

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email editorial.bmjopen@bmj.com

BMJ Open

Association of erythrocyte parameters with metabolic syndrome in the Pearl River Delta region of China

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019792
Article Type:	Research
Date Submitted by the Author:	26-Sep-2017
Complete List of Authors:	Huang, LingLing Dou, Dong-Mei; Henan University, Public Health, School of Nursing Liu, Nan; Guangzhou Medical University, School of Public Health Wang, XiaoXiao; Henan University, Public Health, School of Nursing Fu, Li-Ying; Henan University, Public Health, School of Nursing Wu, Xiao; Henan University, Public Health, School of Nursing wang, peixi; Department of Preventive Medicine, School of Public Health, Guangzhou Medical University,
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Public health, Epidemiology
Keywords:	Erythrocyte parameters, Metabolic syndrome, Pearl River Delta region, China

SCHOLARONE™
Manuscripts

Only

1
2
3 **Association of erythrocyte parameters with metabolic syndrome in the Pearl River Delta region**
4 **of China**
5
6
7
8
9

10 Ling-Ling Huang ¹⁺, Dong-Mei Dou ¹⁺, Nan Liu ², Xiao-Xiao Wang ¹, Li-Ying Fu ¹, Xiao Wu ¹, Pei-Xi
11 Wang ^{1,2*}
12
13

14
15 ¹ Institute of Public Health, School of Nursing, Henan University, Kaifeng, 475004,

16
17 China;
18
19

20 ² School of Public Health, Guangzhou Medical University, Guangzhou, PR China
21
22
23
24

25 E-Mails: HuangLingLing0703@163.com (L.-L.H.); doudongmei1224@126.com (D.-M.D.);
26 LNQ555@126.com (N. L.); xiaoxiao52625@163.com (X.-X. W.); 18317856338@163.com (L.-Y. F);
27 18625832936@163.com (X. W); peixi001@163.com (P.-X. W).
28
29
30
31
32
33

34 * Corresponding author to Pei-Xi Wang, tel: -8618927539896, e-mail: peixi001@163.com
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Objective: Increasing studies have reported that erythrocyte parameters, including red blood cell (RBC), hematocrit (HCT), hemoglobin (Hb), and red blood cell distribution width (RDW), are associated with metabolic syndrome (MetS) in adults worldwide. However, the association, stratified by sex, in populations in the Pearl River Delta region of China remains to be elucidated. Therefore, our aim was to explore the association of erythrocyte parameters with MetS, stratified by sex in the Pearl River Delta region of China.

Methods: In this cross-sectional study, 2161 males and 2511 females were enrolled. MetS was diagnosed using a modified version of the Adult Treatment Panel III (ATP III) criteria. Logistic regression analyses were performed to calculate adjusted odds ratios (ORs) of erythrocyte parameters associated with MetS stratified by sex.

Results: The prevalence of MetS was higher in females than that in males (35.2% vs. 26.7%). RBC, HCT, Hb and RDW values increased linearly with the number of MetS components from zero to five identified in both males and females. Among males, the ORs of MetS risk increased across the tertiles of Hb (Q2: OR=1.687, 95% confidence interval [CI]=1.151~2.472; Q3: OR=2.252, 95%CI=1.550~3.272). Males in the highest tertiles of RDW had a 2.750-fold increased risk of suffering from MetS compared to those in the reference group. Among females, the ORs of MetS risk increased across

1
2
3 the tertiles of HCT (Q2: OR=1.738, 95%CI=1.229~2.458; Q3: OR=1.922, 95%CI=1.337~2.761).
4
5 Females in the highest tertiles of RBC had a 1.785-fold increased risk of experiencing MetS compared
6
7 to those in the reference group.
8

9
10 **Conclusions:** MetS was more prevalent in females than that in males. The association between
11 erythrocyte parameters and MetS differed between sex, whereby RBC and HCT were identified as the
12 risk factors of MetS in females and Hb and RDW as the risk factors in males.
13
14

15
16
17
18 **Keywords:** Erythrocyte parameters, Metabolic syndrome, Pearl River Delta region, China
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34 **Strengths and limitations of this study**

- 35 ● The large sample of subjects was enrolled in our survey.
- 36
- 37 ● This is the first study to explore the the association of erythrocyte parameters with MetS,
38 stratified by sex in the Pearl River Delta region of China.
- 39
- 40 ● The present study was designed as a cross-sectional study; therefore, direct causation cannot be
41 concluded from the results.
- 42
- 43 ● Supplementary information about the lifestyle of the subjects was not collected; therefore, these
44 factors could not be included in the adjustments of our multivariate logistic regression analyses.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

1
2
3 Metabolic syndrome (MetS) is defined as a cluster of multiple correlated metabolic features, including
4 abdominal obesity, hypertension, elevated triglyceride (TG) levels, decreased high-density lipoprotein
5 cholesterol (HDL-C) levels, and hyperglycemia.[1]. It is known to be strongly associated with an
6 increased risk of type 2 diabetes [2], cardiovascular disease (CVD) [2-4], and all-cause mortality [4]. In
7 recent years, MetS has emerged as a global public health issue owing to its increased prevalence
8 around the world, affecting nearly 20-30% of adults in many countries [5-7]. Hence, early
9 identification of individuals at high risk of MetS is essential for the prevention of MetS.
10
11
12
13
14
15

16
17 Currently, the pathogenesis of MetS is not clearly understood. Generally, MetS is often
18 accompanied by insulin resistance and/or chronic low-grade inflammation [8-9]. Numerous
19 investigators previously reported that erythrocyte parameters levels, including red blood cell (RBC),
20 hematocrit (HCT), hemoglobin (Hb) and red blood cell distribution width (RDW) were positively
21 associated with a insulin resistance and chronic low grade inflammation [10-14]. In fact, RBC [14-16],
22 HCT [15-16], Hb [14-15, 17] and RDW[18] were demonstrated in several studies worldwide to
23 correlate with MetS in adults. However, the association between erythrocyte parameters and MetS
24 remains controversial, because the results reported are inconsistent depending on the different ethnic
25 population studied. In addition, discrepancies in the results may be partly attributed to the sexes
26 differences. Many studies simply applied sex as an adjustment variable to investigate the relationship
27 between erythrocyte parameters and MetS, and no studies were conducted in the Pearl River Delta
28 region of China. Therefore, the aim of this study was to explore the association between erythrocyte
29 parameters and MetS stratified by sex in the Pearl River Delta region of China.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

47 **Materials and Methods**

48 **Study participants**

49
50 This cross-sectional study involved participants who underwent a general health examination at the
51 Community Health Service Agencies in the Pearl River Delta region of China in 2015. The health
52 examination included recording of medical history, anthropometric measurements, and laboratory tests.
53
54
55
56
57
58
59
60

Participants with a history of cardiovascular diseases, severe liver and kidney dysfunction, tumors, and severe inflammatory diseases were excluded. In addition, we excluded participants who did not have complete data on their MetS components and erythrocyte parameters. Altogether, a total of 4672 subjects (2161 males and 2511 females) were enrolled in this study. The study was approved by the Ethics Committee of Guangdong Sociological Society. Written informed consent was obtained from all participants.

Data collection and measurements

The medical history of subjects was obtained by review of self-reported questionnaires. Anthropometric parameters were measured by trained staff, following a standardized protocol. Height, weight, waist circumference (WC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured in replicate, and mean values were calculated for this study. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. After an overnight fast, venous blood samples from participants were obtained to measure blood levels of routine laboratory tests, including TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), uric acid (UA), white blood cell (WBC), platelet (PLT), RBC, HCT, Hb, RDW, alanine transaminase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase;(GGT), albumin (ALB) and glycated hemoglobin A1c (HbA1c).

Tertiles of erythrocyte parameters levels

Erythrocyte parameters levels were categorized into tertiles on the basis of the individual distributions for males and females, respectively (in males: RBC, $Q1 < 4.37 \times 10^{12}/L$, $Q2 = 4.37 \sim 4.75 \times 10^{12}/L$, $Q3 \geq 4.76 \times 10^{12}/L$; HCT, $Q1 < 39.8\%$, $Q2 = 39.8 \sim 42.4\%$, $Q3 \geq 42.5\%$; Hb, $Q1 < 137 \text{ g/L}$, $Q2 = 137 \sim 146 \text{ g/L}$, $Q3 \geq 147 \text{ g/L}$; RDW, $Q1 < 12.5\%$, $Q2 = 12.5 \sim 13.1\%$, $Q3 \geq 13.2\%$; in females: RBC, $Q1 < 3.96 \times 10^{12}/L$, $Q2 = 3.96 \sim 4.27 \times 10^{12}/L$, $Q3 \geq 4.28 \times 10^{12}/L$; HCT, $Q1 < 35.2\%$, $Q2 = 35.2 \sim 37.3\%$, $Q3 \geq 37.4\%$; Hb, $Q1 < 120 \text{ g/L}$, $Q2 = 120 \sim 127 \text{ g/L}$, $Q3 \geq 128 \text{ g/L}$; RDW, $Q1 < 12.3\%$, $Q2 = 12.3 \sim 12.8\%$, $Q3 \geq 12.9\%$).

Definition of metabolic syndrome

MetS was diagnosed using a modified version of the Adult Treatment Panel III (ATP III) criteria [1], which included at least three of the following five components: 1) $WC \geq 90 \text{ cm}$ in males and $WC \geq 80 \text{ cm}$

1
2
3 in females; 2) SBP \geq 130 mmHg or DBP \geq 85 mmHg; 3) TG \geq 1.70 mmol/L; 4) HDL-C $<$ 1.03 mmol/L in
4 males and HDL-C $<$ 1.29 mmol/L in females; and 5) FPG \geq 5.6 mmol/L.
5
6
7
8
9

10 **Statistical analysis**

11
12 All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS)
13 version 21.0 (SPSS Inc., Chicago, IL, USA). Data were presented as the mean \pm standard deviation or
14 frequency (percentage). The *t*-test was used to evaluate the differences in characteristics of study
15 subjects with and without MetS stratified by sex. The χ^2 test was performed to compare the proportion
16 of MetS components, from zero to five, between males and females, and compare the prevalence of
17 MetS dependent on the tertiles of RBC, HCT, Hb, and RDW between males and females, respectively.
18 The one-way ANOVA was conducted to test mean levels of erythrocyte parameters according to the
19 number of MetS components in males and females, separately. Multivariate logistic regression analyses
20 were performed to calculate adjusted odds ratios (ORs) of erythrocyte parameters associated with MetS
21 stratified by sex. *P* value $<$ 0.05 was considered to be statistically significant.
22
23
24
25
26
27
28
29
30
31
32
33
34

35 **Results**

36 **Prevalence of MetS**

37
38 In total, there were 2161 males and 2511 females enrolled in this study. Of the subjects, 576 males
39 (26.7%) and 885 females (35.2%) were diagnosed with MetS.
40
41
42
43
44
45
46

47 **Characteristics of study subjects**

48
49 In this study, among the males, the mean age of the MetS group was significantly lower than that of the
50 non-MetS group, whereas the opposite trend was observed among the females ($P<$ 0.001). In the cluster
51 of MetS components, WC, SBP, DBP, TG, and FPG levels were remarkably greater in the MetS group
52 than in the non-MetS group among both males and females, but HDL-C levels were significantly lower
53 in the MetS group than that in the non-MetS group among both males and females ($P<$ 0.001). In the
54 cluster of erythrocyte parameters, we found that RBC, HCT, Hb and RDW were significantly higher in
55
56
57
58
59
60

1
2
3 the MetS group than that in the non-MetS group among both males and females ($P<0.001$). Additional
4 information about the characteristics of study subjects with and without MetS stratified by sex are
5 presented in Table 1.
6
7

8 **Proportion of MetS components**

9
10 Our results revealed that most males experienced one metabolic disorder, and most females suffered
11 from two metabolic disorders. In addition, the proportion of MetS components from two to five was
12 significantly lower in males than that in females (25.5% vs. 27.8%, 18% vs. 21.3%, 7.5% vs. 11.3%,
13 1.1% vs. 2.6, respectively). Additional information is shown in Fig 1.
14
15

16 **Association of erythrocyte parameters with MetS**

17
18 This study showed that the levels of RBC, HCT, Hb and RDW levels clearly increased with the number
19 of MetS components from zero to five identified in both males and females ($P<0.001$, shown in Table
20 2), Figure 2 showed that the prevalence of MetS increased in a dose-dependent manner as the tertiles of
21 RBC, HCT, Hb and RDW levels increased in both males and females, Furthermore, at each tertiles of
22 RBC, HCT, Hb and RDW levels, the prevalence of MetS was lower in males than that in females,
23 except at the highest tertiles of RDW levels (shown in Fig 2).
24
25
26
27
28
29
30
31
32

33 **Logistic regression analysis model**

34
35 The adjusted ORs of MetS risk associated with each tertile of RBC, HCT, Hb, and RDW are listed in
36 Table 3. After adjusting for potential confounders, the significant association of Hb and RDW with
37 MetS was observed in males, but this was not true for RBC and HCT. The ORs of MetS risk increased
38 across the tertiles of Hb in males (Q2: OR=1.687, 95% confidence interval [CI]=1.151~2.472; Q3:
39 OR=2.252, 95%CI= 1.550~3.272). Males in the highest tertiles of RDW had a 2,750-fold increased
40 risk of suffering from MetS in comparison to those in the reference group. Only RBC and HCT levels
41 were observed to associate with MetS in females. The ORs of MetS risk increased across the tertiles of
42 HCT(Q2: OR=1.738, 95% CI=1.229~2.458; Q3: OR=1.922, 95%CI=1.337~2.761). Female in the
43 highest tertiles of RBC had a 1.785-fold increased risk of experiencing MetS in comparison to those in
44 the reference group.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Discussion

Main findings

The prevalence of MetS was higher in females than that in males (35.2% vs. 26.7%). The levels of RBC, HCT, and Hb and RDW increased linearly with the number of MetS components from zero to five identified in both males and females. The association between erythrocyte parameters and MetS differed between sexes, whereby Hb and RDW were identified as the risk factors of MetS in males and RBC and HCT as the risk factors in females.

Comparisons with Previous Studies

Sex has been demonstrated as an important factor in the prediction of MetS development. Several previous studies have showed that females have a higher prevalence of MetS than males [19-20]. A large-scale study conducted in Russia reported that the prevalence of MetS diagnosed using the ATP III criteria, was 9.5% in men and 23.5% in women [19]; A study performed in the seven geographical regions of Turkey showed that the prevalence of MetS as determined by the ATP III criteria, was 28% in men and 39.6% in women [20]. The present study confirmed that the prevalence of MetS was, in fact, higher in females (35.2%) than in males (26.7%). Additionally, it was observed that the mean age of the MetS group in females was higher than that of the MetS group in males (59.78 ± 12.34 years vs. 51.39 ± 12.21 years). We hypothesized that the difference observed in the prevalence of MetS between females and males could be attributed to this age difference. It has been well established that the prevalence of MetS significantly increases with age [20-21].

It is well known that MetS represents a cluster of simultaneously occurring metabolic abnormalities. In fact, previous studies demonstrated that RBC and Hb levels clearly increased in a line fashion with the numbers of MetS components [16, 22]. Our findings were consistent with those of these reports. Moreover, our results showed that HCT and RDW were demonstrated a similar trend in relation to the number of MetS components. It has been shown that a higher number of MetS components is associated with insulin resistance. Several studies demonstrated that levels of RBC, HCT and Hb were significantly associated with insulin resistance [10, 12, 23]. We hypothesized that

1
2
3 the increased levels of the tested erythrocyte parameters in this study may be indicative of the
4 development of insulin resistance.
5
6

7
8 Many studies have demonstrated the association between RBC levels and MetS, implicating RBC
9 as a potential hematological marker for early detection of MetS [14-16]. Our results revealed that the
10 highest tertiles of RBC were associated with MetS in females, consistent with a recent study [24]. The
11 pathogenesis of insulin resistance may, in part, be causative of the association between RBC levels and
12 MetS. Aoki et al reported that insulin can stimulate the proliferation and differentiation of
13 erythropoietic cells by binding receptors upon on the cell surface [25]. It was suggested that insulin
14 and insulin growth factors I-II can promote the proliferation and differentiation of erythroid progenitors
15 in human bone marrow and circulation [26-28]. Alternatively, the relationship between RBC levels and
16 MetS may be a result of iron overload. It was reported that iron overload was associated with insulin
17 resistance [29]. Additionally, it was observed that high body iron storage interfered with
18 insulin-mediated effects and bloodletting improved insulin sensitivity [30].
19
20
21
22
23
24
25
26
27

28
29 It has been reported that high HCT levels correlated with increasing risk of MetS [15-16].
30 Lohsoonthorn et al reported that MetS risk increased across successive quartiles of HCT in women, but
31 not in men [31]; Nebeck et al demonstrated that HCT was significantly associated with MetS in women,
32 but no similar trend was observed in men [16]. Our results confirmed that the ORs of MetS risk
33 increased with the elevation of HCT in females. HCT was a major determinant of blood viscosity [32].
34 Increased blood viscosity causes the blood flow to skeletal muscles and fat tissues to decrease,
35 additionally contributing to insulin resistance [33-34]. Moreover, increased blood viscosity was
36 determined as an independent risk factor of hypertension [35]. It has been reported that HCT positively
37 associated with insulin resistance, which is an important factor in the pathogenesis of MetS [12, 14].
38 Considering the bodies of evidence, we hypothesized that the association between HCT levels and
39 MetS may arise from the increased blood viscosity and insulin resistance linked to HCT.
40
41
42
43
44
45
46
47
48

