Is an elevated hemoglobin concentration a novel risk factor for metabolic syndrome in the Chinese population? a large-scale study

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ABSTRACT

Objectives: The potential role of elevated hemoglobin concentration in the development of metabolic syndrome (MS) has not been adequately evaluated. We aim to determine the role of elevated hemoglobin concentrations as a risk factor in MS during health examination of Chinese individuals.

Methods: 57,510 health check-up (45,451 by cross-sectional study and 12,059 in a longitudinal population) were conducted. Subjects were divided into quartiles for analyses, using multiple logistic analyses. Furthermore, the logistic analyses were used to explore the association between hemoglobin concentration and individual MS components respectively in the cross-sectional study and the longitudinal study.

Results: In this cross-sectional study, 10.4% of 45,451 of the health checkup subjects developed MS. Multiple logistic analysis revealed that higher hemoglobin quartiles were positively associated with the development of MS which was in contrast to the lowest hemoglobin quartiles in the fully adjusted model (OR: 2.171, 95% CI 1.944 - 2.424 in males, P < 0.001; 2.694, 95% CI 2.056 - 3.531 in females, P < 0.001). In addition, during a five-year period, 10.8% of 12,059 health checkup subjects in a longitudinal study were identified. When adjustments were made for compounding factors, the HR for this association between MS and hemoglobin persisted (HR: 2.157, 95% CI 1.635 - 2.847, P < 0.001).

Conclusions: Serum hemoglobin levels were associated with the prevalence of MS in Chinese individuals, suggesting that serum hemoglobin levels might be a novel predictor for the incidence of MS.

INTRODUCTION

Metabolic syndrome (MS), characterized by a cluster of individual cardiovascular disease risk factors including hyperlipemia, glucose intolerance, hypertension and central obesity, has recently been proposed to be a key factor leading to a consistent increase in diabetes, cardiovascular disease and even overall mortality [1-3].

Inflammatory biomarkers such as CRP, interleukin-6, tumor necrosis factor-α and white blood cell count have been shown to be related with MS in the context of low-inflammatory states [4-5]. An elevated hemoglobin concentration has also been recognized as a non-conventional risk factor for chronic obstructive pulmonary disease, nonalcoholic fatty liver disease and cardiac disease [6-8]. Moreover, hemoglobin might play an important role in a low-grade inflammatory clinical state [9].

Until now, a few cross-sectional studies have shown the close association between hemoglobin concentrations or red blood cell counts with MS. Hämäläinen et al. revealed that subjects with MS have elevated hemoglobin and erythropoietin (EPO) [10]. Choi et al. and Mardi et al. demonstrated that increased EPO and subclinical inflammation could be part of the MS in Koreans and Europeans [11-12]. Moreover, Kawamoto et al. and Lohsoonthorn et al. also noticed this association in community-dwelling persons in Japan and workers from Bangkok in Thailand respectively [13-14]. However, Kim et al. found no association between red blood cell subtype counts and MS in females [15]. With only 399 participants included, further large-scale studies are desired to clarify the real correlation of hemoglobin concentration with MS. At present, a few longitudinal studies demonstrated the relevance of hemoglobin in the development of MS. Fu et al. provided hematogram models to predict future MS in elderly (above 65 years) in four-year follow-up at the MJ Health Screening Center in Taiwan [16]. Liu et al. also reported similar models with the sensitivity 58.1% and specificity of 61.4% in elderly woman [17]. However, the correlation had not been explored for all ages of the population and the association with the individual MS components needs to be elaborated.

Our investigation established a large-scale cross-sectional and a longitudinal study to explore the association between serum hemoglobin concentration and MS (and individual components) and determined whether serum hemoglobin level had a predictive value in the incidence of MS.

