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Sex-specific Associations Between Serum Hemoglobin Levels and the Risk of Cause-specific Death in Korea Using the National Health Insurance Service-National Health Screening Cohort (NHIS-HEALS)

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Objectives: The purpose of this study was to determine the associations between blood hemoglobin (Hgb) levels and the risk of death by specific causes.

Methods: Using the National Health Insurance Services-National Health Screening Cohort (n=487 643), we classified serum Hgb levels into 6 sex-specific groups. Cox regression analysis was used to analyze the associations between Hgb levels and the risk of cause-specific death.

Results: Hgb levels in male population showed a U-shaped, J-shaped, or inverse J-shaped association with the risk of death from ischemic heart disease, acute myocardial infarction, liver cancer, cirrhosis and chronic obstructive pulmonary disease (COPD) (all non-linear $p<0.05$; hazard ratio [HR]; 95% confidence interval [CI]) for the lowest and the highest Hgb levels for the risk of each cause of death in male population: HR, 1.14; 95% CI, 0.98 to 1.34; HR, 2.87; 95% CI, 1.48 to 5.57; HR, 1.16; 95% CI, 0.96 to 1.40; HR, 3.05; 95% CI, 1.44 to 6.48; HR, 1.36; 95% CI, 1.18 to 1.56; HR, 2.11; 95% CI, 1.05 to 4.26; HR, 3.64; 95% CI, 2.49 to 5.33; HR, 5.97; 95% CI, 1.44 to 24.82; HR, 1.62; 95% CI, 1.14 to 2.30; HR, 3.84; 95% CI, 1.22 to 12.13, respectively), while in female population, high Hgb levels were associated with a lower risk of death from hypertension and a higher risk of death from COPD (overall $p<0.05$; HR, 1.86; 95% CI, 1.29 to 2.67 for the lowest Hgb levels for hypertension; overall $p<0.01$, HR, 6.60; 95% CI, 2.37 to 18.14 for the highest Hgb levels for COPD). For the risk of lung cancer death by Hgb levels, a linear negative association was found in male population (overall $p<0.01$; the lowest Hgb levels, HR, 1.17; 95% CI, 1.05 to 1.33) but an inverse J-shaped association was found in female population (non-linear $p=0.01$; HR, 1.25; 95% CI, 0.96 to 1.63; HR, 2.58; 95% CI, 1.21 to 5.50).

Conclusions: Both low and high Hgb levels were associated with an increased risk of death from various causes, and some diseases showed different patterns according to sex.

Key words: Hemoglobin, Anemia, Mortalities, Sex, Korea

INTRODUCTION

Blood hemoglobin (Hgb) levels are a non-specific marker for which abnormal findings are found in chronic diseases such as cardiovascular disease (CVD), malignant tumors, and hematological diseases [1,2]. Previous studies have emphasized that low serum Hgb levels increase the risk of death from various

diseases, including CVD and cancer [3-5]. However, very few studies have evaluated the effects of high Hgb levels on various types of cause-specific mortality.

Additionally, the extent to which Hgb levels affect cause-specific mortality differs according to sex. Sex differences in the associations between Hgb levels and the outcomes of CVDs, such as stroke, have been evaluated in a few studies [6-8]. Differences in mortality according to sex might be caused by physiological factors such as menopause, lean body mass, or puberty, which are known to be relevant both for individuals and at the population level [9]. To our best knowledge, although some suggestions have been made regarding possible sex differences in the associations between Hgb levels and patterns of mortality, no studies have evaluated the effect of sex on associations between Hgb levels and mortality from various causes. In order to assess these effects, we conducted an analysis of a large population cohort that contains a relatively large number of instances of cause-specific mortality.

In this study, we hypothesized that the risk of death from some diseases would be affected by low Hgb levels, while the risk of death from other diseases might also be affected by high Hgb levels. Additionally, we hypothesized that these associations between Hgb levels and death would differ according to sex. Therefore, in this study, we evaluated sex-specific associations of both low and high sex-specific Hgb levels with the risk for death from various diseases, including all causes, CVD, and malignancy, stratified by sex.

METHODS

Data Collection and Selection of Study Population

We used the National Health Insurance Service-National Health Screening Cohort (NHIS-HEALS) database, which consists of 514 795 participants who were enrolled in a health screening program provided by the NHIS in Korea. Baseline recruitment was performed in 2002 and 2003; the participants were between 40 years and 79 years of age, and were followed until 2013 [10]. Anthropometric variables (e.g., height and weight), laboratory blood and urine tests (e.g., serum Hgb levels, alanine aminotransferase levels, and aspartate aminotransferase levels), and information based on self-reported questionnaires were gathered in the baseline year. Blood tests, including Hgb levels, were conducted using samples collected at health check-ups at different healthcare centers. We excluded participants who died within 2 years from baseline (n=4522)

because we thought that it would take more than 2 years for baseline serum Hgb levels to affect participants' likelihood of death. Subjects with missing information on serum Hgb levels were excluded (n=584). We further excluded participants with a past history of diseases of blood and blood-forming organs (n=13 429). Additionally, we excluded participants with diseases that are known to possibly affect Hgb levels such as hematopoietic neoplasms (n=44), autoimmune diseases such as rheumatoid arthritis and systematic lupus erythematosus (n=7085), inflammatory bowel diseases such as ulcerative colitis and Crohn's disease (n=1065) and chronic kidney diseases (n=423). We used International Statistical Classification of Diseases and Related Health Problems (ICD)-10 codes to identify the above diseases. In total, we analyzed 487 643 subjects [11]. A schematic illustration of the selection of the study population is presented in Supplemental Material 1.

