Hemoglobin as a response marker of endothelial cell damage in elderly non-overweight non-anemic subjects.

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An independent positive correlation between hemoglobin level and risk of hypertension has been reported for non-anemic non-overweight men and women. Additionally, serum hepatocyte growth factor (HGF) concentration in hypertensive subjects was reported to be significantly higher than in normotensive subjects. However no studies have reported on the correlation between hemoglobin and HGF. A cross-sectional study of 695 elderly non-overweight non-anemic Japanese subjects (231 men and 464 women; range 60-92 years old; Body mass index (BMI)<25kg/m^2; Hemoglobin (Hb)≥13g/dL for men and Hb≥12g/dL for women) who were undergoing general health checkups in 2014 was conducted. Multiple linear regression analysis adjustment for classical cardiovascular risk factors showed a significant positive correlation between hemoglobin and serum HGF concentration (parameter estimate (β) =31.8, P<0.001) for men and (β=21.7, P<0.001) for women. An independent positive correlation between hemoglobin and HGF was observed in elderly non-anemic non-overweight Japanese subjects. Since HGF level may become elevated in response to endothelial cell damage (vascular remodeling), these findings suggest that measuring hemoglobin level is clinically relevant for estimating the response to endothelial cell damage.

Introduction

We previously reported an independent positive correlation between hemoglobin level and the risk of hypertension in both non-anemic Japanese men and women with a body mass index (BMI) of <25 kg/m^2.[1] We also reported an independent positive correlation between hemoglobin level and increased arterial stiffness among non-anemic men and women with a BMI<25kg/m^2.[2]

On the other hand, a previous study of 201 community-dwelling healthy residents reported increased plasma hepatocyte growth factor (HGF) concentration in relation to carotid arterial remodeling.[3] Another study reported significantly higher serum HGF concentration in hypertensive subjects compared to normotensive subjects.[4-5] The measurement of serum HGF concentration in hypertensive patients may be useful for evaluating the presence of complications and degree of endothelial dysfunction.[6]

However, no studies have reported on a possible correlation between hemoglobin and serum HGF concentration.

To investigate this possibility, we conducted a cross-sectional study of 695 Japanese subjects (231 men and 464

women, range 60-92 years old) who were non-overweight (BMI<25kg/m^2) and non-anemic (hemoglobin (Hb)≥13g/dL for men and Hb≥12g/dL for women), and undergoing general health check-ups in 2014.

Subjects and Methods

Subjects

The study was conducted during a medical screening program for members of the general population aged 60-99 years who were living in Goto city, Nagasaki Prefecture, Japan. After obtaining informed consent, 1,262 Japanese subjects (449 men, 813 women) were enrolled. Overweight subjects (BMI≥25kg/m^2) (114 men, 168 women) were excluded. Additionally, to avoid the influence of chronic disease, subjects with anemia (Hb<13g/dL for men and Hb<12g/dL for women) (37 men, 83women) were also excluded, as were subjects without habitual status (drinking, smoking) data (2 men, 2women) and/or without blood sample data (65 men, 96 women), leaving a total of 695 subjects (231 men, 464 women) participating in this cross sectional-study. This study was approved by the Ethics Committee for Human Use of Nagasaki University (project registration number 14051404).

Data collection and laboratory measurements

Height and weight in bare feet and light clothing were measured, and BMI was calculated as weight (kg) / height (m^2). Trained interviewers obtained information on smoking and drinking status. Fasting blood samples were collected in an EDTA-2K tube and a siliconized tube. Samples from the siliconized tube were centrifuged after blood coagulation and the separated serum was collected. Samples from the EDTA-2K tube were used to measure hemoglobin using the sodium lauryl surfate (SLS)-hemoglobin method. Serum triglycerides, serum HDL cholesterol, serum aspartate aminotransferase (AST), serum γ-glutamyltranspeptidase (γ-GTP), HbA$_{1C}$ and serum creatinine were measured using standard laboratory procedures. To measure HGF, serum samples were diluted fourfold with specific Bio-Plex sample diluents. HGF concentration was determined using a fluorescent bead-based immunosorbent assay on a suspension array. Glomerular filtration rate (GFR) was estimated using an established method with three variations that were recently proposed by a working group of the Japanese Chronic Kidney Disease Initiative.[7] According to this adaptation, GFR (mL/min/1.73 m^2) = 194 × (serum creatinine (enzyme method))$^{-1.094}$ × (age)$^{-0.287}$ × (0.739 for women).

Statistical analysis

Sex-specific models were conducted. Difference in mean ± standard deviation (SD) values and the prevalence of potential confounding factors by hemoglobin quartile (Q) were calculated. And p for trends of those variables by hemoglobin quartiles was calculated using a generalized linear regression model.