RESULTS

Subject characteristics

Cross-sectional study

Baseline clinical and biochemical measurements of 45,451 study participants are summarized in Table 1. From the entire study population, 4,709 (10.4%) cases of MS were identified. Compared to the non-MS subjects, the mean age and serum hemoglobin levels were respectively 53.7 ± 12.9 years and 147.6 ± 12.0 g/L for the MS subjects and 43.5 ± 14.4 years and 138.7 ± 14.6 g/L in non-MS. As shown in Supplementary Table 1, the incidence of MS is prevalent prominently in males (14.95% in males and 3.75% in females). In order to analyse the influence of gender, the study population was divided into a male and female group. Next, individuals were classified in four groups by hemoglobin level: Q1 (≤ 142 g/L in males and ≤ 122 g/L in female); Q2 (143 - 148 g/L in males and 123-128 g/L in females); Q3 (149 - 155 g/L in male and 129 - 133 g/L in females); and Q4 (≥ 156 g/L in males and ≥ 134 g/L in females). The incidence of MS increased as distribution of hemoglobin level in quartiles and it was 12.1%, 13.4%, 14.9%, 19.3% in males, and 1.8%, 2.2%, 3.4%, 7.0% in females, respectively. These subjects with higher hemoglobin concentration had higher mean age, male-to-female sex ratio, BMI, SBP, DBP, TG, TC, LDL-C, FPG, Hb, RBCC, WBCC, FBG, ALP, GGT, ALT, AST, BUN, sCr and sUA statistically. No significant statistically difference was observed for PLTC and TB between the four quartiles.

Longitudinal study

From a total of 12059 eligible subjects (mean age 43.8 years, mean hemoglobin 141.6 g/L) with at least three repeated health examination, 10.8% had MS within the 5-years follow-up periods. Similar details of follow-up subjects were presented in Supplementary Table S2. Supplementary Table S3 illustrated the relationship between hemoglobin quartiles and the prevalence of MS and its individual components. All individuals were drawn from different hemoglobin levels in the initial year of health examination, and divided into quartiles: Q1, ≤ 132 g/L, n = 3148; Q2, 133 - 143 g/L, n = 3021; Q3, 144 - 152 g/L, n = 3077 and Q4, ≥ 154 g/L, n = 2813.

Elevated hemoglobin is association with the prevalence of MS

As observed in Table 2, the multivariate analysis revealed that in the study group of people belonging to the 2-4th hemoglobin level quartiles, the risk of MS was higher with OR 1.112 (95% CI 1.015 - 1.239), 1.275 (95% CI 1.161 -1.401), and 1.740 (95% CI 1.584 - 1.911) in males, $P < 0.001$; (1.224 (95% CI 0.919 - 1.632), 1.907 (95% CI 1.454 - 2.501), 4.118 (95% CI 3.237 - 5.239) in females $P < 0.001$,with the lowest quartile of hemoglobin level considered as a reference in the unadjusted model. After adjustment for age, a higher probability of hemoglobin was directly correlated with MS. In addition, the association also persisted even when adjustment was made to control for age, WBCC, PLTC, ALP, GGT, ALT, AST, BUN, sCr, sUA and TB. (Q4 vs. Q1 2.171, 95% CI 1.944 - 2.424 in males $P < 0.001$; 2.694, 95% CI 2.056 - 3.531 in females $P < 0.001$)