Outcome Variables

The outcome variables in this study were all-cause death and deaths caused by specific diseases. Deaths were classified according to ICD-10 codes as follows: (1) all-cause deaths were defined by the ICD-10 codes A00-Z99; (2) CVD deaths were defined by the ICD-10 codes I00-I99, and subdivided into those caused by ischemic heart disease (IHD; I20-I25), acute myocardial infarction (AMI; I21), hypertension (I10-I16), total stroke (I60-I69), hemorrhagic stroke (I60-I62), and ischemic stroke (I63); and (3) total cancer deaths were defined by the ICD-10 codes C00-C97, and subdivided into lung cancer (C34), gastric cancer (C16), colon cancer (C18-C20), and liver cancer (C22). Other diseases, including chronic obstructive pulmonary disease (COPD; J44), and liver cirrhosis including fibrosis (K74) were defined by the respective ICD-10 code. The follow-up period of participants extended from the date of enrollment to the date of death or to December 31, 2013.

Exposure Variables

A total of 487 643 participants underwent blood and urine tests. Our exposure variable was serum Hgb, and we set the lowest range of concentrations (g/dL) as Hgb<14.0 for males and Hgb<12.0 for females. Next, we further separated our participants by Hgb levels of 1 g/dL, resulting in the following classification for males: category 1: Hgb<14.0; category 2: 14.0≤Hgb<15.0; category 3: 15.0≤Hgb<16.0; category 4: 16.0≤Hgb<17.0; category 5: 17.0≤Hgb<18.0; and category 6: Hgb≥18.0 g/dL. For females, the corresponding classifica-

tion was as follows: category 1: Hgb<12.0; category 2: 12.0≤ Hgb<13.0; category 3: 13.0≤Hgb<14.0; category 4: 14.0≤ Hgb<15.0; category 5: 15.0≤Hgb<16.0; and category 6: Hgb≥16.0 g/dL. We decided to set the lower limit of Hgb levels as 14.0 g/dL for male population because research has suggested that the World Health Organization definition of anemia might not be able to distinguish anemic patients with Hgb levels of 13.0-14.0 g/dL [12]. Therefore, we chose a range that would ensure that most anemic patients would be included in the lowest Hgb range in our study. A sex-specific approach to categorization was used because mean serum Hgb levels are different according to sex, making it necessary to separate the exposure variables in this study into sex-specific ranges [9,13] (Supplemental Material 2).

Statistical Analysis

We used the chi-square test for categorical variables and analysis of variance for continuous variables to analyze differences in characteristics according to Hgb levels. The associations between Hgb levels and risk of death by various causes were estimated using Cox proportional-hazards models adjusted for potential confounders including age at enrollment, sex, body mass index (BMI), ever smoking (yes, no, and unknown), ever alcohol drinking (yes, no, and unknown), diastolic blood pressure (DBP), blood glucose, serum gamma-glutamyltransferase, and past history of diabetes and hypertension. Not only did these variables show significant differences according to Hgb levels, as presented in Supplemental Material 3, but they were also directly related to all-cause mortality. Cochran-Mantel-Haenszel analysis was used to obtain logit estimates for categories with not enough participants [14].

We assessed non-linear associations between serum Hgb levels and the respective risk of death by various causes using cubic spline regression models. To evaluate the linearity and non-linearity of these relationships, we calculated overall and non-linear *p*-values [15,16]. When we visualized a non-linear pattern in an association between Hgb and cause-specific mortality, we additionally stratified the findings by sex. All statistical analyses were conducted in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and the cubic spline regression model was performed in R version 3.5.1 (https://cran.r-project.org/ bin/windows/base/old/3.5.1/) using the packages *sas7bdat*, *foreign*, *survival*, *pspline*, *dplyr*, *Greg*, and *magrittr*.

Ethics Statement

This research was approved by the Institutional Review Board of Seoul National University Hospital (No. 1901-016-999).

RESULTS

Baseline Characteristics According to Hgb Levels

In the study population, there were 31 768 deaths by all causes during the follow-up period. Of these deaths, 6502 subjects died due to CVD and 12 386 subjects died from a malignancy (Supplemental Material 4). The participants in category 1 tended to have lower BMI, systolic blood pressure (SBP), and DBP. All the anthropometric and laboratory variables showed significant differences (*p*<0.01) according to Hgb levels.

All-cause, cardiovascular, and total cancer mortality according to Hgb levels

For all-cause death, CVD death, and total cancer death, the lowest Hgb levels were associated with elevated risk in both males and females, but the specific patterns and relationships in the cubic spline analysis showed discrepancies between males and females (Figure 1).