49
50 Hb, another important erythrocyte parameter, has been reported to be associated with MetS in both
51 cross-sectional and cohort studies [14, 16-17, 31]. An 8-year follow-up cohort study conducted in Japan
52 detected that the highest and third quartiles of Hb concentration were associated with increased risk of
53 MetS incidence compared to the lowest quartiles of Hb concentration in men, but there was no
54
55
56
57

1
2
3 association observed in women [17]. In general, our findings were consistent with those of previous
4 reports. In our study, the ORs for MetS increased across the successive tertiles of Hb among males;
5 however, no similar trend was observed among females. The following mechanisms may be regarded
6 as the causes of association between Hb and MetS: Hb is a well-known carrier and buffer of nitric
7 oxide (NO), and can regulate the endothelial function of blood vessels by modulating NO levels in the
8 blood [36]. Furthermore, Hb and various compounds of NO modulate the affinity between Hb and
9 oxygen in blood, which can lead to vascular endothelial dysfunction [37]. It has been found that
10 vascular endothelial dysfunction was associated with MetS [38-39]. In addition, Hb plays a key role in
11 regulating sCD40L levels [40]. And sCD40L has been shown to participate in the thrombus formation
12 and proinflammatory, which was the independent risk factor for atherosclerosis and MetS [41]. Another
13 possibility linking Hb and MetS may be the adiponectin. Previous studies showed that higher Hb levels
14 was closely related to lower adiponectin levels [42-43], and lower levels of adiponectin significantly
15 increased the risk for MetS, respectively. Finally, insulin resistance may also be involved in the
16 association between Hb and MetS [8, 12].
17
18
19
20
21
22
23
24
25
26
27
28
29

30 RDW, an common index of routine blood examination, represents a measurement of the
31 heterogeneity in the size of circulating erythrocytes. A high RDW index indicates a greater
32 heterogeneity in size of circulating erythrocytes in a subject. In this study, males in the highest tertiles
33 of RDW (>13.2%) had a 2.750-fold increase in risk of MetS. Multiple groups previously showed that
34 elevated RDW was associated with MetS [44-45]. For instance, Laufer and colleagues demonstrated
35 that RDW $\geq 14\%$ was independently associated with an increased risk for MetS development [44];
36 Sanchez-Chaparro and colleagues reported that the highest quartile of RDW (>14%) was remarkably
37 linked with MetS after adjusting for potential confounders [45]. Moreover, a recent study illustrated
38 RDW as a potential metabolic marker for the detection of metabolic diseases [46]. To date, the
39 mechanism of association between RDW and MetS remains unknown; however, chronic inflammation
40 linked to RDW may play an important role. It was found that MetS was associated with chronic
41 inflammation [9], and RDW reflects an underlying inflammatory state [13]. Pierce and colleagues have
42 proved that proinflammatory cytokines can inhibit erythropoietin-induced erythrocyte maturation,
43 which may lead to an elevation of RDW [47].
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Conclusions

MetS was more prevalent in females than in males. The association between erythrocyte parameters and MetS differed between sexes, whereby RBC and HCT were identified as the risk factors of MetS in females and Hb and RDW as the risk factors in males. Our study provides sufficient evidence that erythrocyte parameters may serve as effective molecular markers for the early detection of MetS risk on a sex-dependent basis.

Acknowledgments: We gratefully acknowledged the staff of local Community Health Service Agencies, for their kind assistance in data collection

Contributors: LLH and PXW conducted the data analyses. LLH, NL, XXW, LYF, XW and MJJ drafted the manuscript. PXW and DMD finalized the manuscript with inputs from all authors. All authors contributed to the development of the study framework, interpretation of the results, revisions of successive drafts of the manuscript, and approved the version submitted for publication.

Funding This study was supported by the Guangzhou 121 Talents Program (GZRSB-2014-2048), and by the Science and Technology Program of Guangzhou (201607010136).

Conflicts of Interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Ethics Committee of Guangdong Sociological Society..

Informed consent Informed consent was obtained from all individual participants included in the

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

study.

Data sharing statement This database is first used in this study. The database belongs to our team, and if shared, you need to get their permission.

For peer review only

References

1. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735–52.
2. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009;120:1640-5
3. Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;56:1113-32.
4. Gami AS, Witt BJ, Howard DE, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol* 2007;49:403-14.
5. Miller JM, Kaylor MB, Johannsson M, et al. Prevalence of metabolic syndrome and individual criterion in US adolescents: 2001–2010 National Health and Nutrition Examination Survey. *Metab Syndr Relat Disord* 2014;12:527-32.
6. Peer N, Lombard C, Steyn K, et al. High prevalence of metabolic syndrome in the Black population of Cape Town: The Cardiovascular Risk in Black South Africans (CRIBSA) study. *Eur J Prev Cardiol* 2015;22:1036-42.

- 1
2
3 7. Lovre D, Mauvais-Jarvis F. Trends in prevalence of the metabolic syndrome. *JAMA*. 2015; 314(9):
4 950-951. Doi: 10.1001/jama.2015.8625
5
6
- 7 8. González AS, Guerrero DB, Soto MB, et al. Metabolic syndrome, insulin resistance and the
8 inflammation markers C-reactive protein and ferritin. *Eur J Clin Nutr* 2006;60: 802-9.
9
- 10 9. Musani SK, Vasan RS, Bidulescu A, et al. Aldosterone, C-Reactive Protein, and Plasma B-Type
11 Natriuretic Peptide Are Associated With the Development of Metabolic Syndrome and Longitudinal
12 Changes in Metabolic Syndrome Components: Findings from the Jackson Heart Study. *Diabetes Care*
13 2013;36:3084-92.
14
- 15 10. Ellinger VC, Carlini LT, Moreira RO, et al. Relation between insulin resistance and hematological
16 parameters in a Brazilian sample. *Arq Bras Endocrinol Metabol* 2006;50:114-7.
17
- 18 11. Mardi T, Toker S, Melamed S, et al. Increased erythropoiesis and subclinical inflammation as part
19 of the metabolic syndrome. *Diabetes Res Clin Pr* 2005;69:249-55.
20
- 21 12. Tabara Y, Igase M, Saito I, et al: Association of hematological parameters with insulin resistance,
22 insulin sensitivity, and asymptomatic cerebrovascular damage: The J-SHIP Toon Health Study. *Clin*
23 *Hemorheol Microcirc* 2013;55: 297-311.
24
- 25 13. Lippi G, Targher G, Montagnana M, et al. Relation between red blood cell distribution width and
26 inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* 2009;133:
27 628-32.
28
- 29 14. Kawamoto R, Tabara Y, Kohara K, et al: Hematological parameters are associated with metabolic
30 syndrome in Japanese community-dwelling persons. *Endocrine* 2013;43:334-41.
31
- 32 15. Wu S, Lin HY, Zhang CQ, et al. Association between erythrocyte parameters and metabolic
33 syndrome in urban Han Chinese: a longitudinal cohort study. *BMC Public Health* 2013;13: 1-8.
34
- 35 16. Nebeck K, Gelaye B, Lemma S, et al. Hematological parameters and metabolic syndrome: Findings
36 from an occupational cohort in Ethiopia. *Diabetes Metab Syndr* 2012;6:22-7.
37
- 38 17. Hashimoto Y, Tanaka M, Kimura T, et al. Hemoglobin concentration and incident metabolic
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 syndrome: a population-based large-scale cohort study. *Endocrine* 2015;50:390-6.
4
5
6 18. Vayá A, Carmona P, Badia N, et al. Association between high red blood cell distribution width and
7 metabolic syndrome. Influence of abdominal obesity. *Clin Hemorheol Micro* 2011;47: 75-7.
8
9
10 19. Sidorenkov O, Nilssen O, Grjibovski AM. Metabolic syndrome in Russian adults associated factors
11 and mortality from cardiovascular diseases and all causes. *BMC Public Health* 2010;10:1-10.
12
13
14 20. Kozan O, Oguz A, Abaci A, et al. Prevalence of the metabolic syndrome among Turkish adults. Et
15 al 2007;61:548-53.
16
17
18 21. Chen CC, Lin WY, Li CI, et al. The association of alcohol consumption with metabolic syndrome
19 and its individual components: the Taichung community health study. *Nutr Res* 2012;32:24-9.
20
21
22 22. Wang YY, Lin SY, Liu PH, et al. Association between hematological parameters and metabolic
23 syndrome components in a Chinese population. *J Diabetes Complicat* 2004;18:322-27.
24
25
26 23. Choi KM, Lee J, Kim YH, et al. Relation between insulin resistance and hematological parameters
27 in elderly Koreans-Southwest Seoul (SWS) Study. *Diabetes Res Clin Pr* 2003;60:205-12.
28
29
30 24. Wang T, Wang H. Study of the relationship between female metabolic syndrome and its related
31 blood indexes in Guangzhou. *Medical Innovation of China* 2016;13:61-4.
32
33
34 25. Aoki I, Taniyama M, Toyoma K, et al. Stimulatory effects of human insulin on erythroid
35 progenitors (CFU-E and BFU-E) in human CD34 separated bone marrow cells and the relationship
36 between insulin and erythropoietin. *Stem Cells* 1994;12:329-38.
37
38
39 26. Bersch N, Groopman E, Golde DW. Natural and biosynthetic insulin stimulates the growth of
40 human erythroid progenitors in vitro. *Jflirt Endocrinol Metab* 1982;55:1209-11.
41
42
43 27. Miyagawa S, Kobayashi M, Konishi N, et al. Insulin and insulin-like growth factor I support the
44 proliferation of erythroid progenitor cells in bone marrow through the sharing of receptors. *Br J*
45 *Haematol* 2000;109:555-62.
46
47
48 28. Dainiak N, Kreczko S. Interactions of insulin, insulinlike growth factor II, and platelet—derived
49 growth factor in erythropoietic culture. *J Clin Invest* 1985;76:1237-42.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 29. Marti'nez-Garcia MA, Luque-Ramirez M, San-Millan JL, et al. Body iron stores and glucose
4 intolerance in premenopausal women: role of hyperandrogenism, insulin resistance, and genomic
5 variants related to inflammation, oxidative stress, and iron metabolism. *Diabetes Care*. 2009;32:
6 1525-30.
7
8
9
10
11 30. Fernandez-Real JM, Penarroja G, Castro A, et al. Blood letting in high-ferritin type 2 diabetes:
12 effect on insulin sensitivity and h-cell function. *Diabetes* 2002;51:1000-4.
13
14
15 31. Lohsoonthorn V, Jiamjarasrunsi W, Williams M A. Association of hematological parameters with
16 clustered components of metabolic syndrome among professional and office workers in Bangkok,
17 Thailand. *Diabetes Metab Syndr*. 2007;1:143-149.
18
19
20
21
22 32. Perrine SP, Greene MF, Lee PD, et al. Insulin stimulates cord blood erythroid progenitor growth:
23 evidence for an aetiological role in neonatal polycythaemia. *Br.J. Haematol*. 1986;64:503-11.
24
25
26 33. Baron AD. Hemodynamic actions of insulin. *Am J Physiol* 1994;267:E187-E202.
27
28
29 34. Facchini FS, Carantoni M, Jeppesen J, et al. Hematocrit and hemoglobin are independently related
30 to insulin resistance and compensatory hyperinsulinemia in healthy, non-obese men and women.
31 *Metabolism* 1998;47:831-5.
32
33
34
35 35. De SG, Devereux RB, Chien S, et al. Relation of blood viscosity to demographic and physiologic
36 variables and to cardiovascular risk factors in apparently normal adults. *Circulation* 1990;81:107.
37
38
39 36. Schiffrin EL. Oxidative stress, nitric oxide synthase, and superoxide Dismutase: a matter of
40 imbalance underlies endothelial dysfunction in the human coronary circulation. *Hypertension* 2008;
41 51:31-2.
42
43
44
45 37. Zinchuk VV, Pronko TP, Lis MA. Blood oxygen transport and endothelial dysfunction in patients
46 with arterial hypertension. *Clin Physiol Funct. Imaging* 2004;24:205-11.
47
48
49 38. Wei Y, Liu G, Yang J, et al. The association between metabolic syndrome and vascular endothelial
50 dysfunction in adolescents. *Exp Ther Med* 2013;5:1663-6.
51
52
53
54 39. Tsuji S, Node K. Vascular endothelial dysfunction as a mechanistic factor for metabolic syndrome.
55
56
57
58
59
60

1
2
3 Nihon Rinsho Japanese. *J Clin Med* 2011;69: 295.

4
5
6 40. Kutlu M, Sonmez A, Genc H, et al. Relationship between hemoglobin and CD40 ligand in
7 prediabetes. *Clin Invest Med* 2009;32:E244-50.

8
9
10 41. Missiou A, Wolf D, Platzer I, et al. CD40L induces inflammation and adipogenesis in adipose
11 cells--a potential link between metabolic and cardiovascular disease. *Thromb Haemost*
12 2010;103:788-96.

13
14
15 42. Kawamoto R, Tabara Y, Kohara K, et al. Hemoglobin is associated with serum high molecular
16 weight adiponectin in Japanese community-dwelling persons. *J Atheroscler Thromb* 2011;18:182-9.

17
18
19 43. Ali SB, Jemaa R, Ftouhi B, et al. Adiponectin and Metabolic Syndrome in a Tunisian Population.
20
21
22 *Inflammation* 2012;35:828-33.

23
24
25 44. Laufer PM, Havakuk O, Finkelstein A, et al. High red blood cell distribution width is associated
26 with the metabolic syndrome. *Clin Hemorheol Micro* 2015;63:1-9.

27
28
29 45. Sanchez-Chaparro MA, Calvo-Bonacho E, Gonzalez-Quintela A, et al. Higher Red Blood Cell
30 Distribution Width Is Associated With the Metabolic Syndrome. *Diabetes Care* 2010;33: e40.

31
32
33 46. Perna S, Peroni G, Monteferrario F, et al. The Role of Red Blood Cell Distribution Width in
34 Metabolic Syndrome. A Cross-Sectional Study in Elderly. *Clin Nutr* 2014;33:S112.

35
36
37 47. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients implanted
38 with a mechanical circulatory assist device. *Perfusion* 2005;20:83-90.

39 40 41 42 43 44 45 **Figure Legends**

46
47
48 **Fig 1** Proportion of metabolic syndrome (MetS) components from zero to five between males and
49 females.

50
51
52 **Fig 2** Prevalence of metabolic syndrome (MetS) in association with the tertiles of red blood cell (RBC),
53 hematocrit (HCT), hemoglobin (Hb), and red blood cell distribution width (RDW) in males and
54

females, separately.

Table 1 Characteristics of study subjects with and without metabolic syndrome stratified by sex

Variables	Male (n=2161)			Female (n=2511)		
	MetS	Non-MetS	<i>P</i>	MetS	Non-MetS	<i>P</i>
MetS status (n, %)	576 (26.7)	1585 (73.3)		885 (35.2)	885 (35.2)	
Age (years)	51.39 ± 12.21	54.61 ± 13.79	<0.001	59.78 ± 12.34	55.70 ± 12.97	<0.001
Components of MetS						
WC (cm)	89.98 ± 6.79	82.40 ± 7.76	<0.001	85.94 ± 7.21	77.59 ± 8.56	<0.001
SBP (mmHg)	134.93 ± 15.20	127.57 ± 16.32	<0.001	136.49 ± 16.42	124.74 ± 18.39	<0.001
DBP (mmHg)	89.24 ± 10.47	82.93 ± 11.09	<0.001	84.13 ± 10.25	78.51 ± 10.53	<0.001
TG (mmol/L)	2.76 ± 1.77	1.29 ± 0.91	<0.001	2.15 ± 1.41	1.20 ± 1.87	<0.001
HDL-C (mmol/L)	1.00 ± 0.47	1.28 ± 0.44	<0.001	1.17 ± 0.22	1.50 ± 0.34	<0.001
FPG (mmol/L)	5.53 ± 2.01	4.87 ± 1.40	<0.001	5.38 ± 1.86	4.71 ± 0.97	<0.001
Erythrocyte parameters						
RBC (×10 ¹² /L)	4.99 ± 0.80	4.53 ± 0.51	<0.001	4.55 ± 0.84	4.10 ± 0.57	<0.001
HCT (%)	42.27 ± 4.09	40.68 ± 3.63	<0.001	37.35 ± 2.80	35.58 ± 2.83	<0.001
Hb (g/L)	147.11 ± 12.57	139.02 ± 12.68	<0.001	129.68 ± 14.45	121.50 ± 11.82	<0.001
RDW (%)	13.33 ± 0.96	12.87 ± 1.21	<0.001	13.18 ± 1.90	12.88 ± 2.27	<0.001
Liver function parameters						
ALT (u/L)	31.44 ± 18.35	26.31 ± 15.52	<0.001	24.09 ± 13.81	21.14 ± 11.79	<0.001
AST (u/L)	26.37 ± 15.87	24.80 ± 10.00	0.026	23.82 ± 8.90	23.27 ± 8.63	0.129

GGT(u/L)	48.73 ± 39.88	36.04 ± 26.83	<0.001	32.00 ± 22.79	26.12 ± 26.03	<0.001
ALB (g/L)	47.36 ± 3.23	47.32 ± 4.07	0.820	47.48 ± 4.32	47.77 ± 12.28	0.484
Other clinical characteristics						
BMI (kg/m ²)	25.90 ± 2.67	23.61 ± 3.04	<0.001	25.21 ± 3.05	22.85 ± 3.17	<0.001
TC (mmol/L)	4.81 ± 0.95	4.73 ± 0.94	0.059	5.26 ± 1.08	5.08 ± 1.02	<0.001
LDL-C (mmol/L)	2.65 ± 0.70	2.66 ± 2.06	0.885	2.95 ± 1.48	2.75 ± 1.03	<0.001
UA (umol/L)	415.45 ± 143.27	382.19 ± 84.92	<0.001	340.60 ± 83.08	306.95 ± 101.63	<0.001
WBC (×10 ⁹ /L)	6.95 ± 1.40	6.43 ± 1.40	<0.001	6.41 ± 1.35	5.84 ± 1.31	<0.001
PLT (×10 ⁹ /L)	214.70 ± 49.89	201.57 ± 52.17	<0.001	224.04 ± 53.55	216.73 ± 52.14	0.001
HbA1c (%)	5.79 ± 1.37	5.42 ± 0.97	<0.001	5.64 ± 1.22	5.33 ± 0.67	<0.001

Data were presented as mean ± SD or *n* (%); WC, waist circumference; 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; UA, uric acid; WBC, white blood cell; PLT, platelet; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; RDW, red blood cell distribution width; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, γ -glutamyltransferase; ALB, albumin; MetS, metabolic syndrome.