Table 1: Baseline characteristics between subjects with and without MS

Cross-sectional study	Overall	Non-MS	MS	P value
N	45451 (100%)	40742 (89.6%)	4709 (10.4%)	
Age (year)	44.5 ± 14.6	43.5 ± 14.4	53.7 ± 12.9	<0.001
Male (%)	27704 (61.0%)	23616 (58.0%)	4088 (86.8%)	<0.001
BMI (kg/m^2)	23.1 ± 3.3	22.7 ± 3.0	27 ± 2.4	<0.001
SBP (mmHg)	123.2 ± 17	121 ± 15.7	143 ± 14.7	<0.001
DBP (mmHg)	76.9 ± 11.4	75.3 ± 10.5	90.1 ± 9.6	<0.001
TG (mmol/L)	1.6 ± 1.4	1.4 ± 1.2	2.9 ± 2.2	<0.001
TC (mmol/L)	4.7 ± 0.9	4.6 ± 0.9	5.2 ± 1.0	<0.001
HDL-C (mmol/L)	1.3 ± 0.3	1.4 ± 0.3	1.2 ± 0.3	<0.001
LDL-C (mmol/L)	2.6 ± 0.7	2.6 ± 0.7	2.9 ± 0.7	<0.001
FBG (mmol/L)	5.4 ± 1.1	5.3 ± 0.8	6.6 ± 1.9	<0.001
Hb (g/L)	139.6 ± 14.6	138.7 ± 14.6	147.6 ± 12	<0.001
RBCC (10^3/μL)	4.6 ± 0.5	4.6 ± 0.5	4.8 ± 0.4	<0.001
WBCC (10^3/μL)	6.2 ± 1.6	6.1 ± 1.5	6.9 ± 1.6	<0.001
PLTC (10^3/μL)	184.4 ± 43	184.5 ± 42.9	183.5 ± 43.7	0.129
ALP (IU/L)	72.4 ± 23.5	71.6 ± 23.3	79.6 ± 23.8	<0.001
GGT (IU/L)	32.4 ± 37.6	29.3 ± 31.9	59.1 ± 63.7	<0.001
ALT (IU/L)	22 ± 14.6	21 ± 13.8	31.3 ± 18	<0.001
AST (IU/L)	22.8 ± 8.2	22.3 ± 7.8	27.1 ± 10.3	<0.001
BUN (μmol/L)	4.6 ± 1.3	4.5 ± 1.3	4.9 ± 1.3	<0.001
sCr (μmol/L)	84.1 ± 21.5	83.2 ± 21.1	91.7 ± 23.8	<0.001
sUA (μmol/L)	312.9 ± 91.9	306.4 ± 90.1	369.4 ± 88.5	<0.001
TB (μmol/L)	13.2 ± 5	13.2 ± 5	13.3 ± 4.9	0.164

Abbreviation: MS: metabolic syndrome; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglyceride; TC: total cholesterol; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; FPG: fasting plasma glucose; Hb: hemoglobin; RBCC: red blood cell counts; WBCC: white blood cell counts; PLTC: platelet count; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urine nitrogen; sCr: serum creatinine; sUA: serum uric acid; TB: total bilirubin.

Hemoglobin concentration has predictive value for MS

As illustrated in Table 2, our analyses revealed that initial serum hemoglobin levels were correlated in rude model (HR: 1.0, reference; 2.772, 95% CI 2.234 - 3.439; 4.191, 95% CI 3.410 - 5.151 and 4.389, 95% CI 3.566 - 5.402, P < 0.001, across increasing quartiles of hemoglobin). Furthermore, after adjustment for age and sex, the association persisted and became more significant. Controlling for all compounding covariates, the highest hemoglobin quartile (≥ 154 g/L) are associated with increased odds for MS, compared to the lowest hemoglobin quartile (≤ 132 g/L) (HR: 2.157, 95% CI 1.635 -2.847, P < 0.001). HRs for each analysis model increased in our trend analysis.

To determine the association with the cumulative prevalence of MS and serum hemoglobin levels, Kaplan-Meier curves were generated for time to event. As illustrated in Figure 1, baseline serum hemoglobin levels were significantly associated with future incidence of MS, suggesting that increasing levels of serum hemoglobin were progressively associated with an increased incidence of MS.

Table 2: Quartiles serum hemoglobin concentrations and risk of metabolic syndromes

Cross-sectional study	Male		
	Model A	**Model B**	**Model C**
Q1	ref	ref	ref
Q2	1.122 (1.015 - 1.239)†	1.575 (1.419 - 1.749)†	1.422 (1.276 - 1.584)†
Q3	1.275 (1.161 - 1.401)†	2.031 (1.836 - 2.246)†	1.667 (1.500 - 1.852)†
Q4	1.740 (1.584 - 1.911)†	3.034 (2.737 - 3.363)†	2.171 (1.944 - 2.424)†
Cross-sectional study	**Female**		
	Model A	**Model B**	**Model C**
Q1	ref	ref	ref
Q2	1.224 (0.919 - 1.632)	1.262 (0.938 - 1.697)	1.147 (0.842 - 1.563)
Q3	1.907 (1.454 - 2.501)†	2.027 (1.531 - 2.685)†	1.602 (1.192 - 2.153)†
Q4	4.118 (3.237 - 5.239)†	3.901 (3.037 - 5.012)†	2.694 (2.056 - 3.531)†
Longitudinal study	**Model A**	**Model B**	**Model C**
Q1	ref	ref	ref
Q2	2.772 (2.234 - 3.439)†	1.708 (1.344 - 2.171)†	1.615 (1.256 - 2.077)†
Q3	4.191 (3.410 - 5.151)†	2.209 (1.721 - 2.835)†	1.915 (1.471 - 2.493)†
Q4	4.389 (3.566 - 5.402)†	2.694 (2.080 - 3.489)†	2.157 (1.635 - 2.847)†

Model A is univariate analysis for serum hemoglobin;
Model B is adjusted for age and sex;
Model C is adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid and total bilirubin.
†: $P < 0.05$

Which MS components might exert predominate influence on the association?