All-cause death showed an inverse J-shaped pattern for both males (lowest: hazard ratio [HR], 1.28; 95% confidence interval [CI], 1.23 to 1.32; highest: HR, 1.52; 95% CI, 1.22 to 1.91) and females (lowest: HR, 1.31; 95% CI, 1.23 to 1.39; highest: HR, 1.25; 95% CI, 0.98 to 1.59). The cubic spline analysis confirmed these results, with significant overall and non-linear *p*-values. For deaths from CVD, a U-shaped association was observed in males (lowest: HR, 1.13; 95% CI, 1.04 to 1.24; highest: HR, 1.77; 95% CI, 1.11 to 2.82), while for females, only the lowest Hgb level showed elevated risk (HR, 1.23; 95% CI, 1.10 to 1.38). The cubic spline graphs for deaths from CVD in males and females also showed a similar pattern to these results. Total cancer death had elevated risk in only the lowest level for both males and females (males: HR, 1.24; 95% CI, 1.17 to 1.31; females: HR, 1.31; 95% CI, 1.18 to 1.45). Although the highest Hgb level for males did not show a significant risk elevation, the cubic spline of total cancer deaths for male population showed a small increased risk at high levels of Hgb, while no such trend was observed for female population. Since all-cause, CVD, and total cancer deaths included many more specific causes of death within each outcome, varying patterns for these outcomes were expected; however, all of them showed significant overall and non-linear *p*-values.
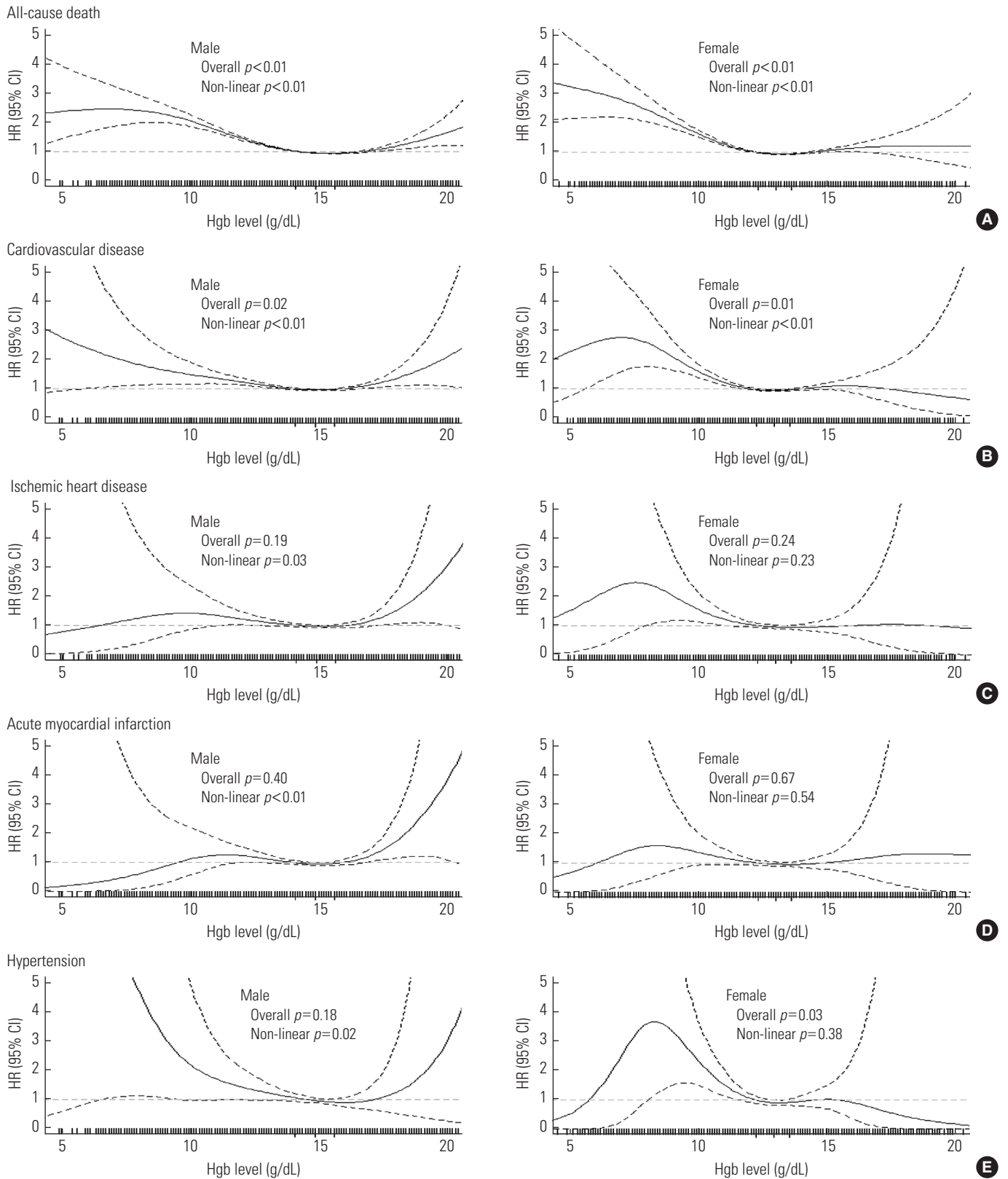
Figure 1. Cubic spline graphs for the sex-specific risk of death by various causes (A) all-cause death, (B) cardiovascular disease, (C) ischemic heart disease, (D) acute myocardial infarction, (E) hypertension, (F) total stroke, (G) hemorrhagic stroke, (H) ischemic stroke, (I) total cancer, (J) gastric cancer, (K) colon cancer, (L) liver cancer, (M) lung cancer, (N) chronic obstructive pulmonary disease, and (O) liver cirrhosis according to hemoglobin (Hgb) levels. Bold line graph represents hazard ratio (HR) between Hgb level and mortalities. Dotted line represents its 95% confidence interval (CI).
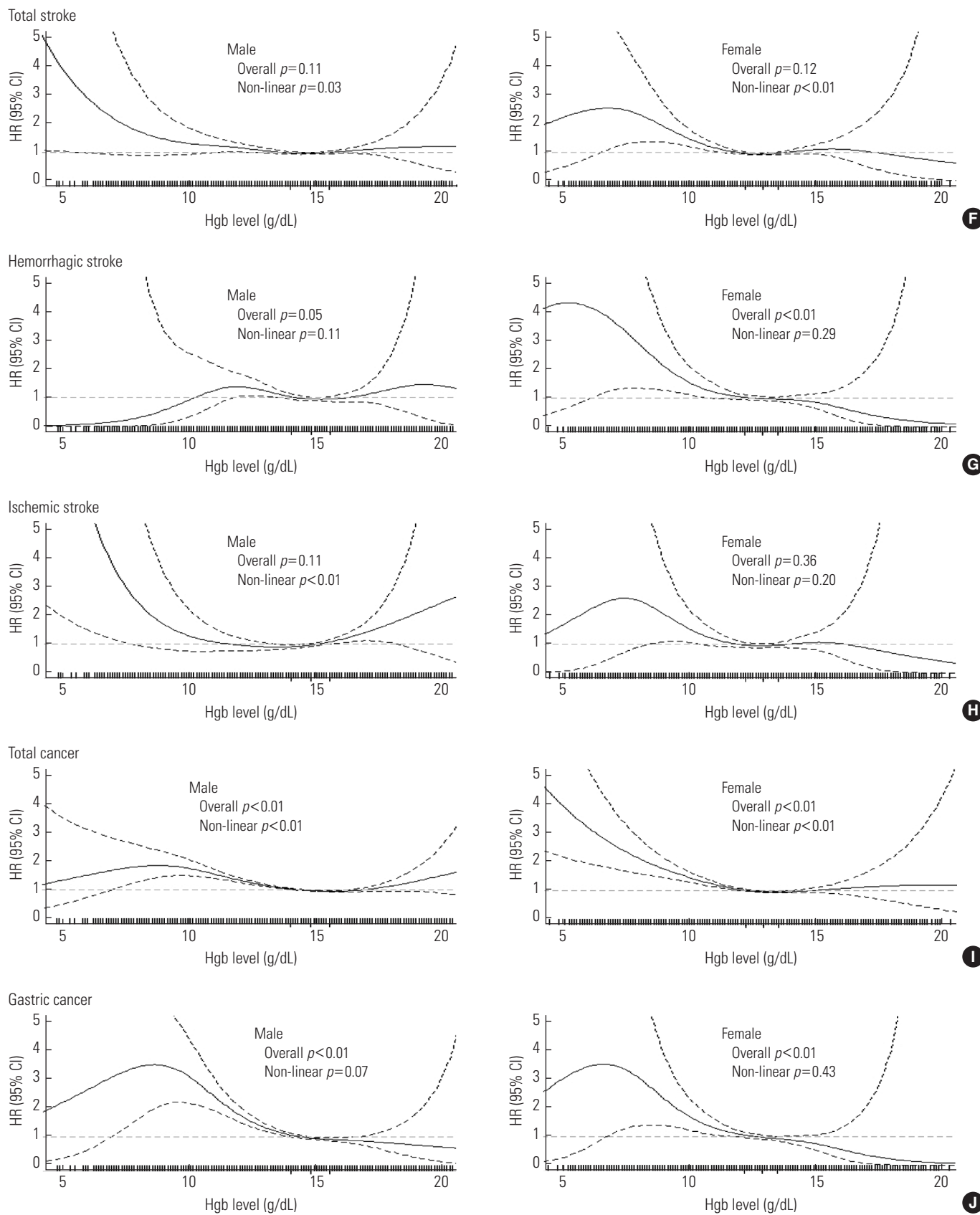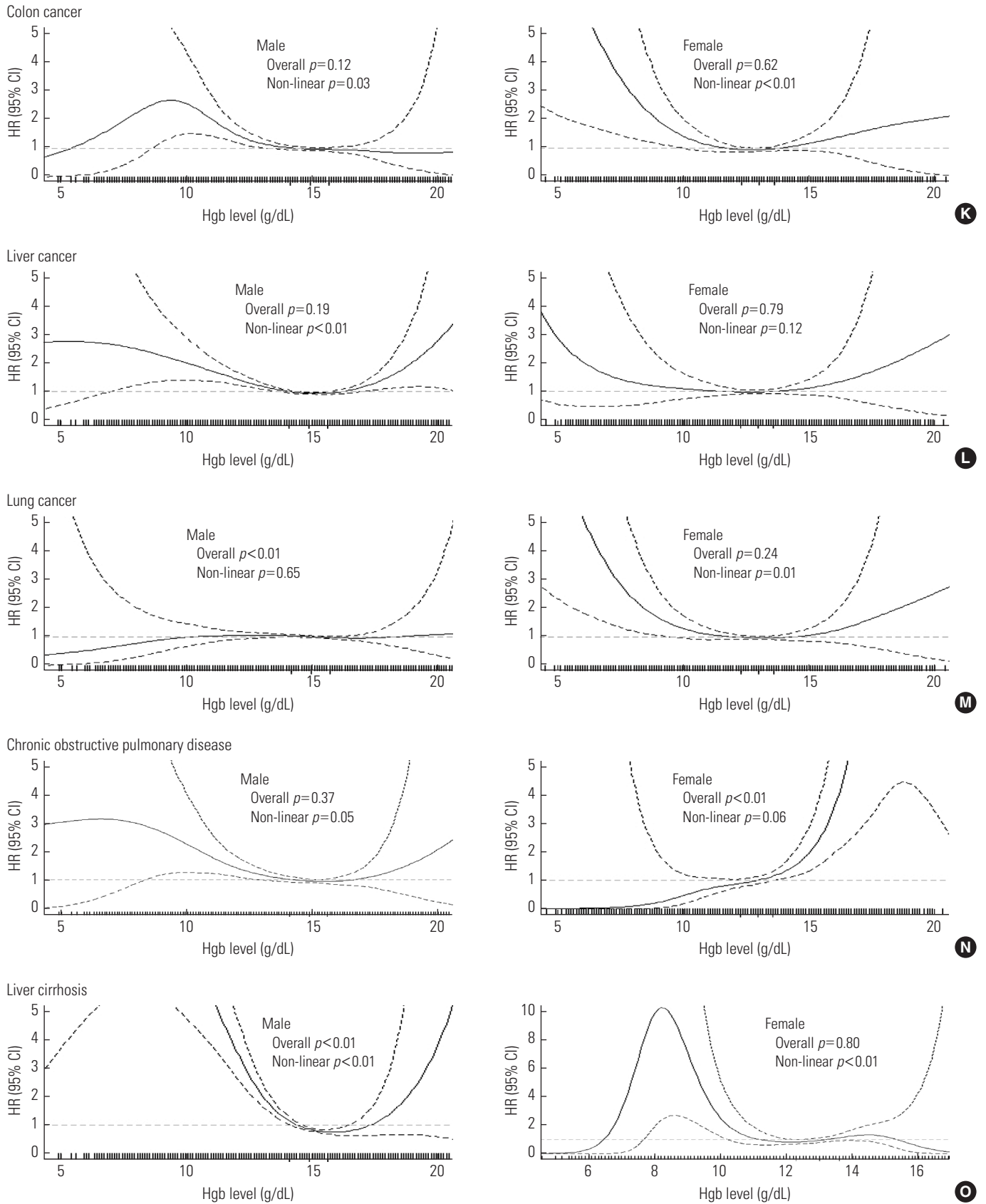
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Cause-specific mortality according to Hgb levels

