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Study on the usefulness of high-molecular-weight (HMW)-Adiponectin level check of Japanese general population upon health check: Comparison of carotid ultrasonography measurement

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ABSTRACT

Objective: High-molecular-weight (HMW)-adiponectin is an active and powerful predictor of atherosclerosis. Unfortunately, there is currently no reliable epidemiological reference level data available in Japan. The current ELISA method for HMW-adiponectin measurement is tedious as it requires several sample pretreatment steps. We studied the reference level of HMW-adiponectin measured by a simple, fully-automated CLEIA.

Design: The study subjects were 858 men and 793 women who underwent health check-ups at a Japanese Red Cross Medical Center. We investigated the relation between HMW-adiponectin and metabolic parameters, including carotid ultrasonography.

Results: The mean values of HMW-adiponectin were 3.11 ± 1.99 μg/ml in men, and 5.52 ± 3.07 μg/ml in women. There were significant differences ($p<0.001$) in relation to gender and age, where levels gradually increased with age. HMW-adiponectin was 3.18 ± 1.45 μg/ml in male subjects without carotid lesions, and 2.28 ± 0.90 μg/ml in male subjects with lesions.

Conclusions: HMW-adiponectin measured by simple CLEIA could be useful for routine risk assessment.

Introduction

Adiponectin, a protein secreted from adipose tissue and found in high-concentrations in blood, has attracted much attention of late. It is considered as a powerful predictor of insulin resistance, diabetes and metabolic syndrome[1, 2]. Although there are three forms of adiponectin, high-molecular-weight (HMW), middle-molecular-weight (MMW), and low-molecular-weight forms (LMW)[3], only HMW-adiponectin requires a post modification (glycosylation) step for production and secretion from adipocytes[4]. Recently, HMW-adiponectin has been acknowledged as a highly reliable predictive factor for metabolic syndrome[5, 6]. Although a number of reports have indicated variations in total adiponectin mean level according to ethnic group, no HMW-adiponectin level comparisons have so far been performed. Therefore, we felt that a study of HMW-adiponectin mean levels in the Japanese population was desirable. [2]

If HMW-adiponectin is validated as a useful predictor or marker of abdominal adipose mass and metabolic syndrome, it may also become a practical tool for physicians at patient consultation or explanation of status. The current ELISA method for the measurement of HMW-adiponectin requires a number of steps before measurement and several hours are needed to obtain results[7, 8]. A simplified measurement method is therefore in order. Recently, a fully-automated (CLEIA: chemiluminescent enzyme immunoassay) system to quantify HMW-adiponectin was introduced with a rapid and low CV% performance[9]. However, it was noticed that the improvement of standard purity from the previous ELISA kit affected sample valuation. Therefore, previous reports based on the ELISA kit might not be suitable for direct comparison. We have evaluated the usefulness of this method for health-check practice. Furthermore, we have compared our values with the carotid ultrasonography measurement value, which represents early abnormality of atherosclerotic events, for correlation.

Subjects and methods

Subjects

The study subjects were 858 men (mean ± SD of age; 55 ± 12 years) and 793 women (mean; 51 ± 12 years) who underwent health check-ups at a Japanese Red Cross Medical Center from October 2009 to

Abbreviations: CLEIA, (chemiluminescent enzyme immunoassay).

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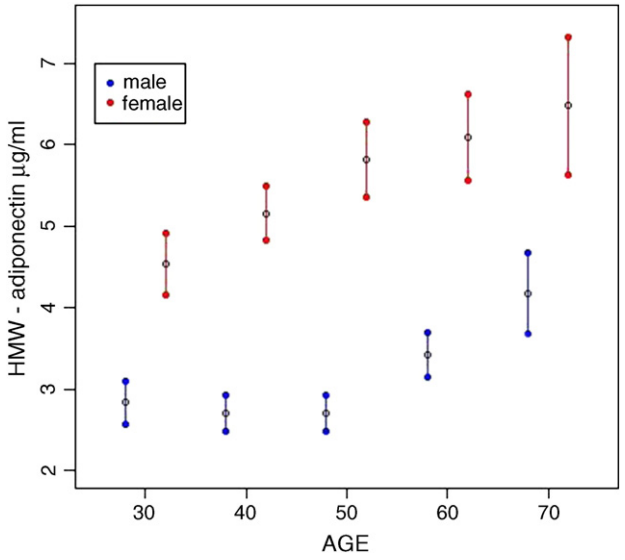

Fig. 1. HMW-adiponectin level (95% confidence interval for mean) in each 10-year age group in all subjects (n = 1651, men 858, women 793). The vertical axis represents HMW-adiponectin level (μg/ml).The mean value of HMW-adiponectin of men was significantly lower than that of women in each 10-year age group, $p<0.001$ (Mann–Whitney test). Values for men versus women were 2.83 vs 4.53 in under 40 age (n = 104, 133 respectively), 2.70 vs 5.16 in 40's age (n = 190, 231 respectively), 2.71 vs 5.81 in 50's age (n = 226, 196 respectively), 3.43 vs 6.08 in 60's age (n = 220, 168 respectively), 4.17 vs 6.47 in 70's age (n = 118, 65 respectively), and 3.11 vs 5.52 in all subjects (n = 858, 793 respectively).There were significant differences in the 10-year age group, $p<0.001$ (ANOVA), in each sex.