We next repeated our multivariate logistic analyses to further explore the association between hemoglobin and individual MS components in the cross-sectional study (Table 3 & Figure 2). Three of individual MS components (dyslipdemia, BMI and hypertension) were found to be associated with serum hemoglobin and showed a statistical significance. Using the Q1 as the reference, ORs for dyslipdemia, BMI, hypertension of Q4 were 1.731 (95% CI 1.589 - 1.885), $P < 0.001$; 1.390 (95% CI 1.277 - 1.513), $P < 0.001$; 1.347 (95% CI 1.231 - 1.474), $P < 0.001$ in males; 1.760 (95% CI 1.525 - 2.031), $P < 0.001$; 1.131 (95% CI 0.976 - 1.312), $P = 0.050$; 1.754 (95% CI 1.496 - 2.056), $P < 0.001$ in females. Elevated FPG at an outpatient clinic tended to be higher in patients with MS, compared to non-MS subjects, although the difference was not statistically (Q4 vs. Q1: OR 1.117, 95% CI 0.997 - 1.253 in males; 1.392, 95% CI 1.139 - 1.701 in females). Moreover, serum hemoglobin levels had no relevance to BMI only in female (Q4 vs. Q1: OR 1.131, 95% CI 0.976 - 1.312). The longitudinal analysis also demonstrated that serum hemoglobin level of all subjects was positively correlated to most of the four individual MS components (Figure 3). However, elevated FPG were not significantly correlated with hemoglobin in all-genders subjects (Q4 vs. Q1: OR 1.188, 95% CI 0.965 - 1.462).

DISCUSSION

The study mainly attempted to quantitatively assess the correlation between serum hemoglobin levels and MS. The positive correlation between hemoglobin and MS were not only identified by a cross-sectional study, but were also observed in a longitudinal cohort study. Our findings are in general agreement with previous reports, supporting that three of the individual MS components (dyslipdemia, BMI and hypertension) exerted the predominated effect on the association. Wu et al. had noticed that RBCC and hemoglobin are associated with MS and might be considered as a potential predictor for the risk of the development of MS on middle-to-upper class urban Han Chinese [18]. However, the research population was derived from the middle-to-upper class and the confounding factors were not adjusted for analysis.

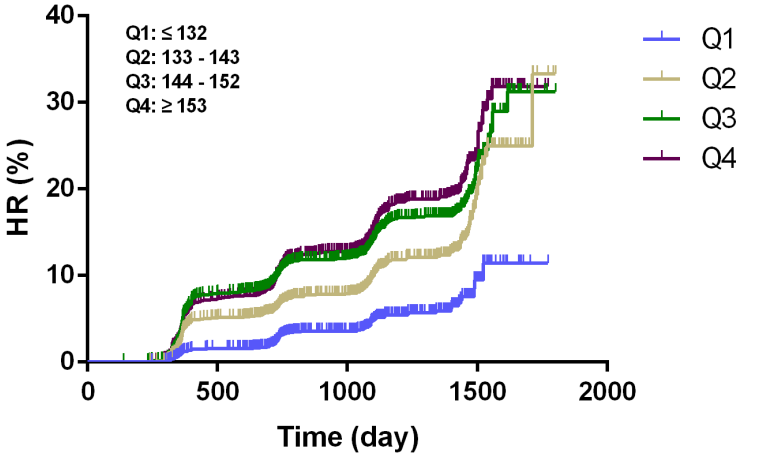

Figure 1: Incidence of metabolic syndrome associated with quartiles of serum hemoglobin in the longitudinal study. All P < 0.05

Table 3: Quartiles serum hemoglobin concentrations and risk of four components of metabolic syndromes