When we further evaluated cause-specific deaths, IHD deaths showed a J-shaped association in males (non-linear $p<0.05$) and AMI deaths also showed J-shaped pattern in males (non-linear $p<0.01$). For deaths due to hypertension, females showed a negative linear association with Hgb levels (overall $p<0.05$, non-linear $p=0.38$). Hemorrhagic stroke deaths yielded a negative linear association in both sexes (overall $p=0.05$ for males, $p<0.01$ for females). Both males and females showed a negative linear association between Hgb levels and risk of gastric cancer death (both overall $p<0.01$). For colon cancer, an inverse J-shaped association was found in both sexes (non-linear $p<0.05$ for males, $p<0.01$ for females; respectively). For liver cancer, male population presented a U-shaped pattern (non-linear $p<0.01$). For lung cancer, males showed a negative linear association (overall $p<0.01$), while female population showed an inverse J-shaped pattern (non-linear $p=0.01$). Lastly, for COPD deaths, males showed a J-shaped pattern (non-linear $p=0.05$) while females presented a positive linear association (overall $p<0.01$). Deaths due to liver cirrhosis showed a U-shaped pattern in the male population (non-linear $p<0.01$).

Similarities and differences in the associations between Hgb levels and cause-specific mortality according to sex

Deaths due to IHD and AMI showed significant associations with both the lowest and the highest Hgb levels only in the male population (for lowest level: HR, 1.14; 95% CI, 0.98 to 1.34; HR, 1.16; 95% CI, 0.96 to 1.40; for highest level: HR, 2.87; 95% CI, 1.48 to 5.57; HR, 3.05; 95% CI, 1.44 to 6.48, respectively). For deaths from hypertension, only females presented a negative linear association (lowest level: HR, 1.86; 95% CI, 1.29 to 2.67). For liver cancer, males showed a U-shaped pattern (lowest level: HR, 1.36; 95% CI, 1.18 to 1.56; highest level: HR, 2.11; 95% CI, 1.05 to 4.26). For lung cancer, a negative linear association was found in the male population (lowest level: HR, 1.17; 95% CI, 1.05 to 1.33), while the female population demonstrated an inverse J-shaped pattern (lowest level: HR, 1.25; 95% CI, 0.96 to 1.63; highest level: HR, 2.58; 95% CI, 1.21 to 5.50). For COPD deaths, males showed an inverse J-shaped pattern (lowest level: HR, 1.62; 95% CI, 1.14 to 2.30; highest level: HR, 3.84; 95% CI, 1.22 to 12.13), while a positive linear association was found in females (highest level: HR, 6.60; 95% CI, 2.37 to 18.41). Finally, for deaths from liver cirrhosis, only the male population presented a U-shaped pattern (lowest level: HR, 3.64; 95% CI, 2.49 to 5.33; highest level: HR, 5.97; 95%

CI, 1.44 to 24.82). However, some outcome variables showed similar patterns in both males and females. The risk of death from hemorrhagic stroke showed a negative linear association with Hgb levels in males and females (males: HR, 1.31; 95% CI, 1.03 to 1.67; females: HR, 1.29; 95% CI, 0.98 to 1.69, respectively). For site-specific cancer deaths, gastric cancer showed a negative linear association in both sexes (males: HR, 1.37; 95% CI, 1.18 to 1.59; females: HR, 1.44; 95% CI, 1.09 to 1.91). For colon cancer, both males and females presented an inverse J-shaped pattern in the cubic spline analysis, but only the lowest Hgb level for males (HR, 1.24; 95% CI, 1.02 to 1.49) and the highest Hgb level for females (HR, 2.74; 95% CI, 1.12 to 6.72) resulted in significant HRs.

We performed sensitivity analyses by removing participants with any CVDs, any malignancies, and with either COPD or liver cirrhosis (n=37 127). Among 450 516 healthy individuals, similar patterns according to sex for the risk of death from various causes were observed. In male population, U-shaped, J-shaped, or inverse J-shaped associations between Hgb and the risk of death from IHD, AMI, liver cancer, cirrhosis, and COPD were shown, while the risk of death from hypertension was inversely associated with Hgb levels in females (Tables 1-4) (Supplemental Materials 5-8).

