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Original Article

Association between Hemoglobin Levels and All-Cause Mortality Rate in Japanese Community-Dwelling Individuals

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SUMMARY

Introduction: This study examined the association between hemoglobin (Hb) levels and all-cause mortality in community-dwelling individuals in Japan.
Methods: Participants were 1,243 males (62 ± 14 years old) and 1,754 females (64 ± 12 years old). The research was based on a follow-up study with 7- and 19-year intervals.
Results: The follow-up survey revealed 448 male deaths (36.0% of male participants) and 419 female deaths (23.9% of female participants). Male participants' hazard ratio (HR) for all-cause mortality was significantly higher for groups whose Hb level was < 11.0 g/dL (HR: 2.39; 95% confidence interval [CI]: 1.28–4.47) or in the range 11.0–11.9 g/dL (2.11; 1.23–3.62) or 12.0–12.9 g/dL (1.66; 1.15–2.41) than for the reference group (14.0–14.9 g/dL). For female participants, the HR for all-cause mortality was significantly higher in groups whose Hb level was < 11.0 g/dL (1.91; 1.19–3.05), in the range 11.0–11.9 g/dL (1.44; 1.03–2.03), or ≥ 15.0 g/dL (1.77; 1.10–2.85) than for the reference group (13.0–13.9 g/dL).
Conclusions: The study revealed a linear positive association between Hb levels and the risk of all-cause mortality for male participants. However, this association formed a J-shaped curve for female participants.

1. Introduction

Blood hemoglobin (Hb) level is a nonspecific marker with abnormal findings in nutritional deficiencies[1] and chronic diseases such as hematological disorders, chronic kidney disease (CKD),[2] cardiovascular disease (CVD),[3] and malignancy.[4–6] Numerous epidemiological studies, in particular, have shown that anemia is associated with an increased risk of mortality. Some studies have found that anemia and relatively low Hb levels are predictors of increased risk of all-cause mortality,[7,8] CVD mortality,[8] and cancer mortality,[8] while others have reported no such association or no relationship after adjusting for CVD risk factors.[9]

Anemia, defined as Hb < 13.0 g/dL in male adults and < 12.0 g/dL in female adults,[10] has no impact on the overall survival of individuals aged younger than 60 years, but significantly impairs the overall survival of individuals aged 60 years and above.[11] In older participants without anemia at baseline, the young-old (65–84 years) and old-old (≥ 80 years) groups exhibited a significant association between mild Hb decline (10.0–11.9/dL in females and 10.0–12.9 g/dL in males) and increased mortality risk over a seven-year period.[12] By contrast, several prospective cohort studies have shown a nonlinear association between Hb levels and all-cause mortality. For example, Hb levels and all-cause mortality form a U-shaped association in the general population aged 20–39 years[8] and ≥ 40 years[13] and in postmenopausal women.[14] Furthermore, few population-based studies have examined the impact of mild anemia on mortality; instead, they have mostly compared specific intervals of Hb concentration with variable reference categories.[12]

Several research findings on the relationship between Hb levels and all-cause mortality remain inconsistent. To address this, the present cohort study investigated the inconsistencies noted above by examining the potentially independent role of Hb in long-term all-cause mortality in community-dwelling individuals using baseline data from the Nomura Cohort Study conducted in Seiyo.

2. Method

2.1. Study design and participants

This study constituted a prospective cohort analysis forming part of the Nomura Study conducted in 2002 (1st cohort) and 2014 (2nd cohort).[15] Participants mainly included individuals from rural Ehime Prefecture and people who received annual community-based health examinations. The 1st and 2nd cohorts respectively comprised 3,164 and 1,832 participants. All participants were aged between 22 and 95 years. A total of 2,507 participants from the 1st cohort and 490 from the 2nd cohort underwent a baseline physical examination and follow-up. Participants' lifestyle, medical history, current condition, and medication usage were obtained using a self-administered questionnaire. A flowchart of the enrolment process and exclusion criteria for participants is available in Figure 1. Follow-up surveys

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were conducted after a 19-year interval for the 1st cohort and a 7-year interval for the 2nd cohort. Participants' survival status was confirmed using Japan's Basic Resident Register. This study analyzed assessment data for the 1st and 2nd cohorts (N = 2,997). The institutional review board (IRB) of Ehime University Hospital reviewed and approved the research proposal (approval no. 1903018). All participants provided written informed consent.