Cross-sectional study	Male			
	Dyslipdemia*	Hyperglycemia**	BMI***	Hypertension****
Q1	ref	ref	ref	ref
Q2	1.375 (1.269 - 1.489)†	1.064 (0.958 - 1.182)	1.148 (1.059 - 1.244)†	1.043 (0.958 - 1.134)
Q3	1.527 (1.412 - 1.652)†	1.121 (1.010 - 1.244)†	1.237 (1.144 - 1.338)†	1.138 (1.047 - 1.237)†
Q4	1.731 (1.589 - 1.885)†	1.117 (0.997 - 1.253)	1.390 (1.277 - 1.513)†	1.347 (1.231 - 1.474)†
Cross-sectional study	**Female**			
	Dyslipdemia*	**Hyperglycemia****	**BMI*****	**Hypertension******
Q1	ref	ref	ref	ref
Q2	1.300 (1.124 - 1.503)†	1.086 (0.880 - 1.339)	1.033 (0.890 - 1.198)	1.299 (1.105 - 1.528)†
Q3	1.420 (1.226 - 1.645)†	1.175 (0.952 - 1.449)	1.075 (0.925 - 1.250)	1.486 (1.262 - 1.750)†
Q4	1.760 (1.525 - 2.031)†	1.392 (1.139 - 1.701)†	1.131 (0.976 - 1.312)	1.754 (1.496 - 2.056)†
Longitudinal study	**Dyslipdemia***	**Hyperglycemia****	**BMI*****	**Hypertension******
Q1	ref	ref	ref	ref
Q2	1.181 (1.033 - 1.350)†	1.140 (0.958 - 1.357)	1.262 (1.065 - 1.496)†	1.233 (1.048 - 1.449)†
Q3	1.396 (1.191 - 1.637)†	1.162 (0.958 - 1.410)	1.493 (1.237 - 1.803)†	1.307 (1.087 - 1.572)†
Q4	1.597 (1.344 - 1.898)†	1.188 (0.965 - 1.462)	1.611 (1.320 - 1.966)†	1.548 (1.271 - 1.885)†

*: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, height and weight.
**: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, systolic blood pressure, diastolic blood pressure, height and weight.
***: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, systolic blood pressure, diastolic blood pressure and fasting plasma glucose.
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†: P < 0.05