DISCUSSION

Prior epidemiological and biological studies of mechanisms based on sex hormones between males and females can sufficiently explain different patterns in deaths between males and females by Hgb levels. Androgens act as a direct stimulant of the production of red cell mass in the bone marrow and erythropoietin in the kidneys, while estrogen acts as a direct inhibitor of these processes [17-19]. Conversely, estrogen is considered to exhibit an endothelial vasodilator function, impacting the cardiovascular system and resulting in a protective effect from CVDs, whereas androgens induce vasoconstriction [13]. Higher Hgb levels, even within the normal range, are clearly associated with the risk of thrombosis [20]. These previous studies have suggested that the increased risk of IHD, including AMI, in male population is mediated by higher Hgb levels, which increase the possibility of thrombosis and vasoconstriction. In addition, low Hgb levels are well known to increase the risk of IHD, including AMI [20]. Thus, the U-shaped association of Hgb levels with the risk of IHD death in male population can be explained for both higher and lower Hgb values [20,21].

Table 1. Associations of Hgb levels with all-cause death and deaths from CVD, IHD, AMI, and hypertension[1]

Cause of death		Six levels of Hgb (g/dL)					
		Lowest (anemia)		Reference			Highest
	Male	Hgb <14.0	14.0-14.9	15.0-15.9	16.0-16.9	17.0-17.9	Hgb ≥18.0
	Female	Hgb <12.0	12.0-12.9	13.0-13.9	14.0-14.9	15.0-15.9	Hgb ≥16.0
All-cause							
Male		1.28 (1.23, 1.32)*	1.06 (1.03, 1.10)*	1.00 (reference)	1.05 (0.99, 1.10)	1.13 (1.02, 1.25)*	1.52 (1.22, 1.91)*
Death (n)		6927	7007	5183	2126	410	77
Female		1.31 (1.23, 1.39)*	1.01 (0.96, 1.06)	1.00 (reference)	1.02 (0.96, 1.09)	1.15 (1.02, 1.29)*	1.25 (0.98, 1.59)
Death (n)		1820	3450	3138	1265	299	66
CVD							
Male		1.13 (1.04, 1.24)*	1.05 (0.97, 1.14)	1.00 (reference)	1.06 (0.95, 1.19)	1.12 (0.89, 1.41)	1.77 (1.11, 2.82)*
Death (n)		1155	1298	970	412	80	18
Female		1.23 (1.10, 1.38)*	0.92 (0.84, 1.01)	1.00 (reference)	0.95 (0.84, 1.08)	1.08 (0.85, 1.36)	1.13 (0.69, 1.85)
Death (n)		468	856	834	318	77	16
IHD							
Male		1.14 (0.98, 1.34)	1.08 (0.93, 1.25)	1.00 (reference)	1.00 (0.81, 1.23)	1.06 (0.70, 1.60)	2.87 (1.48, 5.57)*
Death (n)		343	416	309	124	24	9
Female		1.08 (0.84, 1.38)	0.97 (0.80, 1.19)	1.00 (reference)	0.78 (0.58, 1.05)	1.16 (0.72, 1.86)	0.61 (0.15, 2.47)
Death (n)		93	203	188	59	19	2
AMI							
Male		1.16 (0.96, 1.40)	1.12 (0.94, 1.33)	1.00 (reference)	1.06 (0.83, 1.34)	1.20 (0.76, 1.89)	3.05 (1.44, 6.48)*
Death (n)		249	313	227	97	20	7
Female		0.94 (0.70, 1.27)	1.01 (0.80, 1.27)	1.00 (reference)	0.82 (0.59, 1.14)	1.31 (0.78, 2.20)	0.41 (0.06, 2.96)
Death (n)		63	160	141	46	16	1
Hypertension							
Male		1.16 (0.81, 1.67)	1.01 (0.71, 1.45)	1.00 (reference)	0.77 (0.45, 1.33)	0.72 (0.23, 2.31)	3.18 (0.78, 13.06)
Death (n)		71	71	54	17	3	2
Female		1.86 (1.29, 2.67)*	0.96 (0.68, 1.35)	1.00 (reference)	1.03 (0.66, 1.62)	1.21 (0.55, 2.64)	0.92 (0.13, 6.66)
Death (n)		57	69	64	27	7	1

Values are presented as hazard ratio (95% confidence interval).
Hgb, hemoglobin; CVD, cardiovascular disease; IHD, ischemic heart disease; AMI, acute myocardial infarction.
[1]Adjusted for age, body mass index, cigarette smoking, alcohol drinking, exercise, diastolic blood pressure, blood glucose levels, and gamma-glutamyltransferase levels.
*$p < 0.05$.

Regarding the risk of death from stroke, a retrospective study showed negative linear associations between Hgb levels and death from stroke in both males and females [6]. However, the association between Hgb levels and stroke death risk was similar between males and females [6].

In our results, male population presented a negative linear association with lung cancer mortality, while females demonstrated an inverse J-shaped association. Among the many mechanisms that have been suggested to explain sex differences in lung cancer mortality, sex hormones seem to be the most convincing one. Even though the exact mechanism is still unclear, estrogen acts as an estrogen receptor ligand and may promote cell proliferation. Furthermore, strong evidence has been reported that estrogen stimulates angiogenesis, which is the most important target for lung cancer treatment [22,23].