2.2. Evaluation of risk factors

The participants' weight and height were measured, and their body mass index (BMI) was estimated by dividing their weight (kg) by their height squared (m^2). Smoking status (pack-years) was calculated by multiplying the number of years the participant had been a smoker by the average number of packs they smoked per day. Smoking status categories were nonsmoker, ex-smoker, light smoker (< 20 pack-years), or heavy smoker (> 20 pack-years). Daily alcohol intake categories, which were based on 1 unit of sake (22.9 g of ethanol), were nondrinker, occasional drinker (< 1 unit/day), light daily drinker (1–2 units/day), and heavy daily drinker (2–3 units/day). No participant consumed more than 3 units/day. For systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements, the participants were asked to remain in a seated position for at least five minutes before the measurement was taken. An automated sphygmomanometer was used and an appropriately sized cuff was placed on the right upper arm. Participants were also tested for triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine (Cr), serum uric acid (SUA), and blood glucose (BG), mean corpuscular volume (MCV), and red blood cell count. Participants were required to undergo an overnight fast prior to the tests. The CKD Epidemiology Collaboration (CKD-EPI) formula was modified using Japanese coefficients to estimate the glomerular filtration ratio (eGFR). The following equations were used: for males with Cr ≤ 0.9 mg/dL, $141 \times (Cr/0.9)^{-0.411} \times 0.993^{age} \times 0.813$; for males with Cr > 0.9 mg/dL, $141 \times (Cr/0.9)^{-1.209} \times 0.993^{age} \times 0.813$; for females with Cr ≤ 0.7 mg/dL, $144 \times (Cr/0.7)^{-0.329} \times 0.993^{age} \times 0.813$; for females with Cr > 0.7 mg/dL, $144 \times (Cr/0.7)^{-1.209} \times 0.993^{age} \times 0.813$.[16] Ischemic heart disease, ischemic stroke, and peripheral vascular disease were defined as CVDs. Metabolic syndrome (MetS) was determined according to the modified criteria of the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III).[17] MetS present when three or more of the following five criteria are met: 1) high waist circumference (men ≥ 85 cm and women ≥ 80 cm) according to Japanese modified waist circumference criteria[18] and/or BMI ≥ 25.0 kg/m^2 according to the guidelines of the Japanese Society for the Study of Obesity[19] if waist circumference was not available in the study; 2) elevated BP {systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg, and/ or antihypertensive medication use}; 3) high TG level (≥ 150 mg/dL); 4) low HDL cholesterol (men < 40 mg/dL and women < 50 mg/dL, and/or lipid-lowering medication use); and 5) elevated fasting BG level {≥ 100 mg/dL (equivalent to HbA1c = 5.6%) and/or antidiabetic medication use}.

2.3. Statistical analysis

IBM SPSS Statistics (version 27.0; SPSS, Chicago, IL, USA) was used for the statistical analysis. The mean ± one standard deviation (SD) is used to represent continuous variables. Median and interquartile range are reported for non-normal variables (TG and BG). Log-transformed values were used for non-normally distributed parameters in all analyses. Participants were divided into six groups according to their Hb levels (< 11.0 g/dL; 11.0–11.9 g/dL; 12.0–12.9 g/dL; 13.0–13.9 g/dL; 14.0–14.9 g/dL; ≥ 15.0 g/dL).[20] A chi-squared test was used to compare categorical variables, and analysis of variance (ANOVA) was conducted to compare normally distributed continuous variables. A multivariate analysis based on the Cox proportional hazards model, with age as the main time variable, was used for all confounders; age, obesity, alcohol drinking habits, smoking habits, history of CVD, hypertension, hypertriglyceridemia, low HDL cholesterolemia, hyper LDL cholesterolemia, diabetes, chronic kidney disease, hyperuricemia, and mean corpuscular volume.[12] The forced imputation method was used for covariates. Subgroup analyses were performed to determine the consistency of the observed associations between Hb levels and all-cause mortality. Likelihood ratio tests were used to examine interactions between Hb groups and subgroup variables. The effect variable was assessed using an interaction test adjusted for all significant confounding variables (except the effect variable). Three types of sensitivity analyses were performed to predict the complex association between Hb levels and all-cause mortality. First, participants who died within three years of baseline were excluded, to minimize the possibility of reverse causality. Second, participants with a history of CVD were excluded, to limit spurious increases in mortality. Third, participants with low BMI (< 18.5 kg/m^2) were excluded, to address the effect of malnutrition. All significance tests were two sided, and $p < 0.05$ was