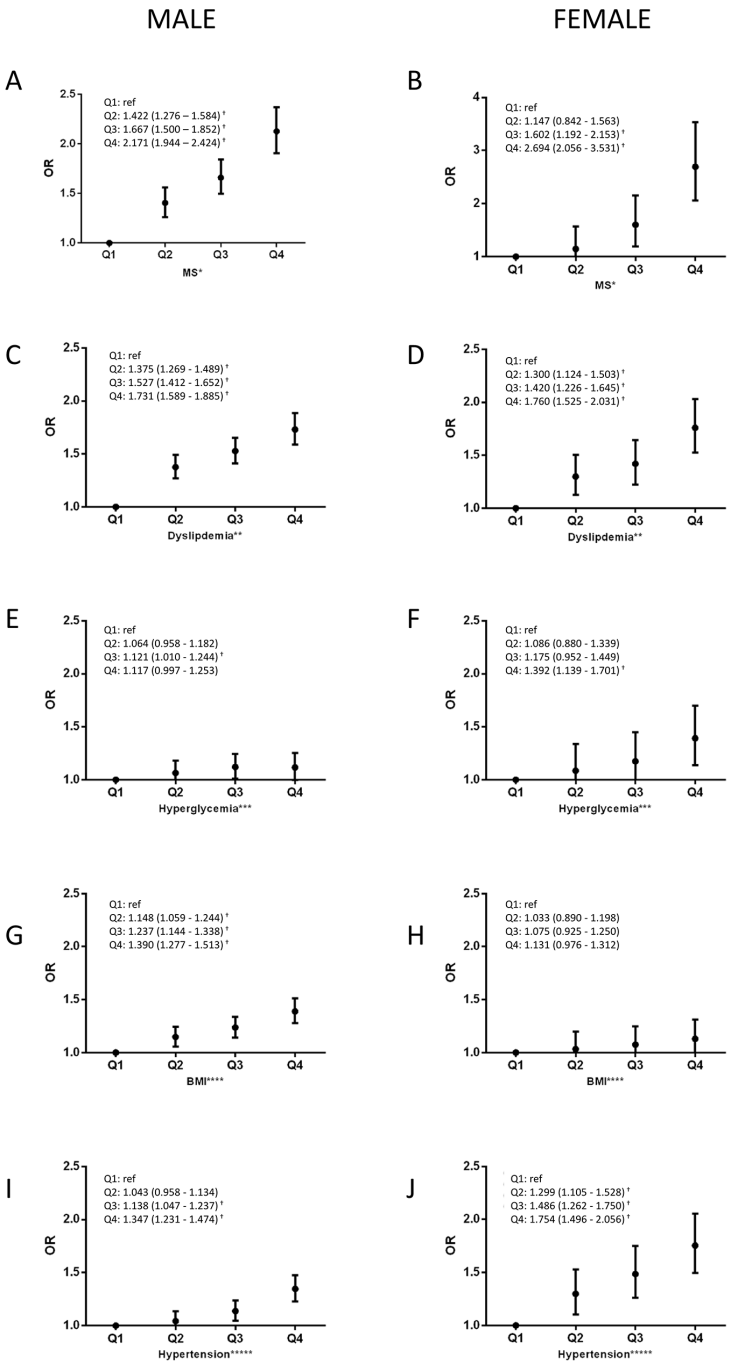

Figure 2: Risks of metabolic syndrome under different status of MS A. and **B.** dyslipdemia **C.** and **D.** hyperglycemia **E.** and **F.** BMI **G.** and **H.** and hypertension **I.** and **J.** associated with quartiles of serum hemoglobin in the cross-sectional study, stratified by genders. *: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid and total bilirubin **: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, height and weight. ***: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, systolic blood pressure, diastolic blood pressure, height and weight. ****: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, systolic blood pressure, diastolic blood pressure and fasting plasma glucose. *****: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, fasting plasma glucose, height and weight. †: $P < 0.05$

Although the detailed mechanisms behind the associations of hemoglobin with MS remain not fully resolved, several potential pathogenesis mechanisms have been suggested. First, a review of laboratory research had demonstrated that insulin and insulin growth factors I and II contribute to EPO, both in vitro and in vivo [19-21]. Moreover, the insulin resistance mechanisms, lying at the core of the MS, might be considered as the linkage between elevated hemoglobin level and the progression of MS [19-20,22]. Secondly, the low-grade inflammatory state and endothelial dysfunction may be related with the development of MS [4-5,23-24]. Nitric oxide (NO) -dependent vascular relaxation seems to have a pivotal core role of the insulin-resistant state [25-26]. Various compounds of hemoglobin with NO were previously shown to be positively associated with hemoglobin-oxygen affinity of the whole blood, leading to endothelial dysfunction, though the L-arginine-NO pathway [5,19,25,27]. Thirdly, higher serum EPO concentrations may suggest underlying adipose tissue hypoxemia in MS [21,28]. Several laboratory investigations announced that reduced adipose tissue oxygenation and cellular hypoxia may produce an insulin resistant state, inhibiting insulin-stimulated glucose transport activity by preventing the progression of insulin receptor phosphorylation, contributed to MS [29-31]. Several researchers speculated that in essential hypertensive patients, evolution to MS increasing blood viscosity is caused by hemoconcentration