Table 2 Levels of Erythrocyte parameters of study subjects according to number of metabolic syndrome components in males and females, respectively

Variables	0	1	2	3	4	5	<i>F</i>	<i>P</i>
Male								
RBC	4.44 ± 0.53	4.55 ± 0.50	4.55 ± 0.52	4.80 ± 0.54	5.31 ± 0.86	5.95 ± 1.23	87.448	<0.001
HCT	39.96 ± 3.39	40.76 ± 3.88	41.00 ± 3.38	42.12 ± 4.10	42.33 ± 4.14	44.21 ± 3.01	19.799	<0.001
Hb	131.60 ± 12.35	138.81 ± 12.86	140.71 ± 12.41	144.51 ± 11.65	151.28 ± 12.87	160.04 ± 9.19	52.445	<0.001

RDW	12.75 ± 0.82	12.83 ± 1.55	13.00 ± 0.80	13.24 ± 0.86	13.41 ± 1.07	14.33 ± 1.13	20.264	<0.001
Female								
RBC	4.03 ± 0.39	4.07 ± 0.52	4.16 ± 0.54	4.45 ± 0.82	4.67 ± 0.88	4.83 ± 0.78	66.453	<0.001
HCT	35.16 ± 2.65	35.43 ± 2.78	35.91 ± 2.91	37.07 ± 2.81	37.74 ± 2.79	37.96 ± 2.36	52.237	<0.001
Hb	119.70 ± 11.54	121.28 ± 11.79	122.49 ± 11.88	128.26 ± 14.04	130.04 ± 14.35	139.61 ± 14.46	59.262	<0.001
RDW	12.71 ± 2.10	12.74 ± 1.40	13.07 ± 2.87	13.11 ± 1.39	13.25 ± 2.70	13.38 ± 1.13	4.493	<0.001

RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; RDW, red blood cell distribution width.

Table 3 Odds ratios of erythrocyte parameters associated with metabolic syndrome stratified by sex

Variables	Male		Female	
	OR (95% CI)	P	OR (95% CI)	P
RBC				
Q1			Reference	
Q2			—	
Q3			1.785 (1.248~2.554)	0.002
HCT				
Q1			Reference	
Q2			1.738 (1.229~2.458)	0.002
Q3			1.922 (1.337~2.761)	<0.001
Hb				
Q1	Reference			
Q2	1.687 (1.151~2.472)	0.007		
Q3	2.252 (1.550~3.272)	<0.001		
RDW				
Q1	Reference			

Q2	—	
Q3	2.750 (1.939~3.899)	<0.001

Statistical analysis by binary logistic regression (adjusted for age, body mass index, total cholesterol, low-density lipoprotein cholesterol, uric acid, white blood cell, platelet, alanine transaminase, aspartate aminotransferase, γ -glutamyltransferase, albumin, and the components of metabolic syndrome); OR, odds ratio; CI, confidence interval; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; RDW, red blood cell distribution width.

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

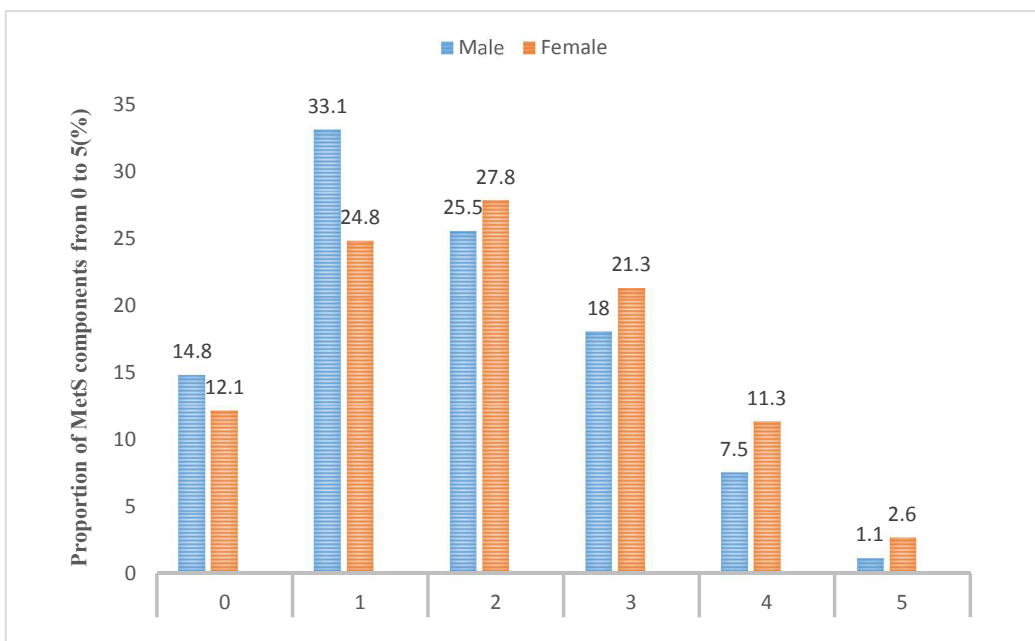


Fig 1

Peer review only

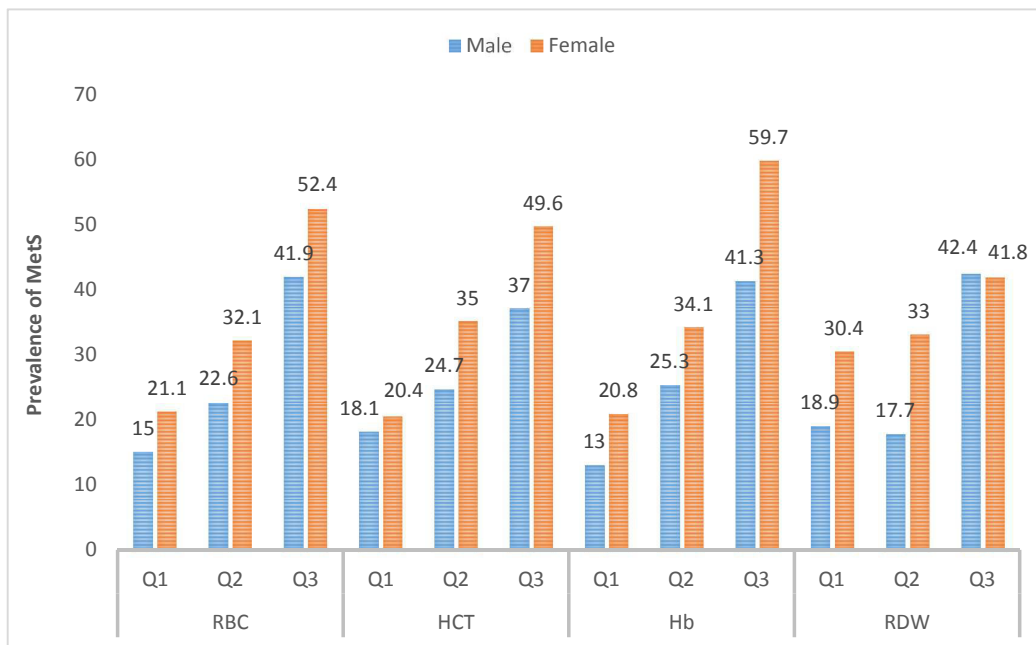


Fig 2

BMJ Open

Association of erythrocyte parameters with metabolic syndrome in the Pearl River Delta region of China: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019792.R1
Article Type:	Research
Date Submitted by the Author:	31-Oct-2017
Complete List of Authors:	Huang, LingLing; Institute of Chronic Disease Risks Assessment, Henan University Dou, Dong-Mei; Institute of Chronic Disease Risks Assessment, Henan University Liu, Nan; Guangzhou Medical University, School of Public Health Wang, XiaoXiao; Institute of Chronic Disease Risks Assessment, Henan University Fu, Li-Ying; Institute of Chronic Disease Risks Assessment, Henan University Wu, Xiao; Institute of Chronic Disease Risks Assessment, Henan University wang, peixi; Department of Preventive Medicine, School of Public Health, Guangzhou Medical University,
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Public health, Epidemiology
Keywords:	Erythrocyte parameters, Metabolic syndrome, Cross-sectional study, China

SCHOLARONE™
Manuscripts

1
2
3 **Association of erythrocyte parameters with metabolic syndrome in the Pearl River Delta region**
4 **of China: a cross-sectional study**
5
6
7
8
9

10 Ling-Ling Huang ¹⁺, Dong-Mei Dou ¹⁺, Nan Liu ², Xiao-Xiao Wang ¹, Li-Ying Fu ¹, Xiao Wu ¹, Pei-Xi
11 Wang ^{1,2*}
12
13

14
15 ¹ Institute of Chronic Disease Risks Assessment, Henan University, Kaifeng, 475004,
16
17 China;
18
19

20 ² School of Public Health, Guangzhou Medical University, Guangzhou, PR China
21
22
23
24

25 E-Mails: HuangLingLing0703@163.com (L.-L.H.); doudongmei1224@126.com (D.-M.D.);
26 LNQ555@126.com (N. L.); xiaoxiao52625@163.com (X.-X. W.); 18317856338@163.com (L-Y. F);
27 18625832936@163.com (X. W); peixi001@163.com (P-X. W).
28
29
30
31
32
33

34 * Corresponding author to Pei-Xi Wang, tel: -8618927539896, e-mail: peixi001@163.com
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Objective: Increasing studies have reported that erythrocyte parameters, including red blood cell (RBC), hematocrit (HCT), hemoglobin (Hb), and red blood cell distribution width (RDW), are associated with metabolic syndrome (MetS) in adults worldwide. However, the association, stratified by sex, remains to be elucidated, particularly in the Pearl River Delta region of China. Therefore, our aim was to explore the association of erythrocyte parameters with MetS, stratified by sex in the Pearl River Delta region of China.

Methods: In this cross-sectional study, 2161 males and 2511 females were enrolled. MetS was diagnosed using a modified version of the Adult Treatment Panel III (ATP III) criteria. Logistic regression analyses were performed to calculate adjusted odds ratios (ORs) of erythrocyte parameters associated with MetS stratified by sex.

Results: The prevalence of MetS was higher in females than that in males (35.2% vs. 26.7%). RBC, HCT, Hb and RDW values increased linearly with the number of MetS components from zero to five identified in both males and females. Among males, the ORs of MetS risk increased across the tertiles of Hb (Q2: OR=1.687, 95% confidence interval [CI]=1.151~2.472; Q3: OR=2.252, 95%CI=1.550~3.272). Males in the highest tertiles of RDW had a 2.750-fold increased risk of suffering from MetS compared to those in the reference group. Among females, the ORs of MetS risk increased across

1
2
3 the tertiles of HCT (Q2: OR=1.738, 95%CI=1.229~2.458; Q3: OR=1.922, 95%CI=1.337~2.761).

4 Females in the highest tertiles of RBC had a 1.785-fold increased risk of experiencing MetS compared
5
6
7 to those in the reference group.

8
9 **Conclusions:** MetS was more prevalent in females than that in males. The association between
10 erythrocyte parameters and MetS differed between sex, whereby RBC and HCT were identified as the
11 risk factors of MetS in females and Hb and RDW as the risk factors in males.
12
13
14

15
16
17
18 **Keywords:** Erythrocyte parameters, Metabolic syndrome, Cross-sectional study, China
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34 **Strengths and limitations of this study**

- 35 ● A large sample of subjects was enrolled in our survey.
- 36
- 37 ● To the best of our knowledge, this is the first study to report the association of erythrocyte
- 38 parameters with MetS, stratified by sex in the Pearl River Delta region of China.
- 39
- 40 ● The present study was designed as a cross-sectional study; therefore, direct causation may not be
- 41 concluded from the results.
- 42
- 43 ● Supplementary information about the lifestyle of the subjects was not collected; therefore, these
- 44 factors could not be included in the adjustments of our multivariate logistic regression analyses.
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

1
2
3 Metabolic syndrome (MetS) is defined as a cluster of multiple correlated metabolic features, including
4 abdominal obesity, hypertension, elevated triglyceride (TG) levels, decreased high-density lipoprotein
5 cholesterol (HDL-C) levels, and hyperglycemia.[1]. It is known to be strongly associated with an
6 increased risk of type 2 diabetes [2], cardiovascular disease (CVD) [2-4], and all-cause mortality [4]. In
7 recent years, MetS has emerged as a global public health issue owing to its increased prevalence
8 around the world, affecting nearly 20-30% of adults in many countries [5-7]. Hence, early
9 identification of individuals at high risk of MetS is essential for the prevention of MetS.
10
11
12
13
14
15

16
17 Currently, the pathogenesis of MetS is not clearly understood. Generally, MetS is often
18 accompanied by insulin resistance and/or chronic low-grade inflammation [8-9]. Numerous
19 investigators previously reported that erythrocyte parameters levels, including red blood cell (RBC),
20 hematocrit (HCT), hemoglobin (Hb) and red blood cell distribution width (RDW) were positively
21 associated with a insulin resistance and chronic low grade inflammation [10-14]. In fact, RBC [14-16],
22 HCT [15-16], Hb [14-15, 17] and RDW[18] were demonstrated in several studies worldwide to
23 correlate with MetS in adults. However, the association between erythrocyte parameters and MetS
24 remains controversial, because the results reported are inconsistent depending on the different ethnic
25 populations studied. In addition, discrepancies in the results may be partly attributed to differences
26 between sexes. Many studies simply applied sex as an adjustment variable to investigate the
27 relationship between erythrocyte parameters and MetS, and no studies were conducted in the Pearl
28 River Delta region of China. Therefore, the aim of this study was to explore the association between
29 erythrocyte parameters and MetS stratified by sex in the Pearl River Delta region of China.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

47 **Materials and Methods**

48 **Study participants**

49
50 This cross-sectional study involved participants who underwent a general health examination at the
51 Community Health Service Agencies in the Pearl River Delta region of China in 2015. The health
52 examination included recording of medical history, anthropometric measurements, and laboratory tests.
53
54
55
56
57
58
59
60

Participants with a history of cardiovascular diseases, severe liver and kidney dysfunction, tumors, and severe inflammatory diseases were excluded. In addition, participants who did not have complete data on their MetS components and erythrocyte parameters were excluded. Altogether, a total of 4672 subjects (2161 males and 2511 females) were enrolled in this study. The study was approved by the Ethics Committee of Guangdong Sociological Society. Written informed consent was obtained from all participants.

Data collection and measurements

The medical history of subjects was obtained by review of self-reported questionnaires. Anthropometric parameters were measured by trained staff, following a standardized protocol. Height, weight, waist circumference (WC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured in replicate, and all the mean values of the above indexes were calculated for this study. Then, body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. After an overnight fast, venous blood samples from participants were obtained by measuring the blood levels of routine laboratory tests, including TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), uric acid (UA), white blood cell (WBC), platelet (PLT), RBC, HCT, Hb, RDW, alanine transaminase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase;(GGT), albumin (ALB) and glycated hemoglobin A1c (HbA1c).

Tertiles of erythrocyte parameters levels

Erythrocyte parameters levels were categorized into tertiles on the basis of individual distributions for males and females, respectively (in males: RBC, $Q1 < 4.37 \times 10^{12}/L$, $Q2 = 4.37 \sim 4.75 \times 10^{12}/L$, $Q3 \geq 4.75 \times 10^{12}/L$; HCT, $Q1 < 39.8\%$, $Q2 = 39.8 \sim 42.4\%$, $Q3 \geq 42.5\%$; Hb, $Q1 < 137$ g/L, $Q2 = 137 \sim 146$ g/L, $Q3 \geq 147$ g/L; RDW, $Q1 < 12.5\%$, $Q2 = 12.5 \sim 13.1\%$, $Q3 \geq 13.2\%$; in females: RBC, $Q1 < 3.96 \times 10^{12}/L$, $Q2 = 3.96 \sim 4.27 \times 10^{12}/L$, $Q3 \geq 4.28 \times 10^{12}/L$; HCT, $Q1 < 35.2\%$, $Q2 = 35.2 \sim 37.3\%$, $Q3 \geq 37.4\%$; Hb, $Q1 < 120$ g/L, $Q2 = 120 \sim 127$ g/L, $Q3 \geq 128$ g/L; RDW, $Q1 < 12.3\%$, $Q2 = 12.3 \sim 12.8\%$, $Q3 \geq 12.9\%$).