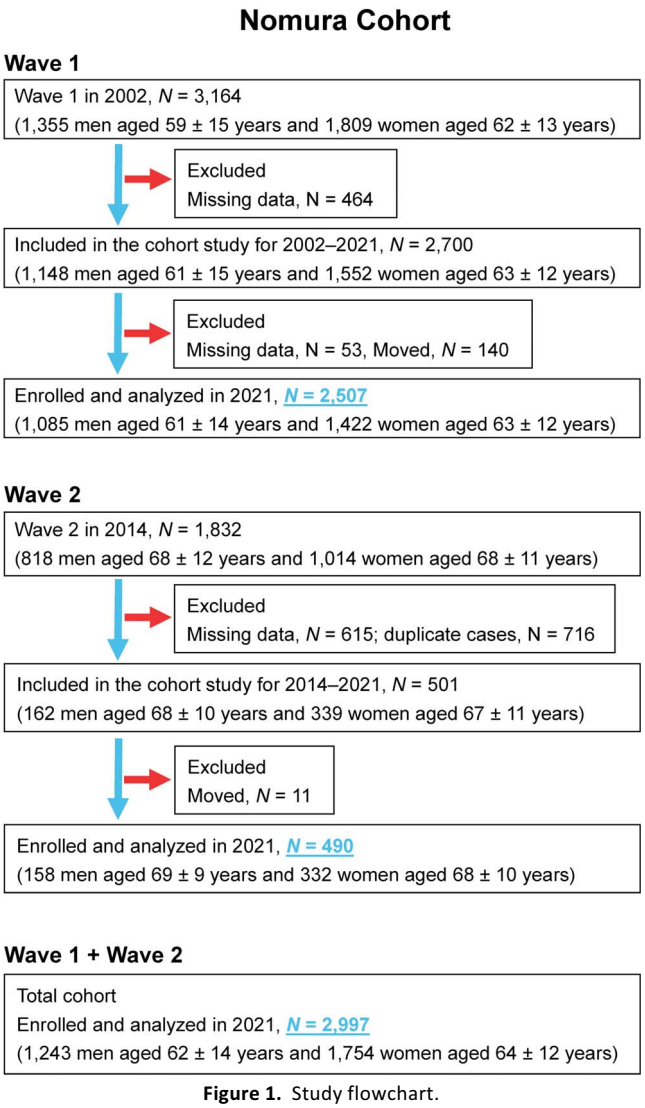

Figure 1. Study flowchart.

considered statistically significant.

3. Results

3.1. Participants' baseline characteristics by Hb category

The sample comprised 1,243 males (62 ± 14 years old) and 1,754 females (64 ± 12 years old). The median (interquartile range) follow-up period was 6,924 (3,410–6,993) days. Follow-up surveys confirmed 867 (28.9%) deaths, of which 448 were male (36.0% of all males) and 419 were female (23.9% of all females). The underlying diseases of the participants by cohort are shown in the Supplementary Table for the items investigated. Table 1 presents the participants' baseline characteristics stratified by Hb categories. The proportion of female participants and mean age decreased with increasing Hb levels. Conversely, participants with higher Hb levels were more likely to be current smokers or drinkers and have higher BMI, TG, prevalence of MetS, eGFR, MCV, and red blood cell count.

3.2. Hb levels and all-cause mortality

Table 2 shows data for the associations between stratified Hb levels and all-cause mortality. Compared with the reference group used for the sample as a whole (13.0–14.9 g/dL), the < 11.0 g/dL, 11.0–11.9 g/dL, and 12.0–12.9 g/dL groups had fully adjusted HRs of 2.05 (95% CI: 1.44–2.91), 1.53 (95% CI: 1.17–2.01), and 1.30 (95% CI: 1.08–1.58), respectively, for all-cause mortality.

Table 1
Baseline characteristics of participants by hemoglobin categories (*N* = 2,997).