Simple correlation coefficients of hemoglobin and other variables were calculated. The partial correlation coefficient between hemoglobin and HGF adjusted for other variables was also calculated. Simple and multiple linear regression analyses were performed to evaluate the correlation between hemoglobin and HGF. Probable values less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed with the SAS system for Windows (version 9.3; SAS Inc., Cary, NC).

Results

Characteristics of the study population based on hemoglobin level are shown in Table 1. For both men and women, a significant positive correlation between hemoglobin level and BMI and HGF were observed. Systolic blood pressure, diastolic blood pressure, and GFR also significantly correlated with hemoglobin level in women only.

Simple correlation coefficients of hemoglobin and other variables are shown in Table 2. For both men and women, significant positive correlation between hemoglobin and diastolic blood pressure, BMI, and HGF was observed. A positive correlation between triglycerides and hemoglobin was observed in men only, while a positive correlation between systolic blood pressure and GFR was observed in women only.

Simple linear regression analysis showed a significant positive correlation between hemoglobin and HGF in men (γ=31.77, P<0.001) and in women (γ=23.06, P<0.001) (Figure 1).

Evaluation of the partial correlation coefficient between hemoglobin and HGF adjusted for age, systolic blood pressure, diastolic blood pressure, BMI, drinking status, smoking status, HDL, triglycerides, AST, γ-GTP, HbA$_{1C}$ and GFR also revealed a significant correlation; adjusted partial correlation coefficients were 0.24 (P<0.001) for men, and 0.18 (P<0.001) for women.

Using multiple linear regression analysis adjustment for known cardiovascular risk factors, significant positive correlation between hemoglobin and HGF was found both in men (β=31.82, P<0.001) and in women (β=21.71, P<0.001) (Table 3).

Table 1. Characteristics of the study population in relation to hemoglobin levels

	Hemoglobin quartiles (Q)				
	Q1 (low)	Q2	Q3	Q4 (high)	p
Men					
Median hemoglobin (Hb) level, g/dL	13.5	14.3	15.0	15.9	
No. at risk	54	64	53	60	
Age, years	71.9 ± 7.0	72.0 ± 7.4	71.0 ± 7.0	70.1 ± 5.5	0.386
Systolic blood pressure, mmHg	135 ± 20	142 ± 19	136 ± 18	138 ± 18	0.201
Diastolic blood pressure, mmHg	80 ± 13	83 ± 11	83 ± 11	84 ± 11	0.233
Body mass index, kg/m^2	21.4 ± 2.1	21.8 ± 1.8	22.2 ± 1.9	22.5 ± 1.7	0.015
Current drinker, %	50.0	54.7	60.4	70.0	0.150
Current smoker, %	13.0	21.9	11.3	25.0	0.164
Serum HDL-cholesterol (HDL), mg/dL	56 ± 15	59 ± 16	58 ± 13	61 ± 16	0.464
Serum triglycerides (TGs), mg/dL	104 ± 74	90 ± 43	103 ± 55	126 ± 115	0.075
Serum aspartate transaminase (AST), IU/L	25 ± 9	24 ± 7	24 ± 6	26 ± 11	0.400
Serum γ-glutamyltranspeptidase (γ GTP), IU/L	28 ± 18	33 ± 29	36 ± 27	39 ± 23	0.120
Hemoglobin A1c (HbA1c), %	5.7 ± 0.6	5.7 ± 0.8	5.6 ± 0.4	5.7 ± 0.8	0.873
Glomerular filtration rate (GFR), mL/min/1.73m^2	67.9 ± 16.6	67.3 ± 13.2	68.5 ± 15.6	71.0 ± 11.8	0.498
Serum hepatocyte growth fcator (HGF), pg/mL	240.05 ± 94.21	259.56 ± 111.71	260.25 ± 98.02	314.81 ± 166.46	0.008
Women					
Median hemoglobin (Hb) level, g/dL	12.4	13.0	13.5	14.3	
No. at risk	123	113	112	116	
Age, years	72.1 ± 7.2	69.8 ± 6.9	70.4 ± 6.9	71.4 ± 7.2	0.052
Systolic blood pressure, mmHg	133 ± 18	134 ± 18	138 ± 17	141 ± 20	0.002
Diastolic blood pressure, mmHg	77 ± 11	79 ± 11	82 ± 10	83 ± 12	<0.001
Body mass index, kg/m^2	21.3 ± 2.1	21.1 ± 2.4	21.2 ± 2.2	21.9 ± 2.1	0.043
Current drinker, %	10.6	22.1	17.9	14.7	0.101
Current smoker, %	2	2	2	6	0.115
Serum HDL-cholesterol (HDL), mg/dL	64 ± 17	65 ± 14	66 ± 17	64 ± 15	0.635
Serum triglycerides (TGs), mg/dL	99 ± 49	105 ± 59	106 ± 73	104 ± 59	0.820
Serum aspartate transaminase (AST), IU/L	23 ± 5	22 ± 5	23 ± 6	23 ± 8	0.739
Serum γ-glutamyltranspeptidase (γ GTP), IU/L	20 ± 13	22 ± 15	21 ± 14	23 ± 15	0.300
Hemoglobin A1c (HbA1c), %	5.6 ± 0.4	5.6 ± 0.3	5.6 ± 0.4	5.6 ± 0.4	0.427
Glomerular filtration rate (GFR), mL/min/1.73m^2	65.0 ± 12.0	68.5 ± 12.5	66.0 ± 10.9	69.7 ± 11.8	0.008
Serum hepatocyte growth fcator (HGF), pg/mL	224.36 ± 82.81	225.80 ± 104.48	230.76 ± 87.56	274.58 ± 103.74	<0.001