Definition of metabolic syndrome

MetS was diagnosed using a modified version of the Adult Treatment Panel III (ATP III) criteria [1], which included at least three of the following five components: 1) $WC \geq 90$ cm in males and $WC \geq 80$ cm

1
2
3 in females; 2) SBP \geq 130 mmHg or DBP \geq 85 mmHg; 3) TG \geq 1.70 mmol/L; 4) HDL-C $<$ 1.03 mmol/L in
4
5 males and HDL-C $<$ 1.29 mmol/L in females; and 5) FPG \geq 5.6 mmol/L.
6
7
8
9

10 **Statistical analysis**

11
12 All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS)
13 version 21.0 (SPSS Inc., Chicago, IL, USA). Data is presented as the mean \pm standard deviation or
14 frequency (percentage). The *t*-test was used to evaluate differences in characteristics of study subjects
15 with and without MetS stratified by sex. The χ^2 test was performed to compare the proportion of MetS
16 components, from zero to five, between males and females; and compare the prevalence of MetS
17 dependent on the tertiles of RBC, HCT, Hb, and RDW between males and females, respectively. A
18 one-way ANOVA was conducted to test mean levels of erythrocyte parameters according to the number
19 of MetS components in males and females, separately. Multivariate logistic regression analyses (the
20 forward selection procedure) were performed to calculate adjusted odds ratios (ORs) of erythrocyte
21 parameters associated with MetS stratified by sex with adjustments for potential confounders (the
22 statistically significant variables in table 1, Male: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG,
23 ALT, AST, GGT, BMI, UA, WBC, PLT and HbA1c; Female: adjusted for age, WC, SBP, DBP, TG,
24 HDL-C, FPG, ALT, GGT, BMI, TC, LDL-C, UA, WBC, PLT and HbA1c). A *P* value $<$ 0.05 was
25 considered to be statistically significant.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43 **Results**

44 **Prevalence of MetS**

45
46 In total, there were 2161 males and 2511 females enrolled in this study. Of the subjects, 576 males
47 (26.7%) and 885 females (35.2%) were diagnosed with MetS.
48
49
50
51
52
53

54 **Characteristics of study subjects**

1
2
3 In this study, among males, the mean age of the MetS group was significantly lower than that of the
4 non-MetS group, whereas the opposite trend was observed among females ($P<0.001$). In the cluster of
5 MetS components, WC, SBP, DBP, TG, and FPG levels were remarkably greater in the MetS group
6 than in the non-MetS group in both males and females, but HDL-C levels were significantly lower in
7 the MetS group than those the non-MetS group in both males and females ($P<0.001$). In the cluster
8 of erythrocyte parameters, we found that RBC, HCT, Hb and RDW were significantly higher in the
9 MetS group than those in the non-MetS group in both males and females ($P<0.001$). Additional
10 information about the characteristics of study subjects with and without MetS stratified by sex are
11 presented in Table 1.
12
13
14
15

16 **Proportion of MetS components**

17 Our results revealed that most males experienced one metabolic disorder, and most females suffered
18 from two metabolic disorders. In addition, the proportion of MetS components from two to five was
19 significantly lower in males than that in females (25.5% vs. 27.8%, 18% vs. 21.3%, 7.5% vs. 11.3%,
20 1.1% vs. 2.6, respectively). Additional information is shown in Fig 1.
21
22
23

24 **Association of erythrocyte parameters with MetS**

25 This study showed that the levels of RBC, HCT, Hb and RDW clearly increased with number of MetS
26 components from zero to five identified in both males and females ($P<0.001$, shown in Table 2), Figure
27 2 showed that the prevalence of MetS increased in a dose-dependent manner as the tertiles of RBC,
28 HCT, Hb and RDW levels increased in both males and females. Furthermore, at each tertile of the
29 above-mentioned parameters, the prevalence of MetS was lower in males than that in females, except at
30 the highest tertiles of RDW levels (shown in Fig 2).
31
32
33
34
35
36
37
38
39
40

41 **Multivariate Logistic regression analysis model**

42 Adjusted ORs of MetS risk associated with each tertile of RBC, HCT, Hb, and RDW are listed in Table
43 3. After adjusting for potential confounders (the statistically significant variables in table 1, Male:
44 adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, AST, GGT, BMI, UA, WBC, PLT and
45 HbA1c; Female: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, GGT, BMI, TC, LDL-C,
46 UA, WBC, PLT and HbA1c). a significant association of Hb and RDW with MetS was observed in
47 males, but this was not the same for RBC and HCT. The ORs of MetS risk increased across the tertiles
48 of Hb in males (Q2: OR=1.687, 95% confidence interval [CI]=1.151~2.472; Q3: OR=2.252, 95%CI=
49 1.550~3.272). Males in the highest tertiles of RDW had a 2.75-fold increased risk of suffering from
50
51
52
53
54
55
56
57
58
59
60

1
2
3 MetS in comparison to those in the reference group. Only RBC and HCT levels were observed to
4 associate with MetS in females. The ORs of MetS risk increased across the tertiles of HCT(Q2:
5 OR=1.738, 95% CI=1.229~2.458; Q3: OR=1.922, 95%CI=1.337~2.761). Females in the highest
6
7 tertiles of RBC had a 1.785-fold increased risk of experiencing MetS in comparison to those in the
8
9 reference group.
10
11
12
13
14
15

16 **Discussion**

17 **Main findings**

18
19 The prevalence of MetS was higher in females than that in males (35.2% vs. 26.7%). Levels of RBC,
20
21 HCT, and Hb and RDW increased linearly with the number of MetS components from zero to five
22
23 identified in both males and females. The association between erythrocyte parameters and MetS
24
25 differed between sexes, whereby Hb and RDW were identified as risk factors of MetS in males and
26
27 RBC and HCT as the risk factors in females.
28
29
30

31 **Comparisons with Previous Studies**

32
33 Sex has been demonstrated to be a predictive factor for MetS development. Several studies have
34
35 showed that females have a higher prevalence of MetS than that of males [19-20]. A large-scale study
36
37 conducted in Russia reported that the prevalence of MetS diagnosed using the ATP III criteria, was
38
39 9.5% in men and 23.5% in women [19]; Another study performed in the seven geographical regions of
40
41 Turkey showed that the prevalence of MetS as determined by the ATP III criteria, was 28% in men and
42
43 39.6% in women [20]. Our study are in accordance with the former reports. However, other studies
44
45 have reported that males have a higher prevalence of MetS than that of females. For example, Tao et al
46
47 found the 5-year cumulative incidence of MetS in Beijing adults was 14.22% for males and 7.59% for
48
49 females [21]; Yang et al revealed that the 5-year cumulative incidence of MetS in Taiwanese adults was
50
51 14.95% for males and 9.89% for females [22]. The difference in the findings might be due to the
52
53 different study design and population.
54

55 It is well known that MetS represents a cluster of simultaneously occurring metabolic
56
57 abnormalities. In fact, previous studies demonstrated that RBC and Hb levels clearly increased with the
58
59

1
2
3 numbers of MetS components [16, 23]. Our findings were consistent with those of these reports. It has
4 also been shown that a higher number of MetS components is associated with insulin resistance. Based
5 on the facts that the levels of RBC, HCT and Hb were significantly associated with insulin resistance
6 [10, 12, 24], we hypothesize that increased levels of erythrocyte parameters tested in this study may be
7 indicative of the development of insulin resistance.
8
9
10
11
12

13 Many studies have demonstrated an association between RBC levels and MetS, indicating RBC is
14 a potential hematological marker for early detection of MetS [14-16]. Our results revealed that the
15 highest tertiles of RBC were associated with MetS in females, consistent with a recent study [24]. The
16 pathogenesis of insulin resistance may, in part, be causative of the association between RBC levels and
17 MetS. Aoki et al reported that insulin can stimulate the proliferation and differentiation of
18 erythropoietic cells by binding receptors upon the cell surface [26]. It was suggested that insulin and
19 insulin growth factors I-II can promote the proliferation and differentiation of erythroid progenitors in
20 human bone marrow and circulation [27-29]. Alternatively, the relationship between RBC levels and
21 MetS may be a result of iron overload. It was reported that iron overload was associated with insulin
22 resistance [30], and high body iron storage interfered with insulin-mediated effects and bloodletting
23 improved insulin sensitivity [31]. Bozzini et al found that iron overload was strongly associated with
24 obesity and dyslipidemia, and serum ferritin would help identify a subgroup of individuals at risk for
25 insulin resistance-associated hepatic iron overload.[32]. Additionally, the erythrocyte fatty acids
26 composition changes may play a major role in the association of RBC levels with MetS.
27 Novgorodtseva et al found that the development of MetS was accompanied by erythrocyte fatty acids
28 composition changes [33], and Zong et al demonstrated that erythrocyte fatty acids in the de novo
29 lipogenesis pathway were independently associated with an elevated risk of MetS [34]. It has been
30 reported that insulin resistance could link fatty acids with MetS [35].
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

47 It has been reported that high HCT levels correlated with increasing risk of MetS [15-16].
48 Lohsoonthorn et al reported that MetS risk increased across successive quartiles of HCT in females, but
49 not in males [36]; Nebeck et al demonstrated that HCT was significantly associated with MetS in
50 females, but no similar trend was observed in males [16]. Our results confirmed that the ORs of MetS
51 risk increased with an elevation in HCT in females. HCT was considered as a major determinant of
52 blood viscosity [37]. Increased blood viscosity causes blood flow to skeletal muscles and fat tissues to
53
54
55
56
57
58
59
60

1
2
3 decrease, additionally contributing to insulin resistance [38-39]. Moreover, increased blood viscosity
4 was determined as an independent risk factor of hypertension [40]. It has been reported that HCT is
5 positively associated with insulin resistance, which is an important factor in the pathogenesis of MetS
6 [12, 14]. Considering these bodies of evidence, we hypothesize that the association between HCT
7 levels and MetS may arise from increased blood viscosity and insulin resistance linked to HCT.
8
9

10
11
12
13 Hb, another important erythrocyte parameter, has been reported to be associated with MetS in both
14 cross-sectional and cohort studies [14, 16-17, 36]. An 8-year follow-up cohort study conducted in Japan
15 detected that the highest and third quartiles of Hb concentration were associated with increased risk of
16 MetS incidence compared to the lowest quartiles of Hb concentration in males, but there was no
17 association observed in females [17]. In general, our findings were consistent with those of the
18 previous reports. In our study, the ORs of MetS increased across the successive tertiles of Hb among
19 males; however, no similar trend was observed among females. The following mechanisms may be
20 regarded as the causes of association between Hb and MetS: Hb is a well-known carrier and buffer of
21 nitric oxide (NO), and can regulate the endothelial function of blood vessels by modulating NO levels
22 in blood [41]. Furthermore, Hb and various compounds of NO modulate the affinity between Hb and
23 oxygen in blood, which can lead to vascular endothelial dysfunction [42]. It has been found that
24 vascular endothelial dysfunction was associated with MetS [43-44]. In addition, Hb plays a key role in
25 regulating sCD40L levels [45], and sCD40L has been shown to participate in thrombus formation and
26 inflammation, which is a independent risk factor for atherosclerosis and MetS [46]. Another possibility
27 linking Hb and MetS may be the adiponectin. Previous studies showed that higher Hb levels were
28 closely related to the lower adiponectin levels [47-48], and lower levels of adiponectin significantly
29 increased the risk for MetS, respectively. Finally, insulin resistance may also be involved in the
30 association between Hb and MetS [8, 12].
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

47 RDW, a common index of routine blood examination, represents a measure of heterogeneity in the
48 size of circulating erythrocytes. A high RDW index indicates greater heterogeneity in size of circulating
49 erythrocytes in a subject. In this study, males in the highest tertiles of RDW (>13.2%) had a 2.75-fold
50 increased risk for MetS. Multiple groups previously showed that elevated RDW was associated with
51 MetS [49-50]. For instance, Laufer and colleagues demonstrated that $RDW \geq 14\%$ was independently
52 associated with an increased risk for MetS development [49]; Sanchez-Chaparro and colleagues
53
54
55
56
57
58
59
60

1
2
3 reported that the highest quartile of RDW (>14%) was remarkably linked with MetS after adjusting for
4 potential confounders [50]. Moreover, a recent study illustrated RDW is a potential metabolic marker
5 for the detection of metabolic diseases [51]. To date, the mechanism of association between RDW and
6 MetS remains unknown; however, chronic inflammation linked to RDW may play an important role. It
7 was found that MetS was associated with chronic inflammation [9], and RDW reflects an underlying
8 inflammatory state [13]. Pierce and colleagues have proved that proinflammatory cytokines can inhibit
9 erythropoietin-induced erythrocyte maturation, which may lead to an elevation of RDW [52].

10
11
12
13
14
15
16
17 However, there were several limitations in this study. First, the present study was designed as a
18 cross-sectional study; therefore, direct causation cannot be concluded from the results. Then,
19 supplementary information about the lifestyle of the subjects was not collected; therefore, these factors
20 could not be included in the adjustments of our multivariate logistic regression analyses. It was found
21 that people from the Pearl River Delta region of China often seldom eat breakfast, stay up late and lack
22 physical exercise [53]. In addition, Han and his colleague reported that the smoking rate of people over
23 15 years old in Shenzhen was higher than that in the whole country (22.32% vs. 22.30%) [54-55]. It is
24 well known that these factors are related to the development of MetS.
25
26
27
28
29
30
31
32
33
34

35 **Conclusions**

36
37 In our study, MetS was more prevalent in females than that in males. The association between
38 erythrocyte parameters and MetS differed between sexes, whereby RBC and HCT were identified as
39 the risk factors of MetS in females and Hb and RDW as the risk factors in males. Our study provides
40 sufficient evidence that erythrocyte parameters may serve as effective molecular markers for the early
41 detection of MetS risk on a sex-dependent basis.
42
43
44
45
46
47
48
49

50 **Acknowledgments:** We gratefully acknowledge the staff of local Community Health Service Agencies,
51 for their kind assistance in data collection
52
53
54
55
56
57
58
59
60

1
2
3 **Contributors:** LLH and PXW conducted the data analyses. LLH, NL, XXW, LYF and XW drafted the
4 manuscript. PXW and DMD finalized the manuscript with inputs from all authors. All authors
5 contributed to the development of the study framework, interpretation of the results, revisions of
6 successive drafts of the manuscript, and approved the version submitted for publication.
7
8
9

10
11
12
13
14 **Funding** This study was supported by the Guangzhou 121 Talents Program (GZRS-2014-2048), the
15 Science and Technology Program of Guangzhou (201607010136, 201510010109) and the National
16 Science Foundation of China (81402716).
17
18

19
20
21
22
23 **Conflicts of Interest** The authors declare that they have no conflicts of interest.
24
25

26
27
28 **Ethical approval** The study was approved by the Ethics Committee of Guangdong Sociological
29 Society..
30
31

32
33
34
35 **Informed consent** Informed consent was obtained from all individual participants included in the
36 study.
37
38

39
40
41
42
43 **Data sharing statement** This database is first used in this study. The database belongs to our team, and
44 if shared, you need to get their permission.
45
46

47 48 49 50 **References**

51
52
53 1. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome.
54 An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement.
55 *Circulation* 2005,112:2735–52.
56
57

- 1
2
3 2. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim
4 statement of the International Diabetes Federation Task Force on Epidemiology and Prevention;
5 National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation;
6 International Atherosclerosis Society; and International Association for the Study of Obesity.
7
8 *Circulation* 2009;120:1640-5
9
10
11
12
13 3. Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk a systematic
14 review and meta-analysis. *J Am Coll Cardiol* 2010;56:1113-32.
15
16
17 4. Gami AS, Witt BJ, Howard DE, et al. Metabolic syndrome and risk of incident cardiovascular events
18 and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol*
19 2007;49:403-14.
20
21
22
23 5. Miller JM, Kaylor MB, Johannsson M, et al. Prevalence of metabolic syndrome and individual
24 criterion in US adolescents: 2001–2010 National Health and Nutrition Examination Survey. *Metab*
25 *Syndr Relat Disord* 2014;12:527-32.
26
27
28
29 6. Peer N, Lombard C, Steyn K, et al. High prevalence of metabolic syndrome in the Black population
30 of Cape Town: The Cardiovascular Risk in Black South Africans (CRIBSA) study. *Eur J Prev Cardiol*
31 2015;22:1036-42.
32
33
34 7. Lovre D, Mauvais-Jarvis F. Trends in prevalence of the metabolic syndrome. *JAMA*. 2015; 314(9):
35 950-951. Doi: 10.1001/jama.2015.8625
36
37
38 8. González AS, Guerrero DB, Soto MB, et al. Metabolic syndrome, insulin resistance and the
39 inflammation markers C-reactive protein and ferritin. *Eur J Clin Nutr* 2006;60: 802-9.
40
41
42 9. Musani SK, Vasan RS, Bidulescu A, et al. Aldosterone, C-Reactive Protein, and Plasma B-Type
43 Natriuretic Peptide Are Associated With the Development of Metabolic Syndrome and Longitudinal
44 Changes in Metabolic Syndrome Components: Findings from the Jackson Heart Study. *Diabetes Care*
45 2013;36:3084-92.
46
47
48 10. Ellinger VC, Carlini LT, Moreira RO, et al. Relation between insulin resistance and hematological
49 parameters in a Brazilian sample. *Arq Bras Endocrinol Metabol* 2006;50:114-7.
50
51
52
53
54
55
56
57
58
59
60

