hirowatari Y, Nakamura K, Ohkubo S, Ikeda H, Okada M, Tozuka M, Nakahara K, Yatomi Y: Platelet release of b-thromboglobulin and platelet factor 4 and serotonin in plasma samples. Clin Biochem, 38: 1023-1026, 2005.
8. Fan J, Watanabe T: Inflammatory reactions in the pathogenesis of atherosclerosis. J Athero Thromb, 10: 63-71, 2003.
9. Wohlfahrt P, Krajcoviechova A, Seidlerova J, Mayer O, Bruthans J, Filpovsky J, Laurent S, Cifkova R: Arterial stiffness parameters: how do they differ? Atherosclerosis, 231: 359-364, 2013.
10. Nagai K, Shibata S, Akishita M, Sudoh N, Obara T, Toba K, Kozaki K: Efficacy of combined use of three non-invasive atherosclerosis tests to predict vascular events in the elderly; carotid intima-media thickness, flow-mediated dilation of brachial artery and pulse wave velocity. Atherosclerosis, 231: 365-370, 2013.
11. Tanaka H, Munakata M, Kawano Y, Ohishi M, Shoji T, Sugawara J, Tomiyama H, Yamashita A, Yasuda H, Sawayama T, Ozawa T: Comparison between carotid-femoral and brachial-ankle pulse wave velocity as measures of arterial stiffness. J Hypertens, 27: 2022-2027, 2009.