Characteristics	Baseline hemoglobin categories, g/dL						*p*-value[a]
	< 11.0 g/dL (N = 105)	11.0–11.9 (N = 221)	12.0–12.9 (N = 588)	13.0–13.9 (N = 759)	14.0–14.9 (N = 634)	≥ 15.0 (N = 690)	
Male gender, n (%)	19 (18.1)	28 (12.7)	73 (12.4)	174 (22.9)	316 (49.8)	633 (91.7)	**< 0.001**
Age (years)	63 ± 16	68 ± 13	65 ± 12	65 ± 11	63 ± 12	59 ± 14	**< 0.001**
Obesity, n (%)	87 (82.9)	190 (86.0)	471 (80.1)	560 (73.8)	439 (69.2)	430 (62.3)	**< 0.001**
Body mass index (kg/m^2)	21.9 ± 3.5	22.1 ± 2.8	22.5 ± 3.1	23.2 ± 3.2	23.6 ± 3.1	24.3 ± 3.1	**< 0.001**
Smoking habits (non = 1, ex = 2, light = 3, heavy = 4) (%)	76.2/21.0/2.9/0	85.5/9.5/2.3/2.7	87.8/9.9/1.4/1.0	81.7/14.4/1.6/2.4	66.2/30.1/0.9/2.7	40.7/55.2/1.3/2.8	**< 0.001**
Alcohol drinking habits (non = 1, occasionally = 2, light = 3, heavy = 4) (%)	63.8/25.7/7.6/2.9	73.8/19.9/5.0/1.4	69.0/25.0/4.1/1.9	60.7/24.2/10.5/4.5	41.5/29.7/19.9/9.0	18.6/29.4/29.6/22.5	**< 0.001**
History of cardiovascular disease, n (%)	10 (9.5)	16 (7.2)	48 (8.2)	48 (6.3)	57 (9.0)	67 (9.7)	0.246
Hypertension, n (%)	56 (53.3)	112 (50.7)	284 (48.3)	430 (56.7)	382 (60.3)	396 (57.4)	0.001
Systolic blood pressure (mmHg)	136 ± 22	135 ± 21	134 ± 23	138 ± 21	140 ± 21	140 ± 20	**< 0.001**
Diastolic blood pressure (mmHg)	76 ± 11	75 ± 10	77 ± 11	80 ± 11	83 ± 11	85 ± 11	**< 0.001**
Antihypertensive medication, n (%)	28 (26.7)	69 (31.2)	156 (26.5)	239 (31.5)	203 (32.0)	175 (25.4)	**0.034**
Hypertriglyceridemia, n (%)	12 (11.4)	16 (7.2)	60 (10.2)	116 (15.3)	128 (20.2)	210 (30.4)	**< 0.001**
Triglyceride (mg/dL)	77 (56–109)	78 (60–101)	83 (62–115)	90 (67-126)	98 (73–135)	111 (81-161)	**< 0.001**
Low HDLcholesterolemia, n (%)	3 (2.9)	12 (5.4)	10 (1.7)	31 (4.1)	29 (4.6)	67 (9.7)	**< 0.001**
HDL cholesterol (mg/dL)	62 ± 17	64 ± 16	66 ± 15	64 ± 16	62 ± 16	58 ± 15	**< 0.001**
High LDL cholesterolemia, n (%)	17 (16.2)	61 (27.9)	179 (30.4)	259 (34.1)	189 (29.8)	172 (24.9)	**< 0.001**
LDL cholesterol (mg/dL)	104 ± 29	113 ± 27	119 ± 30	121 ± 31	119 ± 31	115 ± 34	**< 0.001**
Lipid-lowering medication, n (%)	3 (2.9)	33 (14.9)	53 (9.0)	69 (9.1)	41 (6.5)	34 (4.9)	**< 0.001**
Diabetes, n (%)	7 (6.7)	15 (6.8)	52 (8.8)	62 (8.2)	62 (9.8)	79 (11.4)	0.168
Blood glucose (mg/dL)	96 (87–114)	99 (88–117)	97 (89–110)	97 (89-111)	97 (90–109)	98 (90-110)	0.448
Antidiabetic medication, n (%)	7 (6.7)	12 (5.4)	48 (8.2)	61 (8.0)	58 (9.1)	76 (11.0)	0.112
Metabolic syndrome, n (%)	24 (22.9)	49 (22.2)	122 (20.7)	195 (25.7)	174 (27.4)	232 (33.6)	**< 0.001**
Chronic kidney disease, n (%)	25 (23.8)	26 (11.8)	66 (11.2)	71 (9.4)	56 (8.8)	60 (8.7)	**< 0.001**
eGFR (mL/min/1.73 m^2)	73.5 ± 23.4	75.8 ± 17.3	77.8 ± 16.5	79.3 ± 17.5	78.6 ± 15.6	81.1 ± 16.1	**< 0.001**
Hyperuricemia, n (%)	15 (14.3)	12 (5.4)	28 (4.8)	48 (6.3)	88 (13.9)	201 (29.1)	**< 0.001**
Serum uric acid (mg/dL)	4.7 ± 1.6	4.4 ± 1.3	4.5 ± 1.2	4.8 ± 1.2	5.3 ± 1.4	6.0 ± 1.4	**< 0.001**
Serum uric acid-lowering medication, n (%)	7 (6.7)	5 (2.3)	10 (1.7)	15 (2.0)	28 (4.4)	61 (8.8)	**< 0.001**
Mean corpuscular volume (fL)	85.0 ± 10.8	92.2 ± 6.8	93.4 ± 5.0	93.6 ± 4.6	94.5 ± 4.4	95.1 ± 5.0	**< 0.001**
Red blood cell count (×10^4/μL)	376 ± 55	386 ± 34	408 ± 25	435 ± 25	458 ± 23	497 ± 31	**< 0.001**
Hemoglobin (g/dL)	10.0 ± 1.1	11.6 ± 0.3	12.5 ± 0.3	13.4 ± 0.3	14.4 ± 0.3	15.9 ± 0.7	**< 0.001**

eGFR, estimated glomerular filtration ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data presented as mean ± standard deviation. Data for triglycerides and blood glucose were skewed and were thus presented as median (interquartile range) values and were log-transformed for analysis.
[a] *p*-values are from the ANOVA for continuous variables or from the χ^2 test for categorical variables. Significant values ($p < 0.05$) are presented in bold.

Table 2
Hazard ratios and 95% confidence intervals of baseline hemoglobin categories for all-cause mortality (N = 2,997).