August 2010. All subjects gave their informed consent to participate in the study. This study was conducted in accordance with the Declaration of Helsinki. Fasting state was initially verified prior to performing the health-checks. Measurements of height, weight, and clinical chemistry parameters were routinely carried-out along with carotid ultrasonography and measurement of HMW-adiponectin. Among the subjects, 625 men (53 ± 12 years) and 667 women (50 ± 11 years) were taking no medication, and had no history of hypertension, diabetes, and dyslipidemia.

Research design

All subjects undergoing health-checks were recruited for the purpose of conducting an analysis of distribution of HMW-adiponectin level on gender, and age. Parameters related to metabolic activities, such as waist circumference, blood pressure, glucose parameters, lipid related parameters, were measured. Also, lifestyle information, such as smoking habits, alcohol intake, and others was collected.

One hundred and sixty eight subjects underwent an ultrasound evaluation of the carotid artery as well as HMW-adiponectin measurement. Among them, 47 males 40 to 65 years of age not taking any medication were divided in two groups, atherosclerotic group and non-atherosclerotic group, based on the information of carotid intima-media thickening and/or plaques, and further comparison analyses were carried out.

Methods

HMW-adiponectin was quantified using the CLEIA kit (Fujirebio, Inc. Tokyo JAPAN) on a fully-automated system (*Lumipusle Presto II*). The detailed results have previously been reported[9]. Since the purity of the standard material has been improved, the resulting values were revised from previous ELISA measurement data[8]. Other measurements, such as triglycerides, uric acid, glucose, LDL-cholesterol, HDL-cholesterol, were performed enzymatically. Also, insulin level was measured by immunoassay (*Lumipulse Presto II* system), and HbA1c level was measured by HPLC method at the Japanese Red Cross Medical Center on routine base systems. An ultrasonography unit (TOSHIBA SSA-580A) with a 7.5-8 MHz linear array probe was used for measurement of the maximum and mean intima-media thickness (IMT) of common carotid artery on the right and left side. Localized elevated (> 1.0 mm) lesions (plaques) were determined as abnormal (atherosclerotic).

Statistical analysis

Mann–Whitney test analysis was applied for gender difference comparison of mean value of HMW-adiponectin. ANOVA and Sheffe-test as PostHoc analysis were applied for comparison of age group and lifestyle difference. Comparison with other parameters was carried out using multiple regression analysis. Mann–Whitney test method was used for comparison between atherosclerotic and non-atherosclerotic groups by carotid ultrasonography.

Results

HMW-adiponectin value

The mean values of HMW-adiponectin of all subjects are shown in Fig. 1. The values are depicted separately for men and women within 10-year age groups. Mean ± SD value of men was 3.11 ± 1.99 μg/ml, and that of women, 5.52 ± 3.07 μg/ml. In every 10-year age group, the value of men was significantly lower than that of women, such as 2.83 vs. 4.53 in under 40 age group, 2.70 vs. 5.16 in 40's, 2.71 vs. 5.81 in 50's, 3.43 vs. 6.08 in 60's, 4.17 vs. 6.47 in 70's, ($p<0.001$) respectively. Levels gradually increased with age, especially from 60's in men and 50's in women. The difference between the 70 years and older segment and the 30–60 years segment in men was recognized with statistical significance. In women, the 30 years segment showed a lower value compared with the over 50 years segment with statistical significance. This phenomenon was also observed in the group without medication. The mean value of HMW-adiponectin of the group without medication is shown in Table 1. Mean ± SD value of men was 3.08 ± 1.86 μg/ml, and that of women was 5.56 ± 2.95 μg/ml.

Table 1
HMW- adiponectin level in each 10- year age group without medication.

Age	Men (n = 625)				Women (n = 667)				*p* value
	n	mean ± SD	95% CI	median	n	mean ± SD	95% CI	median	
- 39	102	2.86 ± 1.37	2.59- 3.13	2.67	131	4.54 ± 2.25	4.15- 4.93	4.21	<0.001**
40 - 49	169	2.75 ± 1.53	2.52- 2.98	2.48	217	5.24 ± 2.58	4.90- 5.59	4.80	<0.001**
50 - 59	156	2.93 ± 1.81	2.64- 3.22	2.60	171	5.93 ± 3.38	5.42- 6.44	5.23	<0.001**
60 - 69	138	3.19 ± 1.73	2.90- 3.49	2.64	114	6.49 ± 3.24	5.89- 7.09	6.13	<0.001**
70 -	60	4.53 ± 2.94	3.77- 5.29	3.60	34	6.64 ± 2.72	5.69- 7.59	6.44	0.001**
Total	625	3.08 ± 1.86	2.93- 3.23	2.67	667	5.56 ± 2.95	5.34- 5.79	5.07	<0.001**

** : $p<0.01$ (Mann-Whitney test for gender).