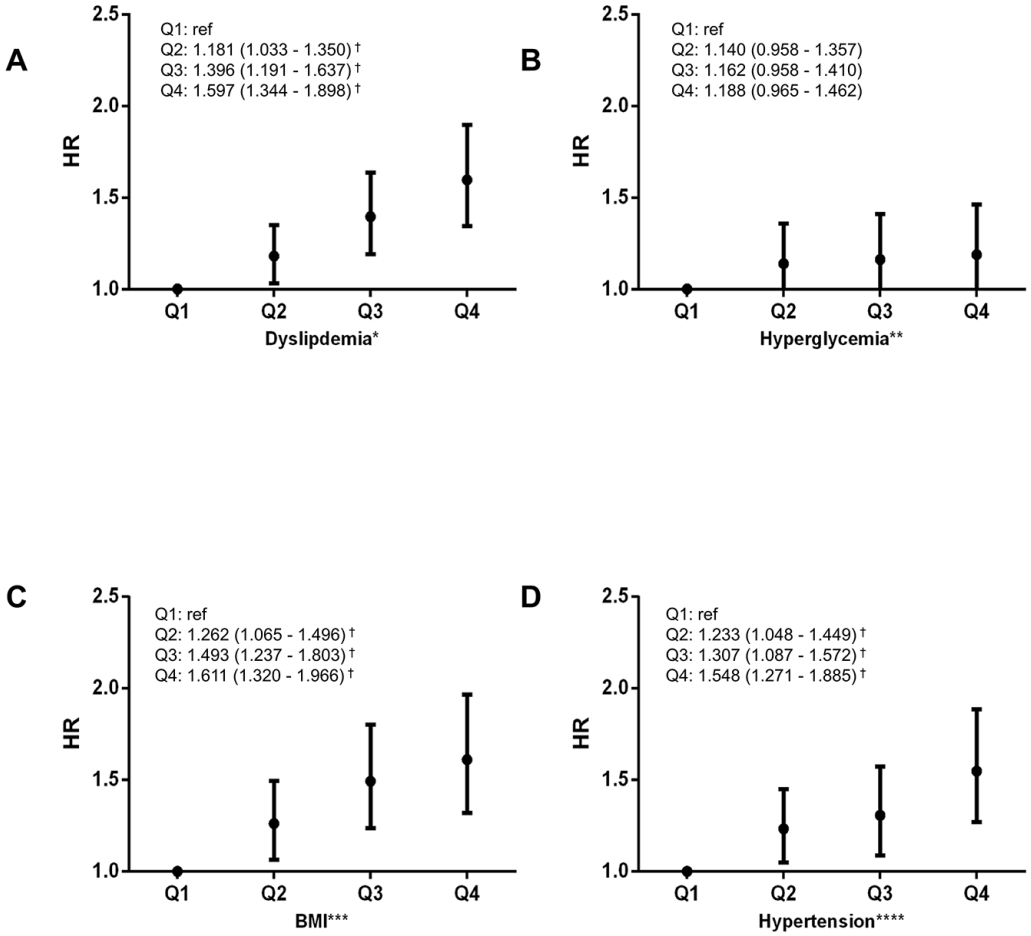

Figure 3: Risks of metabolic syndrome components such as dyslipdemia A. hyperglycemia **B.** BMI **C.** and hypertension **D.** associated with quartiles of serum hemoglobin in the longitudinal study *: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, height and weight. **: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, systolic blood pressure, diastolic blood pressure, height and weight. ***: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, systolic blood pressure, diastolic blood pressure and fasting plasma glucose. ****: adjusted for age, sex, white blood cell counts, platelet count, alkaline phosphatase, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, blood urine nitrogen, serum creatinine, serum uric acid, total bilirubin, triglyceride, high density lipoprotein-cholesterol, fasting plasma glucose, height and weight. †: $P < 0.05$

and increased plasma viscosity [24,32-33]. All these above data, support the linkage between hemoglobin and MS. In addition, the serum hemoglobin levels might be a predictive value for the incidence of MS.

A few limitations of the current study deserve consideration. First, potential selection bias might exist, owing to our study participants based on a general health examination population at two Wenzhou health promotion centers. Second, because of insufficient information gathering, several life habits such as smoking, drinking and psychosocial behaviors, which might be a strong determinant of hemoglobin concentrations, were not analyzed in our study. Additionally, he medication history and medical history, as potential confounding factors, were absent in our database. Thus, all of these factors could confound the relationship. Third, owing to the low incidence of MS, especially in females, the relative scarcity of prior study population induced a statistical uncertainty. It is desired to conduct a longitudinal large-scale multi-center study in a general population in order to evaluate the association between hemoglobin and the incidence of MS, especially in females.

In summary, serum hemoglobin levels are positively correlated with MS in Chinese population during health examination and might be a potential predictor for the incidence of MS. This might be partially explained because of the individual association of hemoglobin with the three of individual MS components (dyslipdemia, BMI and hypertension).

MATERIALS AND METHODS

Study population

Our study population, derived from a cross-sectional and 5-years longitudinal study, was recruited from two separate independent medical centers (Supplementary Figure S1).