Characteristics	N	Event (%)	Non-adjusted		Age-adjusted		Multivariable-adjusted	
			HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
< 11.0 g/dL	105	41 (39.0)	2.00 (1.45–2.76)	**< 0.001**	1.70 (1.23–2.36)	**0.001**	2.05 (1.44–2.91)	**< 0.001**
11.0–11.9	221	69 (31.2)	1.61 (1.24–2.08)	**< 0.001**	1.25 (0.97–1.62)	0.087	1.53 (1.17–2.01)	**0.002**
12.0–12.9	588	172 (29.3)	1.22 (1.02–1.46)	**0.034**	1.10 (0.92–1.32)	0.283	1.30 (1.08–1.58)	**0.006**
13.0–14.9	1,393	374 (26.8)	Reference		Reference		Reference	
≥ 15	690	211 (30.6)	1.09 (0.92–1.29)	0.333	1.61 (1.36–1.90)	< 0.001	1.16 (0.96–1.41)	0.119
p for trend		**0.046**	**< 0.001**		**< 0.001**		**< 0.001**	

CI, confidence interval; HR, hazard ratio.
Multivariable-adjusted HR: adjusted for age, obesity, alcohol drinking habits, smoking habits, history of cardiovascular disease, hypertension, hypertriglyceridemia, low HDL cholesterolemia, hyper LDL cholesterolemia, diabetes, chronic kidney disease, hyperuricemia, and mean corpuscular volume.
Significant values ($p < 0.05$) are presented in bold.

3.3. Kaplan-Meier survival curves for the relationships between the six Hb categories and all-cause mortality

Figure 2 depicts the Kaplan-Meier survival curves for survival days and cumulative survival rates by gender. The curves were plotted to identify patterns in the relationships between the six Hb categories and all-cause mortality by gender. For the male participants, the lower the Hb category, the lower was the survival rate. For the female participants, survival rate was highest at 13.0–13.9 g/dL and lower for the < 13.0 g/dL and ≥ 15.0 g/dL groups.

3.4. Hazard ratios and 95% CIs of baseline Hb category for all-cause mortality by gender

Table 3 presents our further analysis of the association between Hb levels and all-cause mortality by gender and the differences attributable to the varying Hb reference criteria for males and females. For the male participants, the lower the Hb category, the higher was the HR for all-cause mortality when using 14.0–14.9 g/dL as the reference. For female participants, the lower the Hb category, the higher was the HR for all-cause mortality when using 13.0–13.9 g/dL as the reference. Hb categories above 15.0 g/dL were higher than the reference and followed a U-shaped curve.

3.5. Hazard ratios and 95% CIs of baseline Hb categories for all-cause mortality by sub-analysis

Table 4 stratifies participants by cohort (1st cohort and 2nd cohort), gender (male and female), age (< 65 and ≥ 65 years), BMI (< 25 kg/m^2 and ≥ 25 kg/m^2), current smoking habits, current alcohol drinking habits, history of CVD, MetS and CKD (absence and presence). Overall, the results showed that lower Hb levels were associated with a higher risk of all-cause mortality, and this association was particularly significant in participants with BMI < 22.0 kg/m^2 (p = 0.048 for the interaction). The sensitivity analyses showed that the association between Hb levels and all-cause mortality was largely similar to the primary results when excluding individuals who died within three years of the baseline, had a CVD history, or had a low BMI (< 18.5 kg/m^2), based on the fully adjusted model (Table 5).

4. Discussion

This cohort study found that Hb is an independent and significant predictor of all-cause mortality in community-dwelling individuals in Japan. Male participants' HR for all-cause mortality was significantly higher in groups with Hb < 13.0 g/dL than in the reference group (14.0–14.9 g/dL). Female participants' HR for all-cause mortality was significantly higher in groups with Hb < 12.0 g/dL and

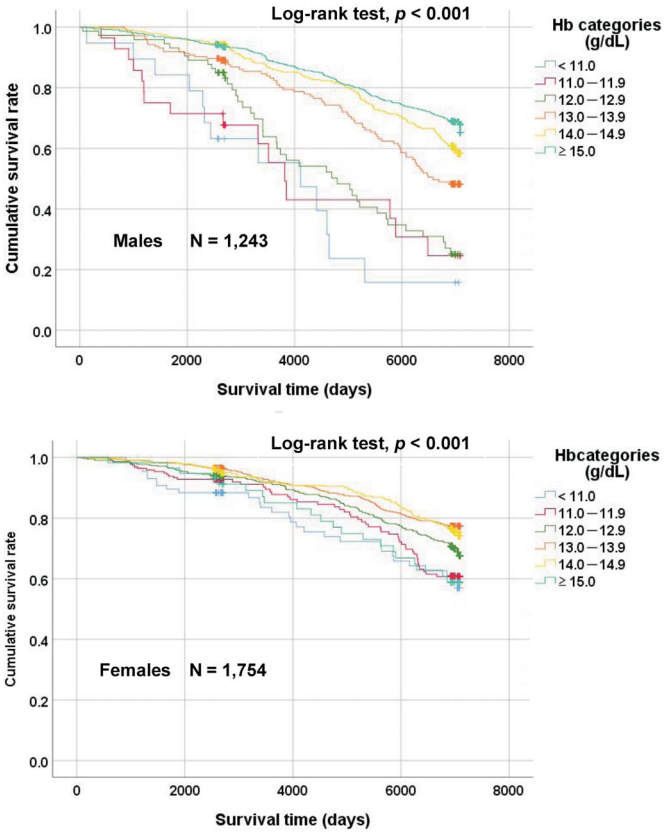

Figure 2. Kaplan-Meier survival analysis of the associations between hemoglobin categories and all-cause mortality. Hb, hemoglobin. Log-rank test: *p*-values were obtained via a log-rank test of equality across various strata.