There are several reasons for sex-specific associations between Hgb levels and the risk for death from specific diseases. Physiologically, healthy males and females have different mean concentrations of Hgb. In particular, Hgb levels are higher in males than in females, and the cut-off levels of anemia are lower for females than for males [17]. Sex differences in Hgb levels are likely to be associated with the risk of death from diseases. The effects of sex hormones on Hgb levels and the vascular system are discussed above. These differences in hormone levels between males and females may play a key role in discrepancies according to sex in our outcome variables. In males, a

Table 2. Associations of hemoglobin (Hgb) levels with death from total stroke, hemorrhagic stroke, and ischemic stroke[1]

Cause of death		Six levels of Hgb (g/dL)					
		Lowest (anemia)	Reference				Highest
	Male	Hgb <14.0	14.0-14.9	15.0-15.9	16.0-16.9	17.0-17.9	Hgb ≥18.0
	Female	Hgb <12.0	12.0-12.9	13.0-13.9	14.0-14.9	15.0-15.9	Hgb ≥16.0
Stroke							
Male		1.09 (0.96, 1.24)	0.99 (0.88, 1.13)	1.00 (reference)	1.07 (0.90, 1.27)	1.08 (0.77, 1.52)	1.03 (0.43, 2.48)
Death (n)		554	586	452	192	36	5
Female		1.17 (1.00, 1.38)	0.91 (0.80, 1.04)	1.00 (reference)	0.93 (0.77, 1.11)	1.07 (0.77, 1.48)	1.22 (0.63, 2.36)
Death (n)		227	438	436	161	40	9
Hemorrhagic stroke							
Male		1.31 (1.03, 1.67)*	1.21 (0.96, 1.52)	1.00 (reference)	1.10 (0.81, 1.51)	1.55 (0.91, 2.65)	1.49 (0.37, 6.04)
Death (n)		149	183	127	58	15	2
Female		1.29 (0.98, 1.69)	0.92 (0.73, 1.15)	1.00 (reference)	0.89 (0.65, 1.21)	0.55 (0.26, 1.17)	0.79 (0.20, 3.20)
Death (n)		83	149	153	54	7	2
Ischemic stroke							
Male		1.01 (0.81, 1.26)	0.93 (0.75, 1.16)	1.00 (reference)	1.40 (1.06, 1.84)*	1.35 (0.78, 2.34)	1.27 (0.31, 5.13)
Death (n)		184	181	143	78	14	2
Female		1.19 (0.88, 1.60)	0.93 (0.72, 1.19)	1.00 (reference)	0.91 (0.65, 1.29)	1.21 (0.68, 2.14)	0.96 (0.24, 3.86)
Death (n)		70	131	123	45	13	2

Values are presented as hazard radio (95% confidence interval).
[1] Adjusted for age, body mass index, cigarette smoking, alcohol drinking, exercise, diastolic blood pressure, blood glucose levels, and gamma-glutamyltransferase levels.
*$p < 0.05$.

high risk for death was mainly found for diseases caused by atherosclerosis. Atherosclerosis is linked to thrombosis, the risk of which increases at high Hgb levels, especially in males, and the risks of high blood viscosity have been emphasized in males [17,24]. As shown in Supplemental Material 3, male population with the highest Hgb levels tended to have high mean SBP, DBP, BMI, and blood sugar levels, unlike what was observed for females. They were also more likely to smoke and drink than females, which could also act as risk factors for increased blood viscosity [17]. These differences between males and females in terms of general characteristics indirectly explain the differences in results by sex at the highest Hgb levels. Despite these hormonal differences, the risk of death from some diseases in our results was not affected by sex-specific Hgb levels. Our data contained participants in a health examination program, so there were relatively many participants with the lowest and highest Hgb levels; however, some categories of cause-specific death did not have enough participants to evaluate the effect of sex-specific Hgb levels on mortality.

In our sensitivity analysis, the patterns found in a healthier sub-population were consistent with those found in the entire study population. This implies that the associations between Hgb levels and risk of cause-specific deaths still existed in healthy participants. Additionally, when we analyzed the population before excluding participants who died within 2 years of baseline, people with low Hgb levels had a higher risk of mortality and those with high Hgb levels had a lower risk of mortality than the final study population (data not shown). Altogether, this might suggest that serum Hgb levels could be a potential biomarker for predicting the risk of mortality not only in the high-risk population, but also in relatively healthy populations.

There are limitations of our research. First, the causes of death from national death records were not fully confirmed by physicians or electronic medical records, meaning that possible discrepancies between our data and the real cause of death could not be ruled out. However, a recent study concluded that information on death certificates from Statistics Korea were reasonably valid [25]. Second, our data did not contain information on stages of cancer. Third, we could not investigate levels of blood viscosity because our dataset did not contain information on hematocrit. Fourth, possible residual confounders such as history of prescription medications or chemotherapy were not included. Fifth, it is well known that pregnancy can affect Hgb levels in females, but our data did

Table 3. Associations of hemoglobin (Hgb) levels with death from total cancer and site-specific cancers (gastric, colon, liver, and lung cancer)[1]