11. Mardi T, Toker S, Melamed S, et al. Increased erythropoiesis and subclinical inflammation as part of the metabolic syndrome. *Diabetes Res Clin Pr* 2005;69:249-55.
12. Tabara Y, Igase M, Saito I, et al: Association of hematological parameters with insulin resistance, insulin sensitivity, and asymptomatic cerebrovascular damage: The J-SHIP Toon Health Study. *Clin Hemorheol Microcirc* 2013;55: 297-311.
13. Lippi G, Targher G, Montagnana M, et al. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* 2009;133: 628-32.
14. Kawamoto R, Tabara Y, Kohara K, et al: Hematological parameters are associated with metabolic syndrome in Japanese community-dwelling persons. *Endocrine* 2013;43:334-41.
15. Wu S, Lin HY, Zhang CQ, et al. Association between erythrocyte parameters and metabolic syndrome in urban Han Chinese: a longitudinal cohort study. *BMC Public Health* 2013;13: 1-8.
16. Nebeck K, Gelaye B, Lemma S, et al. Hematological parameters and metabolic syndrome: Findings from an occupational cohort in Ethiopia. *Diabetes Metab Syndr* 2012;6:22-7.
17. Hashimoto Y, Tanaka M, Kimura T, et al. Hemoglobin concentration and incident metabolic syndrome: a population-based large-scale cohort study. *Endocrine* 2015;50:390-6.
18. Vayá A, Carmona P, Badia N, et al. Association between high red blood cell distribution width and metabolic syndrome. Influence of abdominal obesity. *Clin Hemorheol Micro* 2011;47: 75-7.
19. Sidorenkov O, Nilssen O, Grjibovski AM. Metabolic syndrome in Russian adults associated factors and mortality from cardiovascular diseases and all causes. *BMC Public Health* 2010;10:1-10.
20. Kozan O, Oguz A, Abaci A, et al. Prevalence of the metabolic syndrome among Turkish adults. *Et al* 2007;61:548-53.
21. Tao LX, Li X, Zhu HP, et al. Association of hematological parameters with metabolic syndrome in Beijing adults population: a longitudinal study. *Endocrine* 2014;46:483.
22. Yang X, Tao F, Sun S, et al. The impact of socioeconomic status on the incidence of metabolic

- 1
2
3 syndrome in a Taiwanese health screening population. *Int J Public Health* 2012;57:551-9.
4
5
6 23. Wang YY, Lin SY, Liu PH, et al. Association between hematological parameters and metabolic
7 syndrome components in a Chinese population. *J Diabetes Complicat* 2004;18:322-27.
8
9
10 24. Choi KM, Lee J, Kim YH, et al. Relation between insulin resistance and hematological parameters
11 in elderly Koreans-Southwest Seoul (SWS) Study. *Diabetes Res Clin Pr* 2003;60:205-12.
12
13
14 25. Wang T, Wang H. Study of the relationship between female metabolic syndrome and its related
15 blood indexes in Guangzhou. *Medical Innovation of China* 2016;13:61-4.
16
17
18 26. Aoki I, Taniyama M, Toyoma K, et al. Stimulatory effects of human insulin on erythroid
19 progenitors (CFU-E and BFU-E) in human CD34 separated bone marrow cells and the relationship
20 between insulin and erythropoietin. *Stem Cells* 1994;12:329-38.
21
22
23 27. Bersch N, Groopman E, Golde DW. Natural and biosynthetic insulin stimulates the growth of
24 human erythroid progenitors in vitro. *J Clin Endocrinol Metab* 1982;55:1209-11.
25
26
27 28. Miyagawa S, Kobayashi M, Konishi N, et al. Insulin and insulin-like growth factor I support the
28 proliferation of erythroid progenitor cells in bone marrow through the sharing of receptors. *Br J*
29 *Haematol* 2000;109:555-62.
30
31
32 29. Dainiak N, Kreczko S. Interactions of insulin, insulinlike growth factor II, and platelet—derived
33 growth factor in erythropoietic culture. *J Clin Invest* 1985;76:1237-42.
34
35
36 30. Marti'nez-Garcia MA, Luque-Ramirez M, San-Millan JL, et al. Body iron stores and glucose
37 intolerance in premenopausal women: role of hyperandrogenism, insulin resistance, and genomic
38 variants related to inflammation, oxidative stress, and iron metabolism. *Diabetes Care*. 2009;32:
39 1525-30.
40
41
42 31. Fernandez-Real JM, Penarroja G, Castro A, et al. Blood letting in high-ferritin type 2 diabetes:
43 effect on insulin sensitivity and h-cell function. *Diabetes* 2002;51:1000-4.
44
45
46 32. Bozzini C, Girelli D, Olivieri O, et al. Prevalence of body iron excess in the metabolic syndrome.
47 *Diabetes care* 2005;28:2061-3.
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 33. Novgorodtseva TP, Karaman YK, Zhukova NV, et al. Composition of fatty acids in plasma and
4 erythrocytes and eicosanoids level in patients with metabolic syndrome. *Lipids Health Dis* 2011;10:82.
5
6
7
8 34. Zong G, Zhu J, Sun L, et al. Associations of erythrocyte fatty acids in the de novo lipogenesis
9 pathway with risk of metabolic syndrome in a cohort study of middle-aged and older Chinese. *Am J*
10 *Clin Nutr* 2013;98:319-26.
11
12
13
14 35. Djousse L, Matthan NR, Lichtenstein AH, et al. Red blood cell membrane concentration of
15 cis-palmitoleic and cis-vaccenic acids and risk of coronary heart disease. *Am J Cardiol* 2012;110:539-
16 44.
17
18
19
20 36. Lohsoonthorn V, Jiamjararungsi W, Williams M A. Association of hematological parameters with
21 clustered components of metabolic syndrome among professional and office workers in Bangkok,
22 *Thailand. Diabetes Metab Syndr.* 2007;1:143-149.
23
24
25
26 37. Perrine SP, Greene MF, Lee PD, et al. Insulin stimulates cord blood erythroid progenitor growth:
27 evidence for an aetiological role in neonatal polycythaemia. *Br.J. Haematol.* 1986;64:503-11.
28
29
30
31 38. Baron AD. Hemodynamic actions of insulin. *Am J Physiol* 1994;267:E187-E202.
32
33
34 39. Facchini FS, Carantoni M, Jeppesen J, et al. Hematocrit and hemoglobin are independently related
35 to insulin resistance and compensatory hyperinsulinemia in healthy, non-obese men and women.
36 *Metabolism* 1998;47:831-5.
37
38
39
40 40. De SG, Devereux RB, Chien S, et al. Relation of blood viscosity to demographic and physiologic
41 variables and to cardiovascular risk factors in apparently normal adults. *Circulation* 1990;81:107.
42
43
44 41. Schiffrin EL. Oxidative stress, nitric oxide synthase, and superoxide Dismutase: a matter of
45 imbalance underlies endothelial dysfunction in the human coronary circulation. *Hypertension* 2008;
46 51:31-2.
47
48
49
50 42. Zinchuk VV, Pronko TP, Lis MA. Blood oxygen transport and endothelial dysfunction in patients
51 with arterial hypertension. *Clin Physiol Funct. Imaging* 2004;24:205-11.
52
53
54
55 43. Wei Y, Liu G, Yang J, et al. The association between metabolic syndrome and vascular endothelial
56
57
58
59
60

- dysfunction in adolescents. *Exp Ther Med* 2013;5:1663-6.
44. Tsuji S, Node K. Vascular endothelial dysfunction as a mechanistic factor for metabolic syndrome. *Nihon Rinsho Japanese. J Clin Med* 2011;69: 295.
45. Kutlu M, Sonmez A, Genc H, et al. Relationship between hemoglobin and CD40 ligand in prediabetes. *Clin Invest Med* 2009;32:E244-50.
46. Missiou A, Wolf D, Platzer I, et al. CD40L induces inflammation and adipogenesis in adipose cells--a potential link between metabolic and cardiovascular disease. *Thromb Haemost* 2010;103:788-96.
47. Kawamoto R, Tabara Y, Kohara K, et al. Hemoglobin is associated with serum high molecular weight adiponectin in Japanese community-dwelling persons. *J Atheroscler Thromb* 2011;18:182-9.
48. Ali SB, Jemaa R, Ftouhi B, et al. Adiponectin and Metabolic Syndrome in a Tunisian Population. *Inflammation* 2012;35:828-33.
49. Laufer PM, Havakuk O, Finkelstein A, et al. High red blood cell distribution width is associated with the metabolic syndrome. *Clin Hemorheol Micro* 2015;63:1-9.
50. Sanchez-Chaparro MA, Calvo-Bonacho E, Gonzalez-Quintela A, et al. Higher Red Blood Cell Distribution Width Is Associated With the Metabolic Syndrome. *Diabetes Care* 2010;33: e40.
51. Perna S, Peroni G, Monteferrario F, et al. The Role of Red Blood Cell Distribution Width in Metabolic Syndrome. A Cross-Sectional Study in Elderly. *Clin Nutr* 2014;33:S112.
52. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients implanted with a mechanical circulatory assist device. *Perfusion* 2005;20:83-90.
53. Cross-sectional investigation of dietary pattern and metabolic syndrome in high income population of shenzhen city. Nutrition, metabolism and chronic diseases -- the Tenth Annual Academic Meeting of Danone Nutrition Center. 2007. (In Chinese)
54. Han TG, Chen KY, Zhuang RS, et al. Survey of the Tobacco Epidemic in Shenzhen City in 2012. *Health Edu Health Promot* 2016;11:222-6. (In Chinese)

55. Xu T, Li W, Hu B, et al. Survey of smoking and passive smoking status among Chinese adults in 11 provinces. *Chin J Prev Contr Chron Dis* 2010;18:229-30. (In Chinese)

Figure Legends

Figure 1 Proportion of metabolic syndrome (MetS) components from zero to five between males and females.

Figure 2 Prevalence of metabolic syndrome (MetS) in association with the tertiles of red blood cell (RBC), hematocrit (HCT), hemoglobin (Hb), and red blood cell distribution width (RDW) in males and females, separately.

Table 1 Characteristics of study subjects with and without metabolic syndrome stratified by sex

Variables	Male (n=2161)			Female (n=2511)		
	MetS	Non-MetS	P	MetS	Non-MetS	P
MetS status (n, %)	576 (26.7)	1585 (73.3)		885 (35.2)	885 (35.2)	

1							
2							
3	Age (years)	51.39 ± 12.21	54.61 ± 13.79	<0.001	59.78 ± 12.34	55.70 ± 12.97	<0.001
4							
5							
6	Components of MetS						
7							
8	WC (cm)	89.98 ± 6.79	82.40 ± 7.76	<0.001	85.94 ± 7.21	77.59 ± 8.56	<0.001
9							
10							
11	SBP (mmHg)	134.93 ± 15.20	127.57 ± 16.32	<0.001	136.49 ± 16.42	124.74 ± 18.39	<0.001
12							
13							
14	DBP (mmHg)	89.24 ± 10.47	82.93 ± 11.09	<0.001	84.13 ± 10.25	78.51 ± 10.53	<0.001
15							
16	TG (mmol/L)	2.76 ± 1.77	1.29 ± 0.91	<0.001	2.15 ± 1.41	1.20 ± 1.87	<0.001
17							
18							
19	HDL-C (mmol/L)	1.00 ± 0.47	1.28 ± 0.44	<0.001	1.17 ± 0.22	1.50 ± 0.34	<0.001
20							
21	FPG (mmol/L)	5.53 ± 2.01	4.87 ± 1.40	<0.001	5.38 ± 1.86	4.71 ± 0.97	<0.001
22							
23							
24	Erythrocyte parameters						
25							
26							
27	RBC (×10 ¹² /L)	4.99 ± 0.80	4.53 ± 0.51	<0.001	4.55 ± 0.84	4.10 ± 0.57	<0.001
28							
29	HCT (%)	42.27 ± 4.09	40.68 ± 3.63	<0.001	37.35 ± 2.80	35.58 ± 2.83	<0.001
30							
31							
32	Hb (g/L)	147.11 ± 12.57	139.02 ± 12.68	<0.001	129.68 ± 14.45	121.50 ± 11.82	<0.001
33							
34							
35	RDW (%)	13.33 ± 0.96	12.87 ± 1.21	<0.001	13.18 ± 1.90	12.88 ± 2.27	<0.001
36							
37	Liver function parameters						
38							
39							
40	ALT (u/L)	31.44 ± 18.35	26.31 ± 15.52	<0.001	24.09 ± 13.81	21.14 ± 11.79	<0.001
41							
42	AST (u/L)	26.37 ± 15.87	24.80 ± 10.00	0.026	23.82 ± 8.90	23.27 ± 8.63	0.129
43							
44							
45	GGT(u/L)	48.73 ± 39.88	36.04 ± 26.83	<0.001	32.00 ± 22.79	26.12 ± 26.03	<0.001
46							
47							
48	ALB (g/L)	47.36 ± 3.23	47.32 ± 4.07	0.820	47.48 ± 4.32	47.77 ± 12.28	0.484
49							
50	Other clinical characteristics						
51							
52							
53	BMI (kg/m ²)	25.90 ± 2.67	23.61 ± 3.04	<0.001	25.21 ± 3.05	22.85 ± 3.17	<0.001
54							
55							
56							
57							
58							
59							
60							

TC (mmol/L)	4.81 ± 0.95	4.73 ± 0.94	0.059	5.26 ± 1.08	5.08 ± 1.02	<0.001
LDL-C (mmol/L)	2.65 ± 0.70	2.66 ± 2.06	0.885	2.95 ± 1.48	2.75 ± 1.03	<0.001
UA (umol/L)	415.45 ± 143.27	382.19 ± 84.92	<0.001	340.60 ± 83.08	306.95 ± 101.63	<0.001
WBC (×10 ⁹ /L)	6.95 ± 1.40	6.43 ± 1.40	<0.001	6.41 ± 1.35	5.84 ± 1.31	<0.001
PLT (×10 ⁹ /L)	214.70 ± 49.89	201.57 ± 52.17	<0.001	224.04 ± 53.55	216.73 ± 52.14	0.001
HbA1c (%)	5.79 ± 1.37	5.42 ± 0.97	<0.001	5.64 ± 1.22	5.33 ± 0.67	<0.001

Data were presented as mean ± SD or *n* (%); MetS, metabolic syndrome; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin; RDW, red blood cell distribution width; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, γ -glutamyltransferase; ALB, albumin; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; WBC, white blood cell; PLT, platelet; HbA1c, glycated hemoglobin A1c.

Table 2 Levels of erythrocyte parameters of study subjects according to number of metabolic syndrome components in males and females, respectively

Variables	0	1	2	3	4	5	<i>F</i>	<i>P</i>
Male								
RBC	4.44 ± 0.53	4.55 ± 0.50	4.55 ± 0.52	4.80 ± 0.54	5.31 ± 0.86	5.95 ± 1.23	87.448	<0.001
HCT	39.96 ± 3.39	40.76 ± 3.88	41.00 ± 3.38	42.12 ± 4.10	42.33 ± 4.14	44.21 ± 3.01	19.799	<0.001
Hb	131.60 ± 12.35	138.81 ± 12.86	140.71 ± 12.41	144.51 ± 11.65	151.28 ± 12.87	160.04 ± 9.19	52.445	<0.001
RDW	12.75 ± 0.82	12.83 ± 1.55	13.00 ± 0.80	13.24 ± 0.86	13.41 ± 1.07	14.33 ± 1.13	20.264	<0.001
Female								
RBC	4.03 ± 0.39	4.07 ± 0.52	4.16 ± 0.54	4.45 ± 0.82	4.67 ± 0.88	4.83 ± 0.78	66.453	<0.001

HCT	35.16 ± 2.65	35.43 ± 2.78	35.91 ± 2.91	37.07 ± 2.81	37.74 ± 2.79	37.96 ± 2.36	52.237	<0.001
Hb	119.70 ± 11.54	121.28 ± 11.79	122.49 ± 11.88	128.26 ± 14.04	130.04 ± 14.35	139.61 ± 14.46	59.262	<0.001
RDW	12.71 ± 2.10	12.74 ± 1.40	13.07 ± 2.87	13.11 ± 1.39	13.25 ± 2.70	13.38 ± 1.13	4.493	<0.001

RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin; RDW, red blood cell distribution width.