≥ 15.0 g/dL than in the reference group (13.0–13.9 g/dL). All participants who died within three years of the follow-up period were excluded to address the issue of reverse causality. The effect of doing so on the results was minor. The results highlight an association between lower Hb levels and a higher risk of all-cause mortality, and this association was particularly significant in participants with BMI < 22.0 kg/m^2 (p = 0.048 for the interaction).

To the best of the authors' knowledge, few studies have examined the relationship between Hb levels and all-cause mortality in community-dwelling individuals in Japan, although some cohort studies have examined the impact of anemia and Hb level on all-cause mortality in general. While some show a positive association,[7–9] others suggest U- or inverted-J-shaped associations between Hb levels and all-cause mortality.[8,13,14] Ren et al.[20] studied 1,785 Chinese adults (1,002 female and 783 male) aged ≥ 65 years (mean age: 86.7 years) and reported that Hb levels were inversely and linearly associated with all-cause mortality. Two population-based cohort

Table 3
Hazard ratios and 95% confidence intervals of baseline hemoglobin categories for all-cause mortality by gender (N = 2,997).

Characteristics	N	Event (%)	Non-adjusted		Age-adjusted		Multivariable-adjusted	
			HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Males (N = 1,243)								
< 11.0 g/dL	19	13 (68.4)	4.52 (2.54–8.04)	**< 0.001**	2.49 (1.39–4.64)	**0.002**	2.39 (1.28–4.47)	**0.007**
11.0–11.9	28	16 (57.1)	3.40 (2.01–5.74)	**< 0.001**	1.85 (1.09–3.14)	**0.023**	2.11 (1.23–3.62)	**0.007**
12.0–12.9	73	42 (57.5)	2.72 (1.90–3.88)	**< 0.001**	1.70 (1.19–2.44)	**0.004**	1.66 (1.15–2.41)	**0.007**
13.0–13.9	174	74 (42.5)	1.44 (1.07–1.93)	**0.016**	1.14 (0.85–1.54)	0.369	1.18 (0.88–1.60)	0.272
14.0–14.9	316	113 (35.8)	Reference		Reference		Reference	
≥ 15.0	633	190 (30.0)	0.76 (0.60–0.96)	**0.021**	1.16 (0.92–1.47)	0.204	1.17 (0.92–1.49)	0.205
p for trend		**< 0.001**	**< 0.001**		**0.003**		**0.005**	
Females (N = 1,754)								
< 11.0 g/dL	86	28 (32.6)	2.14 (1.41–3.23)	**< 0.001**	1.77 (1.16–2.70)	**0.008**	1.91 (1.19–3.05)	**0.007**
11.0–11.9	193	53 (27.5)	1.88 (1.36–2.61)	**< 0.001**	1.43 (1.03–1.99)	**0.033**	1.44 (1.03–2.03)	**0.034**
12.0–12.9	515	130 (25.2)	1.40 (1.09–1.80)	**0.009**	1.25 (0.98–1.61)	0.078	1.27 (0.98–1.64)	0.069
13.0–13.9	585	115 (19.7)	Reference		Reference		Reference	
14.0–14.9	318	72 (22.6)	1.07 (0.80–1.44)	0.652	1.09 (0.81–1.46)	0.580	1.04 (0.77–1.40)	0.800
≥ 15.0	57	21 (36.8)	2.05 (1.29–3.26)	**0.002**	1.83 (1.15–2.91)	**0.011**	1.77 (1.10–2.85)	**0.019**
p for trend		**< 0.005**	**< 0.001**		**0.018**		**0.014**	

CI, confidence interval; HR, hazard ratio.
Multivariable-adjusted HR: adjusted for age, obesity, alcohol drinking habits, smoking habits, history of cardiovascular disease, hypertension, hypertriglyceridemia, low HDL cholesterolemia, hyper LDL cholesterolemia, diabetes, chronic kidney disease, hyperuricemia, and mean corpuscular volume.
Significant values ($p < 0.05$) are presented in bold.

Table 4
Hazard ratios and 95% confidence intervals of baseline hemoglobin (continuous) for all-cause mortality by sub-analyses (N = 2,997).