Cause of death		Six levels of Hgb (g/dL)					
		Lowest (anemia)	Reference				Highest
	Male	Hgb <14.0	14.0-14.9	15.0-15.9	16.0-16.9	17.0-17.9	Hgb ≥18.0
	Female	Hgb <12.0	12.0-12.9	13.0-13.9	14.0-14.9	15.0-15.9	Hgb ≥16.0
Total cancers							
Male		1.24 (1.17, 1.31)*	1.07 (1.01, 1.13)*	1.00 (reference)	1.03 (0.96, 1.18)	1.09 (0.93, 1.28)	1.29 (0.89, 1.88)
Death (n)		2764	2994	2238	911	171	28
Female		1.31 (1.18, 1.45)*	1.07 (0.98, 1.16)	1.00 (reference)	0.99 (0.88, 1.11)	1.13 (0.92, 1.40)	1.17 (0.75, 1.82)
Death (n)		556	1162	1041	406	95	20
Gastric cancer							
Male		1.37 (1.18, 1.59)*	0.99 (0.85, 1.15)	1.00 (reference)	0.89 (0.72, 1.11)	0.67 (0.40, 1.12)	0.96 (0.31, 2.98)
Death (n)		435	398	322	113	15	3
Female		1.44 (1.09, 1.91)*	1.04 (0.82, 1.32)	1.00 (reference)	0.82 (0.58, 1.17)	0.99 (0.52, 1.88)	1.31 (0.21, 6.51)[2]
Death (n)		82	147	130	41	10	0
Colon cancer							
Male		1.24 (1.02, 1.49)*	1.02 (0.85, 1.22)	1.00 (reference)	1.01 (0.79, 1.30)	0.71 (0.39, 1.30)	1.38 (0.44, 4.30)
Death (n)		246	266	213	87	11	3
Female		1.06 (0.76, 1.47)	1.08 (0.84, 1.39)	1.00 (reference)	1.05 (0.75, 1.47)	1.33 (0.73, 2.41)	2.74 (1.12, 6.72)*
Death (n)		53	136	117	47	12	5
Liver cancer							
Male		1.36 (1.18, 1.56)*	1.06 (0.92, 1.20)	1.00 (reference)	1.19 (1.01, 1.41)*	1.50 (1.10, 2.04)*	2.11 (1.05, 4.26)*
Death (n)		446	494	403	200	45	8
Female		1.10 (0.80, 1.52)	1.01 (0.79, 1.29)	1.00 (reference)	1.00 (0.72, 1.38)	1.33 (0.77, 2.32)	0.93 (0.23, 3.77)
Death (n)		56	131	126	51	14	2
Lung cancer							
Male		1.17 (1.05, 1.33)*	1.06 (0.95, 1.17)	1.00 (reference)	0.96 (0.82, 1.12)	0.99 (0.71, 1.36)	0.88 (0.37, 2.13)
Death (n)		767	862	612	219	39	5
Female		1.25 (0.96, 1.63)	1.10 (0.89, 1.35)	1.00 (reference)	1.02 (0.77, 1.36)	0.82 (0.44, 1.50)	2.58 (1.21, 5.50)*
Death (n)		86	199	168	67	11	7

Values are presented as hazard radio (95% confidence interval).
[1]Adjusted for age, body mass index, cigarette smoking, alcohol drinking, exercise, diastolic blood pressure, blood glucose levels, and gamma-glutamyltransferase levels.
[2]Logit estimates not available.
*$p<0.05$.

not contain information on pregnancy. According to statistics in Korea, we estimated that 6.0% of 40-year-old to 44-year-old femlaes were pregnant, corresponding to 2906 females in our study, and that 0.2% of 45-year-old to 49-year-old females were pregnant, corresponding to 77 females in our study [26]. Lastly, the method of Hgb sample collection might have differed in various healthcare centers, leading to measurement errors that could have affected our exposure variables.

Despite the above limitations, our study has strengths. First, we used public data with a large study population to increase generalizability. Second, the follow-up period of our data (up to 11 years) was relatively long.

Our results both demonstrate the effects of higher and lower levels of Hgb on various types of cause-specific mortality risk and show sex-specific differences in the association between Hgb levels and cause-specific mortality. Moreover, these patterns regarding Hgb levels and the risk of mortality were found in a relatively healthy sub-population, as well as in the entire population of health examinees. This implies that serum Hgb levels could serve as a potential biomarker for predicting the risk of mortality in the healthy population. Further studies are required to expand upon our results.

Table 4. Associations of Hgb levels with death from COPD and liver cirrhosis[1]

Cause of death		Six levels of Hgb (g/dL)					
		Lowest (anemia)	Reference				Highest
	Male	Hgb <14.0	14.0-14.9	15.0-15.9	16.0-16.9	17.0-17.9	Hgb ≥18.0
	Female	Hgb <12.0	12.0-12.9	13.0-13.9	14.0-14.9	15.0-15.9	Hgb ≥16.0
COPD							
Male		1.62 (1.14, 2.30)*	1.14 (0.88, 1.47)	1.00 (reference)	0.96 (0.74, 1.24)	0.82 (0.30, 2.22)	3.84 (1.22, 12.13)*
Death (n)		174	166	91	48	4	3
Female		0.70 (0.41, 1.19)	0.77 (0.50, 1.17)	1.00 (reference)	1.12 (0.64, 1.96)	2.49 (1.17, 5.30)*	6.60 (2.37, 18.41)*
Death (n)		21	44	43	17	8	4
Liver cirrhosis							
Male		3.64 (2.49, 5.33)*	1.73 (1.17, 2.57)*	1.00 (reference)	1.22 (0.70, 2.13)	2.55 (1.14, 5.72)*	5.97 (1.44, .24.82)*
Death (n)		117	75	37	20	7	2
Female		1.10 (0.61, 2.00)	0.52 (0.29, 0.92)	1.00 (reference)	0.99 (0.52, 1.88)	0.73 (0.17, 3.05)	4.89 (0.75, 26.67)[2]
Death (n)		19	19	32	13	2	0

Values are presented as hazard radio (95% confidence interval).
Hgb, hemoglobin; COPD, chronic obstructive pulmonary disease.
[1]Adjusted for age, body mass index, cigarette smoking, alcohol drinking, exercise, diastolic blood pressure, blood glucose levels, and gamma-glutamyltransfer-ase levels.
[2]Logit estimates not available.
*$p<0.05$.

SUPPLEMENTAL MATERIALS

CONFLICT OF INTEREST

The authors have no conflicts of interest associated with the material presented in this paper.

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AUTHOR CONTRIBUTIONS

Conceptualization: SKP, YA. Data curation: YA, SM. Formal analysis: YA. Funding acquisition: SKP. Methodology: SKP, YA, JJ. Project administration: SKP. Visualization: SL, JJ, SM, YA. Writing - original draft: YA, SL, SM. Writing - review & editing: YA, JJ, SKP.

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