Age: mean ± standard deviation. p: p for trend. Hemoglobin level quartiles: 13.0-13.9g/dL (Q1), 14.0-14.6g/dL (Q2), 14.7-15.2g/dL (Q3), and >15.2g/dL (Q4) for men, and 12.0-12.7g/dL (Q1), 12.8-13.2g/dL (Q2), 13.3-13.8g/dL (Q3), and >13.9g/dL (Q4) for women.

**Table 2.** Simple Correlation Coefficient of Hemoglobin and Other Variables

	Men		Women	
		p		p
No. of participants	231		464	
Age	0.24	< 0.001	-0.03	0.559
Systolic blood pressure	0.03	0.657	0.14	0.002
Diastolic blood pressure	0.13	0.048	0.18	< 0.001
Body mass index (BMI)	0.23	0.001	0.13	0.007
Drinking status	0.10	0.143	0.03	0.525
Smoking status	0.09	0.152	0.08	0.007
Serum HDL-cholesterol (HDL)	0.05	0.454	-0.02	0.728
Serum triglycerides (TG)	0.17	0.010	0.04	0.346
Serum aspartate aminotransferase (AST)	0.01	0.926	0.07	0.152
Serum γ-glutamyltranspeptidase (γ-GTP)	0.09	0.151	0.08	0.087
Hemoglobin A1c (HbA1c)	0.10	0.133	0.05	0.319
Glomerular filtration rate (GFR)	0.06	0.375	0.11	0.017
Hepatocyte growth factor (HGF)	0.24	< 0.001	0.19	< 0.001

Alcohol consumption [never-drinker, former drinker, current drinker (<23g/week , 23-46g/week, 46-69g/week, >69g/week)], smoking status (never-smoker, former smoker, current smoker).

Table 3. Multiple Linear Regression Analysis of Hepatocyte Growth Factor (HGF) with Relevant Factors adjusted for Confounding Factors

	Men			Women		
	β	95%CI	p	β	95%CI	p
No. of participants		231			464	
Age	1.43	(-1.28, 4.14)	0.299	3.6	(2.20, 5.00)	<0.001
Systolic blood pressure	-0.03	(-1.37, 1.31)	0.968	-0.58	(-1.25, 0.08)	0.087
Diastolic blood pressure	0.4	(-1.80, 2.59)	0.712	0.81	(-0.26, 1.89)	0.138
Body mass index (BMI)	6.95	(-2.00, 15.91)	0.127	1.71	(-2.34, 5.76)	0.407
Drinking status	-7.23	(-15.39, 0.93)	0.082	-2.19	(-8.33, 3.94)	0.483
Smoking status	21.86	(-5.94, 49.65)	0.123	23.42	(0.01, 46.82)	0.050
Serum HDL-cholesterol (HDL)	-0.6	(-1.82, 0.61)	0.328	-0.75	(-1.34, -0.16)	0.013
Serum triglycerides (TG)	-0.13	(-0.36, 0.11)	0.295	-0.13	(-0.29, 0.02)	0.095
Serum aspartate aminotransferase (AST)	-0.37	(-2.55, 1.81)	0.735	-0.45	(-1.92, 1.02)	0.550
Serum γ-glutamyltranspeptidase (γ-GTP)	0.15	(-0.64, 0.94)	0.708	0.35	(-0.28, 0.98)	0.272
Hemoglobin A1c (HbA1c)	-13.47	(-39.90, 12.95)	0.316	14.51	(-7.67, 36.69)	0.199
Glomerular filtration rate (GFR)	0.02	(-1.12, 1.17)	0.968	0.19	(-0.57, 0.95)	0.624
Hemoglobin (Hb)	31.82	(14.26, 49.38)	<0.001	21.71	(10.40, 33.02)	<0.001