Characteristics	N	Multivariable-adjusted		*p*-value	*p* for interaction
		Events (%)	HR (95% CI)		
Cohort					---
1st cohort	2,507	834 (33.3)	0.94 (0.89–1.00)	**0.047**	
2nd cohort	490	33 (6.7)	0.66 (0.53–0.83)	**< 0.001**	
Gender					0.115
Male	1,243	448 (36.0)	0.94 (0.87–1.01)	0.070	
Female	1,754	419 (23.9)	0.90 (0.82–0.98)	**0.020**	
Age					0.390
< 65 years	1,369	134 (9.8)	0.83 (0.72–0.96)	**0.014**	
≥ 65 years	1,628	733 (45.0)	0.84 (0.79–0.89)	**< 0.001**	
Body mass index					**0.048**
< 22.0 kg/m^2	1,090	315 (28.9)	0.84 (0.76–0.93)	**0.001**	
≥ 22.0 kg/m^2	1,907	552 (27.6)	0.98 (0.92–1.06)	0.660	
Current smoking habits					0.689
No	2,888	861 (29.8)	0.93 (0.88–0.98)	**0.011**	
Yes	109	552 (28.9)	0.88 (0.28–2.34)	0.823	
Current alcohol drinking habits					0.988
No	2,281	624 (27.4)	0.92 (0.86–0.99)	**0.020**	
Yes	716	243 (33.9)	0.93 (0.84–1.03)	0.175	
History of cardiovascular disease					0.689
No	2,751	717 (26.1)	0.92 (0.87–0.98)	**0.010**	
Yes	246	150 (61.0)	0.91 (0.80–1.03)	0.148	
Metabolic syndrome					0.362
No	2,201	638 (29.0)	0.90 (0.84–0.96)	**0.001**	
Yes	796	229 (28.8)	1.00 (0.90–1.11)	0.966	
Chronic kidney disease					0.193
No	2,693	709 (26.3)	0.94 (0.88–1.00)	0.064	
Yes	304	158 (52.0)	0.85 (0.76–0.94)	**0.003**	

CI, confidence interval; HR, hazard ratio.
Multivariable-adjusted HR: adjusted for gender, age, obesity, alcohol drinking habits, smoking habits, history of cardiovascular disease, hypertension, hypertriglyceridemia, low HDL cholesterolemia, hyper LDL cholesterolemia, diabetes, chronic kidney disease, hyperuricemia, and mean corpuscular volume.
Significant values ($p < 0.05$) are presented in bold.

studies on 4,494 young-old (65–84 years) and 1,842 old-old (≥ 80 years) participants showed that all-cause mortality risk over the 15- and 11-year follow-up periods was significantly higher in individuals with mild anemia (Hb levels: 10.0–11.9/12.9 g/dL in women/men) than in those without (young-old, fully adjusted HR: 1.35; 95% CI: 1.15–1.58; old-old, fully adjusted HR: 1.28; 95% CI: 1.14–1.44).[12] An examination of 138,670 subjects aged 18–93 years indicated that 5,510 (4.0%) had anemia. Further, anemia was present in 516 (2.8%) in the 18,667 individuals older than 60 years. While anemia had no impact on the overall survival of individuals younger than 60 years old, it significantly impaired the overall survival of individuals older than 60 years.[11] A study by the Women's Health Initiative, in which

Table 5
Hazard ratios and 95% confidence intervals of baseline hemoglobin categories for all-cause mortality by sensitivity analysis (N = 2,997).

Characteristics	Multivariable-adjusted HR (95% CI)								
	N	Event (%)	Excluding participants who died within 3 years	N	Event (%)	Excluding participants with a history of CVD	N	Event (%)	Excluding participants with low BMI (< 18.5 kg/m^2)
< 11.0 g/dL	100	36 (36.0)	1.98 (1.36–2.87)	95	34 (35.8)	2.43 (1.66–3.56)	91	34 (37.4)	2.11 (1.45–3.08)
11.0–11.9	211	59 (28.0)	1.45 (1.09–1.93)	205	59 (28.8)	1.55 (1.16–2.08)	202	62 (30.7)	1.52 (1.15–2.02)
12.0–12.9	575	159 (27.7)	1.29 (1.06–1.58)	540	145 (26.9)	1.30 (1.06–1.61)	548	160 (29.2)	1.35 (1.11–1.64)
13.0–14.9	1,375	356 (25.9)	Reference	1,288	311 (24.1)	Reference	1,330	352 (26.5)	Reference
≥ 15	675	196 (29.0)	1.14 (0.93–1.39)	623	168 (27.0)	1.20 (0.97–1.48)	675	206 (30.5)	1.19 (0.98–1.45)
p for trend		0.182	**0.001**		0.080	**< 0.001**		0.087	**< 0.001**

CI, confidence interval; HR, hazard ratio.
Multivariable-adjusted HR: adjusted for age, obesity, alcohol drinking habits, smoking habits, history of cardiovascular disease, hypertension, hypertriglyceridemia, low HDL cholesterolemia, hyper LDL cholesterolemia, diabetes, chronic kidney disease, hyperuricemia, and mean corpuscular volume.
Significant values ($p < 0.05$) are presented in bold.

baseline Hb and Hb in year 3 was measured in 75,658 participants, showed that both low deciles and high deciles of baseline Hb were associated with increased all-cause mortality.[14] The present study concluded that the relationship between Hb and all-cause mortality was related to the presence of obesity but not to gender, age, or history of CVD. This relationship was significantly stronger in the thin group (BMI < 22.0 kg/m^2).