Table 3 Odds ratios of erythrocyte parameters associated with metabolic syndrome stratified by sex

Variables	Male		Female	
	OR (95% CI)	P	OR (95% CI)	P
Age	—	0.918	—	0.161
WC	1.149 (1.123~1.176)	<0.001	1.139 (1.119~1.160)	<0.001
SBP	1.027 (1.015~1.039)	<0.001	1.044 (1.035~1.054)	<0.001
DBP	1.024 (1.006~1.042)	0.007	1.024 (1.009~1.040)	0.002
TG	3.401 (2.844~4.068)	<0.001	1.538 (1.287~1.838)	<0.001
HDL-C	0.026 (0.013~0.054)	<0.001	0.001 (0.001~0.003)	<0.001
FPG	1.551 (1.415~1.700)	<0.001	2.214 (1.832~2.676)	<0.001
ALT	—	0.249	—	0.702
AST	—	0.163	—	—
GGT	—	0.230	1.006 (1.001~1.010)	0.014
BMI	—	0.627	—	0.747
TC	—	—	1.274 (1.115~1.457)	<0.001
LDL-C	—	—	—	0.776
UA	—	0.272	—	0.156
WBC	—	0.205	—	0.202
PLT	—	0.088	—	0.636

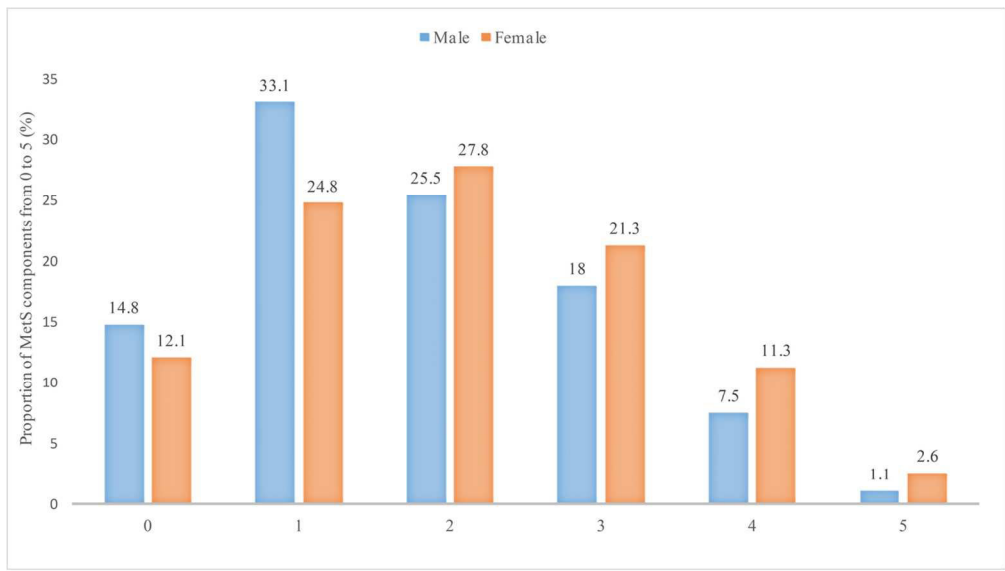
1					
2					
3	HbA1c	—	0.189	0.761 (0.584~0.992)	0.044
4					
5	RBC				
6					
7	Q1	Reference		Reference	
8					
9	Q2	—	0.148	1.129 (0.792~1.610)	0.502
10					
11	Q3	—	0.099	1.785 (1.248~2.554)	0.002
12					
13	HCT				
14					
15	Q1	Reference		Reference	
16					
17	Q2	—	0.134	1.738 (1.229~2.458)	0.002
18					
19	Q3	—	0.268	1.922 (1.337~2.761)	<0.001
20					
21	Hb				
22					
23	Q1	Reference		Reference	
24					
25	Q2	1.687 (1.151~2.472)	0.007	—	0.515
26					
27	Q3	2.252 (1.550~3.272)	<0.001	—	0.173
28					
29	RDW				
30					
31	Q1	Reference		Reference	
32					
33	Q2	1.110 (0.758~1.625)	0.593	—	0.068
34					
35	Q3	2.750 (1.939~3.899)	<0.001	—	0.398

OR, odds ratio; CI, confidence interval; Statistical analysis by binary logistic regression with adjustments for potential confounders (the statistically significant variables in table 1, Male: adjusted for age, WC [waist circumference], SBP [systolic blood pressure], DBP [diastolic blood pressure], TG [triglyceride], HDL-C [high-density lipoprotein cholesterol], FPG [fasting plasma glucose], ALT [alanine transaminase], AST [aspartate aminotransferase], GGT [γ -glutamyltransferase], BMI [body mass index], UA [uric acid], WBC [white blood cell], PLT [platelet] and HbA1c [glycated hemoglobin A1c]; Female: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, GGT, BMI, TC [total cholesterol], LDL-C [low-density lipoprotein cholesterol], UA, WBC, PLT and HbA1c); RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin; RDW, red blood cell distribution width.

For peer review only

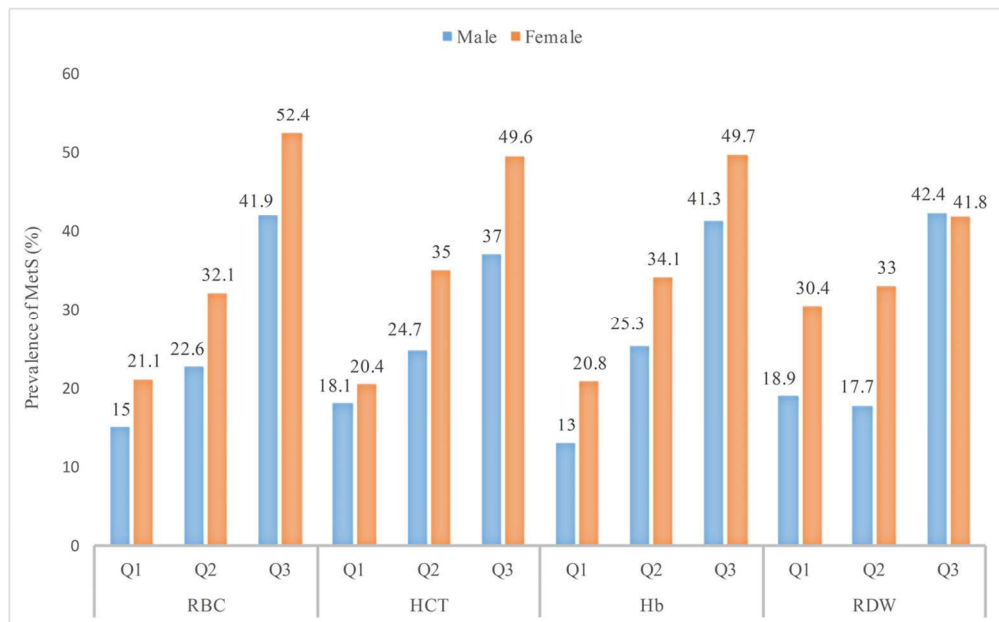
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



119x67mm (300 x 300 DPI)

Review only



118x73mm (300 x 300 DPI)

Review only

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (Page 1) (b) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 4)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 4)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 4)
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants (Page 4-5)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 5-6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 5)
Bias	9	Describe any efforts to address potential sources of bias (Page 5)
Study size	10	Explain how the study size was arrived at (Page 4-5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (5-6)
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (Page 6) (b) Describe any methods used to examine subgroups and interactions (Page 6) (c) Explain how missing data were addressed (Page 4-5) (d) If applicable, describe analytical methods taking account of sampling strategy (not applicable) (e) Describe any sensitivity analyses (Page 6)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Table 1) (b) Give reasons for non-participation at each stage (not applicable) (c) Consider use of a flow diagram (Not applicable)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 6-7, Table 1-2, Figure 1-2) (b) Indicate number of participants with missing data for each variable of interest (Not applicable)
Outcome data	15*	Report numbers of outcome events or summary measures (Page 6, Table 1)
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were

		adjusted for and why they were included (Page 7, Table 3)
		(b) Report category boundaries when continuous variables were categorized (Page 5)
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not applicable)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 7)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 10-11)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 8-10)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 11)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 11)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association of erythrocyte parameters with metabolic syndrome in the Pearl River Delta region of China: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019792.R2
Article Type:	Research
Date Submitted by the Author:	14-Nov-2017
Complete List of Authors:	Huang, LingLing; Institute of Chronic Disease Risks Assessment, Henan University Dou, Dong-Mei; Institute of Chronic Disease Risks Assessment, Henan University Liu, Nan; Guangzhou Medical University, School of Public Health Wang, XiaoXiao; Institute of Chronic Disease Risks Assessment, Henan University Fu, Li-Ying; Institute of Chronic Disease Risks Assessment, Henan University Wu, Xiao; Institute of Chronic Disease Risks Assessment, Henan University wang, peixi; Department of Preventive Medicine, School of Public Health, Guangzhou Medical University,
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Public health, Epidemiology
Keywords:	Erythrocyte parameters, Metabolic syndrome, Cross-sectional study, China

SCHOLARONE™
Manuscripts

1
2
3 **Association of erythrocyte parameters with metabolic syndrome in the Pearl River Delta region**
4 **of China: a cross-sectional study**
5
6
7

8 Ling-Ling Huang ¹⁺, Dong-Mei Dou ¹⁺, Nan Liu ², Xiao-Xiao Wang ¹, Li-Ying Fu ¹, Xiao Wu ¹, Pei-Xi
9 Wang ^{1,2*}
10

11
12 ¹Institute of Chronic Disease Risks Assessment, Henan University, Kaifeng, 475004,
13 China;
14

15
16 ²School of Public Health, Guangzhou Medical University, Guangzhou, PR China
17

18
19 E-Mails: HuangLingLing0703@163.com (L.-L.H.); doudongmei1224@126.com (D.-M.D.);
20 LNQ555@126.com (N. L.); xiaoxiao52625@163.com (X.-X.W.); 18317856338@163.com (L-Y. F);
21 18625832936@163.com (X. W); peixi001@163.com (P-X. W).
22
23
24
25

26
27 *Corresponding author to Pei-Xi Wang, tel: -8618927539896, e-mail:peixi001@163.com
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Objective: Increasing studies have reported that erythrocyte parameters, including red blood cell (RBC), hematocrit (HCT), hemoglobin (Hb), and red blood cell distribution width (RDW), are associated with metabolic syndrome (MetS) in adults worldwide. However, the association, stratified by sex, remains to be elucidated, particularly in the Pearl River Delta region of China. Therefore, our aim was to explore the association of erythrocyte parameters with MetS, stratified by sex in the Pearl River Delta region of China.

Methods: In this cross-sectional study, 2161 males and 2511 females were enrolled. MetS was diagnosed using a modified version of the Adult Treatment Panel III (ATP III) criteria. Logistic regression analyses were performed to calculate adjusted odds ratios (ORs) of erythrocyte parameters associated with MetS stratified by sex.

Results: The prevalence of MetS was higher in females than that in males (35.2% vs.26.7%). RBC, HCT, Hb and RDW values increased linearly with the number of MetS components from zero to five identified in both males and females. Among males, the ORs of MetS risk increased across the tertiles of Hb (Q2: OR=1.921, 95% confidence interval [CI]=1.170~3.151; Q3: OR=1.992, 95%CI=1.198~3.312). Males in the highest tertiles of RDW had a 2.752-fold increased risk of suffering from MetS compared to those in the reference group. Among females, the ORs of MetS risk also increased across the tertiles of Hb (Q2: OR=1.538, 95%CI=1.008~2.348; Q3: OR=1.665, 95%CI=1.075~2.578). Females in the highest tertiles of RBC had a 1.718-fold increased risk of experiencing MetS compared to those in the reference group.

Conclusions: MetS was more prevalent in females than that in males. The association between erythrocyte parameters and MetS differed between sex, whereby RBC and Hb were identified as the risk factors of MetS in females and Hb and RDW as the risk factors in males.

Keywords: Erythrocyte parameters, Metabolic syndrome, Cross-sectional study, China

Strengths and limitations of this study

- A large sample of subjects was enrolled in our survey.
- To the best of our knowledge, this is the first study to report the association of erythrocyte parameters with MetS, stratified by sex in the Pearl River Delta region of China.
- The present study was designed as a cross-sectional study; therefore, direct causation may not be concluded from the results.
- Supplementary information about the lifestyle of the subjects was not collected; therefore, these factors could not be included in the adjustments of our multivariate logistic regression analyses.

Introduction

Metabolic syndrome (MetS) is defined as a cluster of multiple correlated metabolic features, including abdominal obesity, hypertension, elevated triglyceride (TG) levels, decreased high-density lipoprotein cholesterol (HDL-C) levels, and hyperglycemia [1]. It is known to be strongly associated with an increased risk of type 2 diabetes [2], cardiovascular disease (CVD) [2-4], and all-cause mortality [4]. In recent years, MetS has emerged as a global public health issue owing to its increased prevalence around the world, affecting nearly 20-30% of adults in many countries [5-7]. Hence, early identification of individuals at high risk of MetS is essential for the prevention of MetS.

Currently, the pathogenesis of MetS is not clearly understood. Generally, MetS is often accompanied by insulin resistance and/or chronic low-grade inflammation [8-9]. Numerous investigators previously reported that erythrocyte parameters, including red blood cell count (RBC), hematocrit (HCT), hemoglobin (Hb) and red blood cell distribution width (RDW) were positively associated with insulin resistance and chronic low-grade inflammation [10-14]. In fact, RBC [14-16], HCT [15-16], Hb [14-15, 17] and RDW [18] were demonstrated in several studies worldwide to correlate with MetS in adults. However, the association between erythrocyte parameters and MetS remains controversial, because the results reported are inconsistent depending on the different ethnic populations studied. In addition, discrepancies in the results may be partly attributed to differences between sexes. Many studies simply applied sex as an adjustment variable to investigate the relationship between erythrocyte parameters and MetS, and no studies were conducted in the Pearl River Delta region of China. Therefore, the aim of this study was to explore the association between erythrocyte parameters and MetS stratified by sex in the Pearl River Delta region of China.

Materials and Methods

Study participants

This cross-sectional study involved participants who underwent a general health examination at the Community Health Service Agencies in the Pearl River Delta region of China in 2015. The health examination included recording of medical history, anthropometric measurements, and laboratory tests. Participants with a history of cardiovascular diseases, severe liver and kidney dysfunction, tumors, and severe inflammatory diseases were excluded. In addition, participants who did not have complete data on their MetS components and erythrocyte parameters were excluded. Altogether, a total of 4672

1
2
3 subjects (2161 males and 2511 females) were enrolled in this study. The study was approved by the
4
5 Ethics Committee of Guangdong Sociological Society. Written informed consent was obtained from all
6
7 participants.
8
9

10 11 **Data collection and measurements**

12 The medical history of subjects was obtained by review of self-reported questionnaires. Anthropometric
13 parameters were measured by trained staff, following a standardized protocol. Height, weight, waist
14 circumference (WC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured
15 in replicate, and all the mean values of the above indexes were calculated. Then, body mass index (BMI)
16 was calculated as weight in kilograms divided by height in meters squared. After an overnight fast,
17 venous blood samples from participants were obtained and analysed for TG, total cholesterol (TC),
18 HDL-C, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), uric acid (UA),
19 white blood cell (WBC), platelet (PLT), RBC, HCT, Hb, RDW, alanine transaminase (ALT), aspartate
20 aminotransferase (AST), γ -glutamyl transferase (GGT), albumin (ALB) and glycated hemoglobin A1c
21 (HbA1c).
22
23
24
25

26 27 **Quality control**

28 All data are collected by trained doctors or nurses. They are strict in checking the data of every
29 participant. In addition, several supervisors were arranged to verify the authenticity of the data.
30
31

32 33 **Tertiles of erythrocyte parameters levels**

34 Erythrocyte parameters levels were categorized into tertiles on the basis of individual distributions for
35 males and females, respectively (in males: RBC, $Q1 < 4.37 \times 10^{12}/L$, $Q2 = 4.37 \sim 4.75 \times 10^{12}/L$, $Q3 \geq 4.76$
36 $\times 10^{12}/L$; HCT, $Q1 < 39.8\%$, $Q2 = 39.8 \sim 42.4\%$, $Q3 \geq 42.5\%$; Hb, $Q1 < 137$ g/L, $Q2 = 137 \sim 146$ g/L, $Q3 \geq 147$
37 g/L; RDW, $Q1 < 12.5\%$, $Q2 = 12.5 \sim 13.1\%$, $Q3 \geq 13.2\%$; in females: RBC, $Q1 < 3.96 \times 10^{12}/L$,
38 $Q2 = 3.96 \sim 4.27 \times 10^{12}/L$, $Q3 \geq 4.28 \times 10^{12}/L$; HCT, $Q1 < 35.2\%$, $Q2 = 35.2 \sim 37.3\%$, $Q3 \geq 37.4\%$; Hb, $Q1 < 120$
39 g/L, $Q2 = 120 \sim 127$ g/L, $Q3 \geq 128$ g/L; RDW, $Q1 < 12.3\%$, $Q2 = 12.3 \sim 12.8\%$, $Q3 \geq 12.9\%$).
40
41
42
43
44
45

46 47 **Definition of metabolic syndrome**

48 MetS was diagnosed using a modified version of the Adult Treatment Panel III (ATP III) criteria [1],
49 which included at least three of the following five components: 1) $WC \geq 90$ cm in males and $WC \geq 80$ cm
50 in females; 2) $SBP \geq 130$ mmHg or $DBP \geq 85$ mmHg; 3) $TG \geq 1.70$ mmol/L; 4) $HDL-C < 1.03$ mmol/L in
51 males and $HDL-C < 1.29$ mmol/L in females; and 5) $FPG \geq 5.6$ mmol/L.
52
53
54
55
56
57

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA). Data is presented as the mean \pm standard deviation or frequency(percentage). The *t*-test was used to evaluate differences in characteristics of study subjects with and without MetS stratified by sex. The χ^2 test was performed to compare the proportion of MetS components, from zero to five, between males and females; and compare the prevalence of MetS dependent on the tertiles of RBC, HCT, Hb, and RDW between males and females, respectively. A one-way ANOVA was conducted to test mean levels of erythrocyte parameters according to the number of MetS components in males and females, separately. Multivariate logistic regression analyses (the enter selection procedure) were performed to calculate adjusted odds ratios (ORs) of erythrocyte parameters associated with MetS stratified by sex with adjustments for potential confounders (the statistically significant variables in table 1, Male: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, AST, GGT, BMI, UA, WBC, PLT and HbA1c; Female: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, GGT, BMI, TC, LDL-C, UA, WBC, PLT and HbA1c). A *P* value <0.05 was considered to be statistically significant.

Results

Prevalence of MetS

In total, there were 2161 males and 2511 females enrolled in this study. Of the subjects, 576 males (26.7%) and 885 females (35.2%) were diagnosed with MetS.

Characteristics of study subjects

In this study, among males, the mean age of the MetS group was significantly lower than that of the non-MetS group, whereas the opposite trend was observed among females ($P<0.001$). In the cluster of MetS components, WC, SBP, DBP, TG, and FPG levels were remarkably greater in the MetS group than in the non-MetS group in both males and females, but HDL-C levels were significantly lower in the MetS group than that those the non-MetS group in both males and females ($P<0.001$). In the cluster of erythrocyte parameters, we found that RBC, HCT, Hb and RDW were significantly higher in the MetS group than those in the non-MetS group in both males and females ($P<0.001$). Additional information about the characteristics of study subjects with and without MetS stratified by sex are presented in Table 1.