Alcohol consumption [never-drinker, former drinker, current drinker (<23g/week , 23-46g/week, 46-69g/week, >69g/week)], current heavy drinker (every day)], smoking status (never-smoker, former smoker, current smoker).
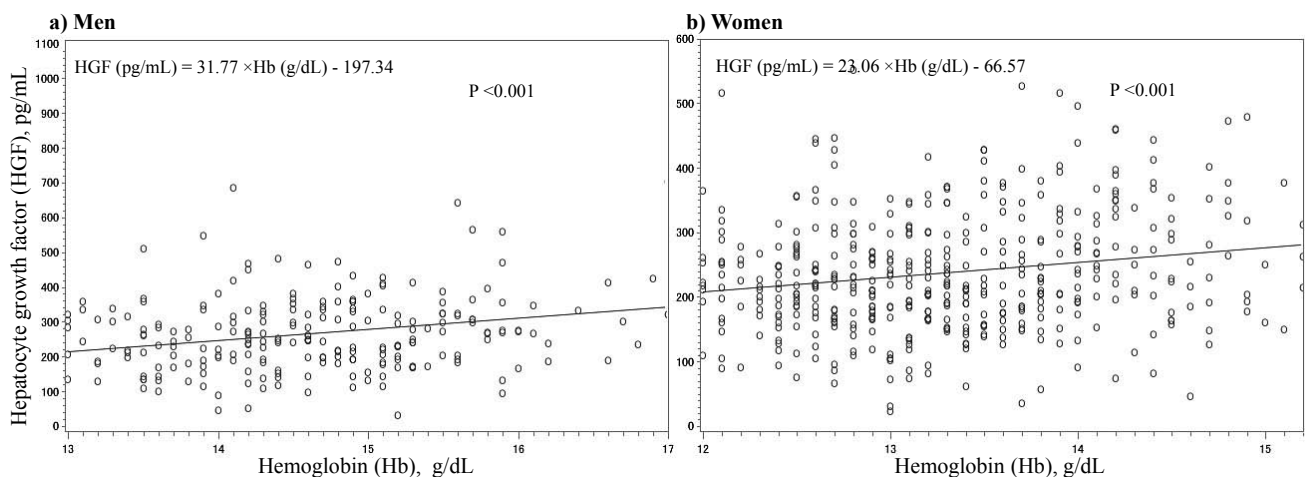
Figure 1. Simple Linear Regression Analysis of Hepatocyte Growth Factor (HGF) with Hemoglobin (Hb) in a) men and b) women.

Discussion

A major finding of the present study was a significant positive correlation between hemoglobin and HGF independent of other known cardiovascular risk factors in elderly non-anemic non-overweight Japanese men and women.

Our previous study found a positive correlation between hemoglobin and hypertension[1] in non-overweight (BMI <25kg/m^{2}) non-anemic subjects. Morishita et al reported that HGF may be considered as an index of the severity of hypertension.[6] In the present study, even after adjustment for systolic and diastolic blood pressures, an independent positive correlation between hemoglobin and HGF was observed in non-anemic non-overweight subjects.

We also previously reported a positive correlation between hemoglobin and increased arterial stiffness in non-anemic non-overweight (BMI<25kg/m^{2}) subjects.[2] Another study reported an association between increased serum HGF concentration and carotid atherosclerosis independent of known atherosclerosis risk factors.[8] Since HGF is suggested to play an important role in tissue regeneration,[9-11] serum HGF level may become elevated in response to endothelial cell damage (vascular remodeling),[6] which is the initial mechanism involved in atherosclerosis. Other recent studies

have reported associations between bone metabolism and vascular homeostasis[12-19] based on the fact that hematopoietic stem cells derived from the bone marrow play a major role in vascular homeostasis.[13-15] Since the side population of hematopoietic stem cells in the bone marrow decreases as individuals age[20,21] and this decline may be associated with an increase in the frequency of anemia and other hematopoietic disorders that are seen in the elderly,[22] hemoglobin levels in the elderly may indicate bone marrow activity. Therefore, an independent positive correlation between hemoglobin and HGF was observed through vascular remodeling activity. Observations by Takai et al that HGF is constitutively produced by bone marrow stromal cells and that it enhances hematopoiesis may support these mechanisms.[23]

Our findings should be interpreted with caution. Although statistical power demonstrated significance, the simple and multi adjusted values of the correlation coefficient of Hemoglobin and HGF in women were lower; the corresponding values were 0.19 (P<0.001) and 0.18 (P<0.001), respectively. However, serum HGF shows a significant positive correlation for quartiles of hemoglobin concentration. Also, since this study was a cross sectional, we were not able to establish any causal relationships.

In conclusion, an independent positive correlation between hemoglobin and HGF was observed in elderly non-anemic non-overweight Japanese subjects. Since HGF level may become elevated in response to endothelial cell damage (vascular remodeling),[6] these findings suggest that measuring hemoglobin level is clinically relevant for estimating the response to endothelial cell damage.

Acknowledgments

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