As noted above, research findings in the literature are inconsistent regarding the relationship between Hb and all-cause mortality. These inconsistencies can be attributed to the influence of confounding variables such as gender, age, history of CVD, obesity, and metabolic syndrome. Further, the mechanisms of increased all-cause mortality in participants with high and low Hb are yet to be fully understood.

This study found that lower Hb levels (< 12.0 g/dL in female and < 13.0 g/dL in male participants) were associated with an increased risk of all-cause mortality. Chronic anemia with Hb < 10 g/dL is known to cause left ventricular hypertrophy due to increased cardiac output, and is commonly observed in anemic patients with CKD.[21] Anemia reduces oxygen delivery,[22] resulting in hypoxia and eventually multiple organ dysfunction. The dysfunction may also be a marker for an underlying inflammatory process that may increase the risk of CVD events.[23] Oh et al. showed that the higher risk of all-cause mortality reflects not only susceptibility to infection in patients with a history of anemia but also the poorer prognosis of these patients after hospitalization for treatment of infection.[24] Furthermore, the risk of all-cause mortality could be greater in female patients with higher Hb levels (≥ 14.0 g/dL). A possible explanation for this is that higher Hb levels are associated with metabolic syndrome,[25,26] including insulin resistance,[27] nonalcoholic fatty liver disease (NAFLD),[28] dyslipidemia,[28,29] hypertension,[30] and hyperuricemia.[28] In the present study, participants with higher Hb levels were more likely to be current smokers or drinkers and to have higher BMI, SBP, DBP, TG levels, and SUA levels, but lower levels of HDL-C. Additional mediators are known to be hyperviscosity or changes in plasma volume,[13] endothelial cell dysfunction,[31] and higher iron/ferritin levels.[32]

The sensitivity analyses revealed patterns in the healthier subpopulation consistent with those observed in the study population as a whole. This indicates that the association between Hb levels and risk of all-cause mortality still exists in healthy individuals. Furthermore, after excluding participants who died within three years of the baseline, those with lower Hb levels tended to have a higher risk of death, while those with higher Hb levels were at a lower risk. Thus, the baseline Hb levels may be a potential biomarker for predicting mortality risk not only in high-risk populations, but also in relatively healthy populations.

The key strengths of this study were that it used a community-based population, incorporated detailed information on potentially related factors, and conducted a comprehensive analysis to examine the relationship between Hb levels and all-cause mortality. The study provides key insights on Japan's rural population, although certain concerns remain regarding the association between Hb concentrations and all-cause mortality. First, the baseline included many confounding factors (e.g., medications, underlying diseases, and lifestyle modifications) that may have falsely increased the estimated risk of all-cause mortality.[33] After adjusting for potential confounders, including demographic characteristics, lifestyle factors, disease conditions, and biochemical indicators, the analysis found a significant relationship between Hb levels and mortality. Second, baseline characteristics and Hb levels were measured only during the first visit. However, Hb values and certain covariates vary over time and may have changed during the follow-up period.[13] Third, this survey covered all deaths (whether related to internal or external factors) registered in the Basic Resident Registry. It is possible that people who moved during the survey period were excluded. Finally, the relatively small sample size may have resulted in the low statistical power of certain subgroup analyses.

5. Conclusions

This study found that abnormally low (in both genders) and high (in females, but not in males) Hb levels were related to all-cause mortality in community-dwelling individuals in Japan. Furthermore, risk reduction was stronger among participants with a BMI < 22 kg/m^2. Further large-scale cohort studies with long-term follow-up periods are needed to examine the association between Hb levels and mortality in greater detail.

Availability of data and materials

The survey data supporting this study's conclusions are not publicly available to protect participant confidentiality, but they can be made available by the corresponding author upon reasonable request.

Conflict interests

The authors declare no competing interests.

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Authors' contribution

RK participated in the study design, performed the statistical analyses, and drafted the manuscript. RK, AK, YT, and TK contributed to data acquisition and interpretation. RK and AK contributed to the conception and design of the statistical analyses. RK conceived the study, participated in its design, coordinated the writing of and helped with the drafting of the manuscript. All authors read and approved the manuscript.

Supplementary materials

Supplementary materials for this article can be found at http://www.sgecm.org.tw/ijge/journal/view.asp?id=25.

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