Proportion of MetS components

Our results revealed that most males experienced one metabolic disorder, and most females suffered from two metabolic disorders. In addition, the proportion of MetS components from two to five was significantly lower in males than that in females (25.5% vs. 27.8%, 18% vs. 21.3%, 7.5% vs. 11.3%, 1.1% vs. 2.6, respectively). Additional information is shown in Fig 1.

Association of erythrocyte parameters with MetS

This study showed that the levels of RBC, HCT, Hb and RDW clearly increased with number of MetS components from zero to five identified in both males and females ($P<0.001$, shown in Table 2), Figure 2 showed that the prevalence of MetS increased in a dose-dependent manner as the tertiles of RBC, HCT, Hb and RDW levels increased in both males and females. Furthermore, at each tertile of the above-mentioned parameters, the prevalence of MetS was lower in males than that in females, except at the highest tertiles of RDW levels (shown in Fig 2).

Multivariate Logistic regression analysis model

Adjusted ORs of MetS risk associated with each tertile of RBC, HCT, Hb, and RDW are listed in Table 3. After adjusting for potential confounders (the statistically significant variables in table 1, Male: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, AST, GGT, BMI, UA, WBC, PLT and HbA1c; Female: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, GGT, BMI, TC, LDL-C, UA, WBC, PLT and HbA1c). A significant association of Hb and RDW with MetS was observed in males, but this was not the same for RBC and HCT. The ORs of MetS risk increased across the tertiles of Hb (Q2: OR=1.921, 95% confidence interval [CI]=1.170~3.151; Q3: OR=1.992, 95%CI= 1.198~3.312). Males in the highest tertiles of RDW had a 2.752-fold increased risk of suffering from MetS compared to those in the reference group. Only RBC and Hb levels were observed to associate with MetS in females. The ORs of MetS risk also increased across the tertiles of Hb (Q2: OR=1.538, 95%CI=1.008~2.348; Q3: OR=1.665, 95%CI=1.075~2.578). Females in the highest tertiles of RBC had a 1.718-fold increased risk of experiencing MetS in comparison to those in the reference group.

Discussion

Main findings

The prevalence of MetS was higher in females than that in males (35.2% vs. 26.7%). Levels of RBC,

1
2
3 HCT, Hb and RDW increased linearly with the number of MetS components from zero to five
4 identified in both males and females. The association between erythrocyte parameters and MetS
5 differed between sexes, whereby Hb and RDW were identified as risk factors of MetS in males and
6 RBC and Hb as the risk factors in females.
7
8
9

10 11 12 **Comparisons with Previous Studies** 13

14 Sex has been demonstrated to be a predictive factor for MetS development. Several studies have
15 showed that females have a higher prevalence of MetS than males [19-20]. A large-scale study
16 conducted in Russia reported that the prevalence of MetS diagnosed using the ATP III criteria, was
17 9.5% in men and 23.5% in women [19]; Another study performed in the seven geographical regions of
18 Turkey showed that the prevalence of MetS as determined by the ATP III criteria, was 28% in men and
19 39.6% in women [20]. Our study outcomes are in accordance with these former reports. However, other
20 studies have reported that males have a higher prevalence of MetS than that of females. For example,
21 Tao et al found that the 5-year cumulative incidence of MetS in Beijing adults was 14.22% for males
22 and 7.59% for females [21]; Yang et al revealed that the 5-year cumulative incidence of MetS in
23 Taiwanese adults was 14.95% for males and 9.89% for females [22]. Difference in the findings might
24 be due to a different study design and/or the selected population.
25
26
27
28
29
30
31
32
33

34 It is well known that MetS represents a cluster of simultaneously occurring metabolic
35 abnormalities. In fact, previous studies demonstrated that RBC and Hb levels clearly increased with the
36 numbers of MetS components [16, 23], and this is demonstrated in our outcomes. It has also been
37 shown that a higher number of MetS components is associated with insulin resistance. Based on the
38 facts that levels of RBC, HCT and Hb are significantly associated with insulin resistance [10, 12, 24],
39 we hypothesize that increased levels of erythrocyte parameters tested in this study may be indicative of
40 the development of insulin resistance.
41
42
43
44
45
46

47 Several studies have demonstrated an association between RBC levels and MetS, indicating the
48 RBC variable is a potential hematological marker for early detection of MetS [14-16]. Our results
49 revealed that the highest tertiles of RBC were associated with MetS in females, consistent with a recent
50 study [25]. The pathogenesis of insulin resistance may, in part, be causative of the association between
51 RBC levels and MetS. Aoki et al reported that insulin can stimulate the proliferation and differentiation
52 of erythropoietic cells by binding receptors on the cell surface [26]. It was suggested that insulin and
53
54
55
56
57
58
59
60

1
2
3 insulin growth factors I and II can promote the proliferation and differentiation of erythroid progenitors
4 in human bone marrow and circulation [27-29]. Alternatively, the relationship between RBC levels and
5 MetS may be a result of iron overload. It was reported that iron overload was associated with insulin
6 resistance [30], and excessive body iron storage interfered with insulin-mediated effects, while
7 bloodletting improved insulin sensitivity [31]. Bozzini et al found that iron overload was strongly
8 associated with obesity and dyslipidemia, and serum ferritin tests would help identify a subgroup of
9 individuals at risk for insulin resistance-associated hepatic iron overload.[32]. Additionally, erythrocyte
10 fatty acids maybe another linking factor between RBC levels and MetS. Novgorodtseva et al found that
11 the development of MetS was accompanied by changes to the composition of erythrocyte fatty acids
12 [33]. Zong et al also demonstrated that erythrocyte fatty acids in the de novo lipogenesis pathway were
13 independently associated with an elevated risk of MetS [34]. Fatty acid composition in erythrocytes
14 may affect insulin sensitivity in individuals with MetS. This may be the underlying mechanism linking
15 insulin resistance to changes in fatty acid composition of RBCs in individuals with MetS [35].

16
17
18
19
20
21
22
23
24
25
26
27 Hb, another important erythrocyte parameter, has been reported to be associated with MetS in both
28 cross-sectional and cohort studies [14, 16-17, 36]. An 8-year follow-up cohort study conducted in Japan
29 detected that the highest and third quartiles of Hb concentration were associated with increased risk of
30 MetS incidence compared to the lowest quartiles of Hb concentration in males, but there was no
31 association observed in females [17]. In general, our findings were consistent with those of the
32 previous reports. In our study, the ORs of MetS increased across the successive tertiles of Hb among
33 males; however, no similar trend was observed among females. The following mechanisms may be
34 regarded as the causes of association between Hb and MetS: Hb is a well-known carrier and buffer of
35 nitric oxide (NO), and can regulate the endothelial function of blood vessels by modulating NO levels
36 in blood [37]. Furthermore, Hb and various compounds of NO modulate the affinity between Hb and
37 oxygen in blood, which can lead to vascular endothelial dysfunction [38]. It has been found that
38 vascular endothelial dysfunction was associated with MetS [39-40]. In addition, Hb plays a key role in
39 regulating sCD40L levels [41], and sCD40L has been shown to participate in thrombus formation and
40 inflammation, which is an independent risk factor for atherosclerosis and MetS [42]. Another
41 possibility linking Hb and MetS may be adiponectin. Previous studies showed that higher Hb levels
42 were closely related to lower adiponectin levels [43-44], and lower levels of adiponectin significantly
43 increased the risk for MetS, respectively. Finally, insulin resistance may also be involved in the
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 association between Hb and MetS [8, 12].
4

5 RDW, a common index of routine blood examination, represents a measure of heterogeneity in the
6 size of circulating erythrocytes. A high RDW index indicates greater heterogeneity in size of circulating
7 erythrocytes in a subject. In this study, males in the highest tertiles of RDW (>13.2%) had a 2.75-fold
8 increased risk for MetS. Multiple groups previously showed that elevated RDW was associated with
9 MetS [45-46]. For instance, Laufer and colleagues demonstrated that RDW \geq 14% was independently
10 associated with an increased risk for MetS development [45]; Sanchez-Chaparro and colleagues
11 reported that the highest quartile of RDW (>14%) was remarkably linked with MetS after adjusting for
12 potential confounders [46]. Moreover, a recent study illustrated RDW is a potential metabolic marker
13 for the detection of metabolic diseases [47]. To date, the mechanism of association between RDW and
14 MetS remains unknown; however, chronic inflammation linked to RDW may play an important role.
15 MetS has previously been associated with chronic inflammation [9], and RDW reflects an underlying
16 inflammatory state [13]. Pierce and colleagues have proved that proinflammatory cytokines can inhibit
17 erythropoietin-induced erythrocyte maturation, which may lead to an elevation of RDW [48].
18
19
20
21
22
23
24
25
26
27
28

29 Our study was conducted in the Pearl River Delta region of China, and it may imply that the
30 generalisability of our results is limited to this region. Additionally, participants with a history of
31 cardiovascular diseases, severe liver and kidney dysfunction, tumors, and severe inflammatory diseases
32 were excluded, so our results are not applicable to these subjects.
33
34
35

36 There were several limitations in this study. First, the present study was designed as a
37 cross-sectional study; therefore, direct causation cannot be concluded from the results. Then,
38 supplementary information about the lifestyle of the subjects was not collected; therefore, these factors,
39 such as smoking, physical exercise and dietary, could not be included in the adjustments of our
40 multivariate logistic regression analyses.
41
42
43
44
45
46

47 **Conclusions**

48 In our study, MetS was more prevalent in females than that in males. The association between
49 erythrocyte parameters and MetS differed between sexes, whereby RBC and Hb were identified as the
50 risk factors of MetS in females and Hb and RDW as the risk factors in males. This has important
51 clinical implications for health makers that erythrocyte parameters may serve as effective indices for
52 the early detection of MetS risk and the treatment of MetS on a sex-dependent basis.
53
54
55
56
57
58
59
60

1
2
3
4
5 **Acknowledgments:** We gratefully acknowledge the staff of local Community Health Service Agencies,
6
7 for their kind assistance in data collection.
8
9

10
11 **Contributors:** LLH and PXW conducted the data analyses. LLH, NL, XXW, LYF and XW drafted the
12 manuscript. PXW and DMD finalized the manuscript with input from all authors. All authors
13 contributed to the development of the study framework, interpretation of the results, revisions of
14 successive drafts of the manuscript, and approved the version submitted for publication.
15
16
17

18
19
20 **Funding:** This study was supported by the Guangzhou 121 Talents Program (GZRS-2014-2048), the
21 Science and Technology Program of Guangzhou (201607010136, 201510010109) and the National
22 Science Foundation of China (81402716).
23
24
25

26
27 **Conflicts of Interest:** The authors declare that they have no conflicts of interest.
28
29

30
31 **Ethical approval:** The study was approved by the Ethics Committee of Guangdong Sociological
32 Society.
33
34
35

36
37 **Informed consent:** Informed consent was obtained from all individual participants included in the
38 study.
39
40

41
42 **Data sharing statement:** This database is first used in this study. The database belongs to our team,
43 and if shared, you need to get their permission.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735–52.
2. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009;120:1640-5
3. Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;56:1113-32.
4. Gami AS, Witt BJ, Howard DE, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol* 2007;49:403-14.
5. Miller JM, Kaylor MB, Johannsson M, et al. Prevalence of metabolic syndrome and individual criterion in US adolescents: 2001–2010 National Health and Nutrition Examination Survey. *Metab Syndr Relat Disord* 2014;12:527-32.
6. Peer N, Lombard C, Steyn K, et al. High prevalence of metabolic syndrome in the Black population of Cape Town: The Cardiovascular Risk in Black South Africans (CRIBSA) study. *Eur J Prev Cardiol* 2015;22:1036-42.
7. Lovre D, Mauvais-Jarvis F. Trends in prevalence of the metabolic syndrome. *JAMA* 2015;314(9):950-951. Doi: 10.1001/jama.2015.8625
8. González AS, Guerrero DB, Soto MB, et al. Metabolic syndrome, insulin resistance and the inflammation markers C-reactive protein and ferritin. *Eur J Clin Nutr* 2006;60: 802-9.
9. Musani SK, Vasan RS, Bidulescu A, et al. Aldosterone, C-Reactive Protein, and Plasma B-Type Natriuretic Peptide Are Associated With the Development of Metabolic Syndrome and Longitudinal Changes in Metabolic Syndrome Components: Findings from the Jackson Heart Study. *Diabetes Care* 2013;36:3084-92.
10. Ellinger VC, Carlini LT, Moreira RO, et al. Relation between insulin resistance and hematological

- parameters in a Brazilian sample. *Arq Bras Endocrinol Metabol* 2006;50:114-7.
11. Mardi T, Toker S, Melamed S, et al. Increased erythropoiesis and subclinical inflammation as part of the metabolic syndrome. *Diabetes Res Clin Pr* 2005;69:249-55.
12. Tabara Y, Igase M, Saito I, et al: Association of hematological parameters with insulin resistance, insulin sensitivity, and asymptomatic cerebrovascular damage: The J-SHIP Toon Health Study. *Clin Hemorheol Microcirc* 2013;55: 297-311.
13. Lippi G, Targher G, Montagnana M, et al. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* 2009;133:628-32.
14. Kawamoto R, Tabara Y, Kohara K, et al: Hematological parameters are associated with metabolic syndrome in Japanese community-dwelling persons. *Endocrine* 2013;43:334-41.
15. Wu S, Lin HY, Zhang CQ, et al. Association between erythrocyte parameters and metabolic syndrome in urban Han Chinese: a longitudinal cohort study. *BMC Public Health* 2013;13: 1-8.
16. Nebeck K, Gelaye B, Lemma S, et al. Hematological parameters and metabolic syndrome: Findings from an occupational cohort in Ethiopia. *Diabetes Metab Syndr* 2012;6:22-7.
17. Hashimoto Y, Tanaka M, Kimura T, et al. Hemoglobin concentration and incident metabolic syndrome: a population-based large-scale cohort study. *Endocrine* 2015;50:390-6.
18. Vayá A, Carmona P, Badia N, et al. Association between high red blood cell distribution width and metabolic syndrome. Influence of abdominal obesity. *Clin Hemorheol Micro* 2011;47: 75-7.
19. Sidorenkov O, Nilssen O, Grjibovski AM. Metabolic syndrome in Russian adults associated factors and mortality from cardiovascular diseases and all causes. *BMC Public Health* 2010;10:1-10.
20. Kozan O, Oguz A, Abaci A, et al. Prevalence of the metabolic syndrome among Turkish adults. *Et al* 2007;61:548-53.
21. Tao LX, Li X, Zhu HP, et al. Association of hematological parameters with metabolic syndrome in Beijing adults population: a longitudinal study. *Endocrine* 2014;46:483.
22. Yang X, Tao F, Sun S, et al. The impact of socioeconomic status on the incidence of metabolic syndrome in a Taiwanese health screening population. *Int J Public Health* 2012;57:551-9.
23. Wang YY, Lin SY, Liu PH, et al. Association between hematological parameters and metabolic syndrome components in a Chinese population. *J Diabetes Complicat* 2004;18:322-27.
24. Choi KM, Lee J, Kim YH, et al. Relation between insulin resistance and hematological parameters

- in elderly Koreans-Southwest Seoul (SWS) Study. *Diabetes Res Clin Pr* 2003;60:205-12.
25. Wang T, Wang H. Study of the relationship between female metabolic syndrome and its related blood indexes in Guangzhou. *Medical Innovation of China* 2016;13:61-4.
26. Aoki I, Taniyama M, Toyoma K, et al. Stimulatory effects of human insulin on erythroid progenitors (CFU-E and BFU-E) in human CD34 separated bone marrow cells and the relationship between insulin and erythropoietin. *Stem Cells* 1994;12:329-38.
27. Bersch N, Groopman E, Golde DW. Natural and biosynthetic insulin stimulates the growth of human erythroid progenitors in vitro. *J Clin Endocrinol Metab* 1982;55:1209-11.
28. Miyagawa S, Kobayashi M, Konishi N, et al. Insulin and insulin-like growth factor I support the proliferation of erythroid progenitor cells in bone marrow through the sharing of receptors. *Br J Haematol* 2000;109:555-62.
29. Dainiak N, Kreczko S. Interactions of insulin, insulinlike growth factor II, and platelet-derived growth factor in erythropoietic culture. *J Clin Invest* 1985;76:1237-42.
30. Marti'nez-Garcia MA, Luque-Ramirez M, San-Millan JL, et al. Body iron stores and glucose intolerance in premenopausal women: role of hyperandrogenism, insulin resistance, and genomic variants related to inflammation, oxidative stress, and iron metabolism. *Diabetes Care*. 2009;32:1525-30.
31. Fernandez-Real JM, Penarroja G, Castro A, et al. Blood letting in high-ferritin type 2 diabetes: effect on insulin sensitivity and h-cell function. *Diabetes* 2002;51:1000-4.
32. Bozzini C, Girelli D, Olivieri O, et al. Prevalence of body iron excess in the metabolic syndrome. *Diabetes care* 2005;28:2061-3.
33. Novgorodtseva TP, Karaman YK, Zhukova NV, et al. Composition of fatty acids in plasma and erythrocytes and eicosanoids level in patients with metabolic syndrome. *Lipids Health Dis* 2011;10:82.
34. Zong G, Zhu J, Sun L, et al. Associations of erythrocyte fatty acids in the de novo lipogenesis pathway with risk of metabolic syndrome in a cohort study of middle-aged and older Chinese. *Am J Clin Nutr* 2013;98:319-26.
35. Djousse L, Matthan NR, Lichtenstein AH, et al. Red blood cell membrane concentration of cis-palmitoleic and cis-vaccenic acids and risk of coronary heart disease. *Am J Cardiol* 2012;110:539-44.
36. Lohsoonthorn V, Jiamjarasrunsi W, Williams M A. Association of hematological parameters with

1 clustered components of metabolic syndrome among professional and office workers in Bangkok,
2
3 Thailand. *Diabetes Metab Syndr*. 2007;1:143-149.