In the cross-sectional study, we derived patient data from 45,451 Chinese people (27,704 males and 17,747 females) who attended a routine clinical health examination at the First Affiliated Hospital of Wenzhou Medical University between January 2007 and December 2009. Only subjects with no data of components of MS or hemoglobin were excluded. The current longitudinal study involved the data from a five-year follow-up from the health examination center of the third People's Hospital of Wenzhou from January 2010 to December 2014. Baseline examinations of the longitudinal population were carried out in 2010 and follow-up health examinations were performed every one year since (Supplementary Figure S2). None of the participants met the study exclusion criteria: 1) Less than three repeated health examination within five years; 2) Missing data of examinational date; 3) Missing data of components of MS or hemoglobin.

Finally, a total of 57,510 subjects (45,451 in the cross-sectional study and 12,059 in the longitudinal population) were eligible for analysis (Supplementary Figure 1). In addition, all individuals had obtained the detailed health examination. Verbal informed consent was obtained from each subject before their participation in the study. The research protocol of the study was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University and Wenzhou People's Hospital, respectively.

Physical assessment

Of all subjects, the clinical research coordinators collected physicalassessment data, including age, sex, height and weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP). At every visit, height and weight were measured by a team of trained nurses, by a standard protocol. The body mass index (BMI) was computed by dividing the measured body weight in kilograms (kg) by the square of the measured height in meters (m). Blood pressure was measured with an automatic instrument (Omron, model 705cp, Kyoto, Japan) in seated position after at least a fifteen-minute rest.

Biochemical laboratory test

Laboratory parameters including hemoglobin concentration, red blood cell counts (RBCC), white blood cell counts (WBCC), triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), fasting plasma glucose (FPG), serum creatinine (sCr), serum uric acid (sUA), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) were subsequently analyzed by an automated analyzer (Abbott AxSYM, Park, IL). Blood samples were collected and analyzed from antecubital vein sampling.

Definition

In this study, MS was diagnosed according to the China Diabetes Federation [34]. Subjects were diagnosed as MS when at least three of the following criteria were satisfied: 1) Central obesity: BMI $\geq$ 25 in both genders. 2) Hypertriglyceridemia: TG $\geq$1.7 mmol/L; HDL-C of< 0.9 mmol/L in males and < 1.0 mmol/L in females. 3) Hypertension: SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg or previously diagnosed. 4) Hyperglycemia: FPG $\geq$ 6.1 mmol/L, or hyperglycemia previously diagnosed.

Statistical analysis

Data analyses were performed with SPSS software (SPSS version 19 for Windows, SPSS software). All clinical data were represented as mean ± SD for continuous variables

and as number (%) for incidence rates. Statistical significance of continuous variables and categorical variables, were expressed by independent student's T test and Chi-Square tests respectively. In order to assess the correlation of baseline variables with baseline hemoglobin concentrations, the study population was subdivided into subgroups based on the serum hemoglobin concentrations. Multivariable logistic regression analysis was computed to estimate odds ratios (ORs) and 95% confidence intervals (CI) for exploring the relative risks for each category of hemoglobin level with the incidence of MS. Cox's proportional hazards regression model was performed for the analysis of survival data from longitudinal studies. A two-tailed P value of < 0.05 was considered as statistical significance.

Abbreviation

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; DBP: diastolic blood pressure; EPO: erythropoietin; FPG: fasting plasma glucose; GGT: gamma-glutamyl transferase; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; MS: metabolic syndrome; PLTC: platelet count; RBCC: red blood cell counts; SBP: systolic blood pressure; sCr: serum creatinine; sUA: serum uric acid; TC: total cholesterol;TG: triglyceride; WBCC: white blood cell counts.

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Authors' contributions: Zhou XD, Wu SJ, Huang WJ and Zheng MH designed the study. Zhou XD, Wang LR, Liu WY, Zhang DC collected data. Shi KQ, Zhou XD did the statistical analyses. Liu WY and Zheng JN prepared figures. Zhou XD, Wu SJ, Wang LR, Poucke SV, Huang WJ and Zheng MH reviewed the results, interpreted data, and wrote the manuscript. All authors have made an intellectual contribution to the manuscript and approved the submission.

CONFLICTS OF INTEREST

The authors report no declarations of interest.

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