4
5
6
7 37. Schiffrin EL. Oxidative stress, nitric oxide synthase, and superoxide Dismutase: a matter of
8 imbalance underlies endothelial dysfunction in the human coronary circulation. *Hypertension* 2008;
9 51:31-2.

10
11
12 38. Zinchuk VV, Pronko TP, Lis MA. Blood oxygen transport and endothelial dysfunction in patients
13 with arterial hypertension. *Clin Physiol Funct. Imaging* 2004;24:205-11.

14
15
16 39. Wei Y, Liu G, Yang J, et al. The association between metabolic syndrome and vascular endothelial
17 dysfunction in adolescents. *Exp Ther Med* 2013;5:1663-6.

18
19
20 40. Tsuji S, Node K. Vascular endothelial dysfunction as a mechanistic factor for metabolic syndrome.
21 Nihon Rinsho Japanese. *J Clin Med* 2011;69: 295.

22
23 41. Kutlu M, Sonmez A, Genc H, et al. Relationship between hemoglobin and CD40 ligand in
24 prediabetes. *Clin Invest Med* 2009;32:E244-50.

25
26
27 42. Missiou A, Wolf D, Platzer I, et al. CD40L induces inflammation and adipogenesis in adipose
28 cells--a potential link between metabolic and cardiovascular disease. *Thromb Haemost*
29 2010;103:788-96.

30
31
32 43. Kawamoto R, Tabara Y, Kohara K, et al. Hemoglobin is associated with serum high molecular
33 weight adiponectin in Japanese community-dwelling persons. *J Atheroscler Thromb* 2011;18:182-9.

34
35
36 44. Ali SB, Jemaa R, Ftouhi B, et al. Adiponectin and Metabolic Syndrome in a Tunisian Population.
37 *Inflammation* 2012;35:828-33.

38
39
40 45. Laufer PM, Havakuk O, Finkelstein A, et al. High red blood cell distribution width is associated
41 with the metabolic syndrome. *Clin Hemorheol Micro* 2015;63:1-9.

42
43
44 46. Sanchez-Chaparro MA, Calvo-Bonacho E, Gonzalez-Quintela A, et al. Higher Red Blood Cell
45 Distribution Width Is Associated With the Metabolic Syndrome. *Diabetes Care* 2010;33: e40.

46
47
48 47. Perna S, Peroni G, Monteferrario F, et al. The Role of Red Blood Cell Distribution Width in
49 Metabolic Syndrome. A Cross-Sectional Study in Elderly. *Clin Nutr* 2014;33:S112.

50
51
52 48. Pierce CN, Larson DF. Inflammatory cytokine inhibition of erythropoiesis in patients implanted
53 with a mechanical circulatory assist device. *Perfusion* 2005;20:83-90.

54
55
56 **Figure Legends**

1
2
3 **Figure 1** Proportion of metabolic syndrome (MetS) components from zero to five between males and
4 females.
5

6 **Figure 2** Prevalence of metabolic syndrome (MetS) in association with the tertiles of red blood cell
7 (RBC), hematocrit (HCT), hemoglobin (Hb), and red blood cell distribution width (RDW) in males and
8 females, separately.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

Table 1 Characteristics of study subjects with and without metabolic syndrome stratified by sex

Variables	Male (n=2161)			Female (n=2511)		
	MetS	Non-MetS	<i>P</i>	MetS	Non-MetS	<i>P</i>
MetS status (n, %)	576 (26.7)	1585 (73.3)		885 (35.2)	885 (35.2)	
Age (years)	51.39 ± 12.21	54.61 ± 13.79	<0.001	59.78 ± 12.34	55.70 ± 12.97	<0.001
Components of MetS						
WC (cm)	89.98 ± 6.79	82.40 ± 7.76	<0.001	85.94 ± 7.21	77.59 ± 8.56	<0.001
SBP (mmHg)	134.93 ± 15.20	127.57 ± 16.32	<0.001	136.49 ± 16.42	124.74 ± 18.39	<0.001
DBP (mmHg)	89.24 ± 10.47	82.93 ± 11.09	<0.001	84.13 ± 10.25	78.51 ± 10.53	<0.001
TG (mmol/L)	2.76 ± 1.77	1.29 ± 0.91	<0.001	2.15 ± 1.41	1.20 ± 1.87	<0.001
HDL-C (mmol/L)	1.00 ± 0.47	1.28 ± 0.44	<0.001	1.17 ± 0.22	1.50 ± 0.34	<0.001
FPG (mmol/L)	5.53 ± 2.01	4.87 ± 1.40	<0.001	5.38 ± 1.86	4.71 ± 0.97	<0.001
Erythrocyteparameters						
RBC (×10 ¹² /L)	4.99 ± 0.80	4.53 ± 0.51	<0.001	4.55 ± 0.84	4.10 ± 0.57	<0.001
HCT (%)	42.27 ± 4.09	40.68 ± 3.63	<0.001	37.35 ± 2.80	35.58 ± 2.83	<0.001
Hb (g/L)	147.11 ± 12.57	139.02 ± 12.68	<0.001	129.68 ± 14.45	121.50 ± 11.82	<0.001
RDW (%)	13.33 ± 0.96	12.87 ± 1.21	<0.001	13.18 ± 1.90	12.88 ± 2.27	<0.001
Liver function parameters						
ALT (u/L)	31.44 ± 18.35	26.31 ± 15.52	<0.001	24.09 ± 13.81	21.14 ± 11.79	<0.001
AST (u/L)	26.37 ± 15.87	24.80 ± 10.00	0.026	23.82 ± 8.90	23.27 ± 8.63	0.129
GGT(u/L)	48.73 ± 39.88	36.04 ± 26.83	<0.001	32.00 ± 22.79	26.12 ± 26.03	<0.001
ALB (g/L)	47.36 ± 3.23	47.32 ± 4.07	0.820	47.48 ± 4.32	47.77 ± 12.28	0.484
Other clinical characteristics						
BMI (kg/m ²)	25.90 ± 2.67	23.61 ± 3.04	<0.001	25.21 ± 3.05	22.85 ± 3.17	<0.001
TC (mmol/L)	4.81 ± 0.95	4.73 ± 0.94	0.059	5.26 ± 1.08	5.08 ± 1.02	<0.001
LDL-C (mmol/L)	2.65 ± 0.70	2.66 ± 2.06	0.885	2.95 ± 1.48	2.75 ± 1.03	<0.001
UA (umol/L)	415.45 ± 143.27	382.19 ± 84.92	<0.001	340.60 ± 83.08	306.95 ± 101.63	<0.001
WBC (×10 ⁹ /L)	6.95 ± 1.40	6.43 ± 1.40	<0.001	6.41 ± 1.35	5.84 ± 1.31	<0.001
PLT (×10 ⁹ /L)	214.70 ± 49.89	201.57 ± 52.17	<0.001	224.04 ± 53.55	216.73 ± 52.14	0.001

HbA1c (%)	5.79 ± 1.37	5.42 ± 0.97	<0.001	5.64 ± 1.22	5.33 ± 0.67	<0.001
-----------	-------------	-------------	--------	-------------	-------------	--------

Data were presented as mean ± SD or *n* (%); MetS, metabolic syndrome; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin; RDW, red blood cell distribution width; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, γ -glutamyl transferase; ALB, albumin; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; WBC, white blood cell; PLT, platelet; HbA1c, glycated hemoglobin A1c.

Table 2 Levels of erythrocyte parameters of study subjects according to number of metabolic syndrome components in males and females, respectively

Variables	0	1	2	3	4	5	<i>F</i>	<i>P</i>
Male								
RBC	4.44 ± 0.53	4.55 ± 0.50	4.55 ± 0.52	4.80 ± 0.54	5.31 ± 0.86	5.95 ± 1.23	87.448	<0.001
HCT	39.96 ± 3.39	40.76 ± 3.88	41.00 ± 3.38	42.12 ± 4.10	42.33 ± 4.14	44.21 ± 3.01	19.799	<0.001
Hb	131.60 ± 12.35	138.81 ± 12.86	140.71 ± 12.41	144.51 ± 11.65	151.28 ± 12.87	160.04 ± 9.19	52.445	<0.001
RDW	12.75 ± 0.82	12.83 ± 1.55	13.00 ± 0.80	13.24 ± 0.86	13.41 ± 1.07	14.33 ± 1.13	20.264	<0.001
Female								
RBC	4.03 ± 0.39	4.07 ± 0.52	4.16 ± 0.54	4.45 ± 0.82	4.67 ± 0.88	4.83 ± 0.78	66.453	<0.001
HCT	35.16 ± 2.65	35.43 ± 2.78	35.91 ± 2.91	37.07 ± 2.81	37.74 ± 2.79	37.96 ± 2.36	52.237	<0.001
Hb	119.70 ± 11.54	121.28 ± 11.79	122.49 ± 11.88	128.26 ± 14.04	130.04 ± 14.35	139.61 ± 14.46	59.262	<0.001
RDW	12.71 ± 2.10	12.74 ± 1.40	13.07 ± 2.87	13.11 ± 1.39	13.25 ± 2.70	13.38 ± 1.13	4.493	<0.001

RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin; RDW, red blood cell distribution width.

Table 3 Odds ratios of erythrocyte parameters associated with metabolic syndrome stratified by sex

Variables	Male		Female	
	<i>OR</i> (95% <i>CI</i>)	<i>P</i>	<i>OR</i> (95% <i>CI</i>)	<i>P</i>
Age	0.999 (0.986~1.013)	0.930	1.012 (0.998~1.025)	0.088
WC	1.157 (1.121~1.194)	<0.001	1.130 (1.103~1.158)	<0.001
SBP	1.025 (1.012~1.039)	<0.001	1.041 (1.031~1.051)	<0.001

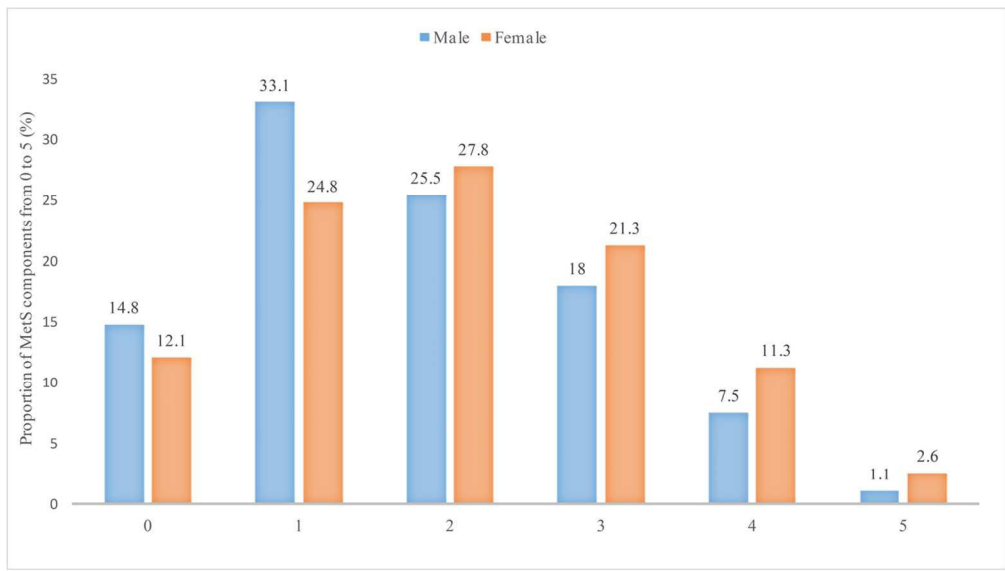
1					
2					
3	DBP	1.025 (1.006~1.045)	0.011	1.030 (1.013~1.047)	0.001
4					
5	TG	3.240 (2.697~3.893)	<0.001	1.518 (1.269~1.817)	<0.001
6					
7	HDL-C	0.026 (0.012~0.054)	<0.001	0.001 (0.001~0.003)	<0.001
8					
9	FPG	1.725 (1.410~2.111)	<0.001	2.221 (1.836~2.687)	<0.001
10					
11	ALT	0.996 (0.980~1.012)	0.620	1.002 (0.990~1.014)	0.757
12					
13	AST	1.016 (0.988~1.045)	0.260		
14					
15	GGT	1.001 (0.997~1.006)	0.492	1.005 (1.000~1.010)	0.064
16					
17	BMI	0.976 (0.907~1.049)	0.506	1.015 (0.958~1.076)	0.620
18					
19	TC			1.243 (1.061~1.455)	0.007
20					
21	LDL-C			0.992 (0.903~1.090)	0.867
22					
23	UA	1.001 (0.999~1.003)	0.291	1.001 (0.999~1.002)	0.303
24					
25	WBC	1.044 (0.935~1.165)	0.447	1.063 (0.959~1.178)	0.248
26					
27	PLT	1.002 (0.999~1.005)	0.202	1.001 (0.998~1.003)	0.639
28					
29	HbA1c	0.856 (0.649~1.130)	0.273	0.747 (0.572~0.976)	0.032
30					
31	RBC				
32	Q1	Reference		Reference	
33	Q2	0.940 (0.613~1.443)	0.779	1.070 (0.743~1.541)	0.716
34	Q3	1.207 (0.771~1.889)	0.410	1.718 (1.173~2.515)	0.005
35					
36	HCT				
37	Q1	Reference		Reference	
38	Q2	0.771 (0.482~1.234)	0.279	1.419 (0.933~2.159)	0.102
39	Q3	0.968 (0.606~1.547)	0.893	1.407 (0.896~2.208)	0.138
40					
41	Hb				
42	Q1	Reference		Reference	
43	Q2	1.921 (1.170~3.151)	0.010	1.538 (1.008~2.348)	0.046
44	Q3	1.992 (1.198~3.312)	0.008	1.665 (1.075~2.578)	0.022
45					
46	RDW				
47	Q1	Reference		Reference	
48	Q2	1.114 (0.757~1.639)	0.583	0.787 (0.571~1.085)	0.144
49					
50					
51					
52					
53					
54					
55					
56					
57					
58					
59					
60					

Q3	2.725 (1.915~3.878)	<0.001	1.057 (0.753~1.484)	0.750
----	---------------------	------------------	---------------------	-------

OR, odds ratio; CI, confidence interval; Statistical analysis by binary logistic regression with adjustments for potential confounders (the statistically significant variables in table 1, Male: adjusted for age, WC [waist circumference], SBP [systolic blood pressure], DBP [diastolic blood pressure], TG [triglyceride], HDL-C [high-density lipoprotein cholesterol], FPG [fasting plasma glucose], ALT [alanine transaminase], AST [aspartate aminotransferase], GGT [γ -glutamyl transferase], BMI [body mass index], UA [uric acid], WBC [white blood cell], PLT [platelet] and HbA1c [glycated hemoglobin A1c]; Female: adjusted for age, WC, SBP, DBP, TG, HDL-C, FPG, ALT, GGT, BMI, TC [total cholesterol], LDL-C [low-density lipoprotein cholesterol], UA, WBC, PLT and HbA1c); RBC, red blood cell; HCT, hematocrit; Hb, hemoglobin; RDW, red blood cell distribution width.

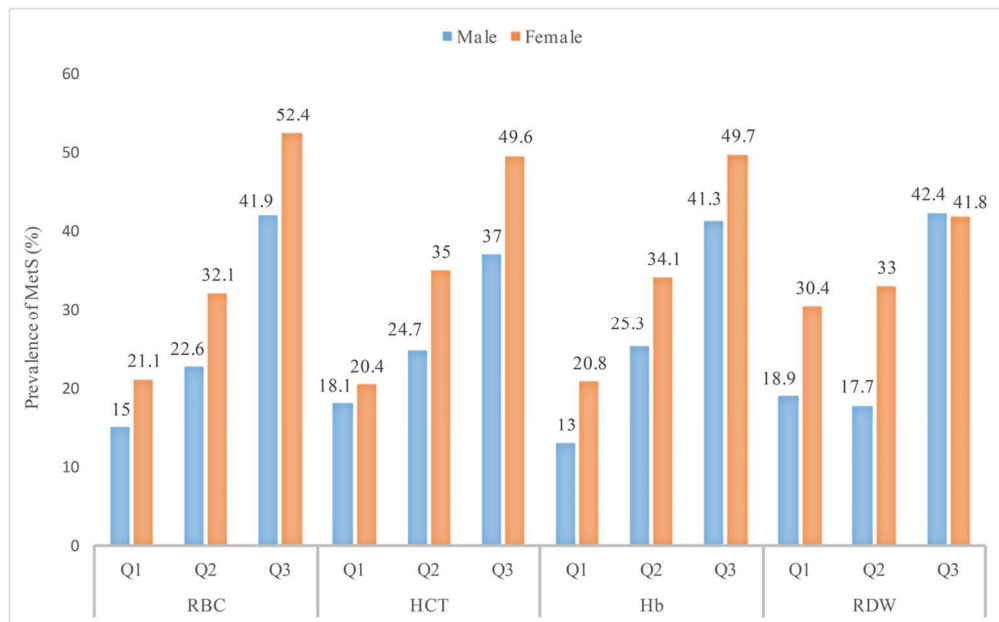
For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



119x67mm (300 x 300 DPI)

Review only



118x73mm (300 x 300 DPI)

Review only