Advance Publication
Ningen Dock International
Accepted for publication: October 29, 2019
Published online: December 20, 2019

Original Article

Ningen Dock International 0000 ; 7 : 00–00

Transitions in Blood Hemoglobin Levels and Platelet Counts in 32–65-year-old Female Examinees Undergoing Periodic Health Check-ups

Atsushi Hioki, Rie Kitayama, Hisakazu Kawai, Makoto Hayashi

Abstract

Objective: Cross-sectional surveys have shown blood hemoglobin (Hb) levels to be low in women in their 30s and 40s. This study investigated secular transitions in blood Hb levels and relevant laboratory data in women aged in their 30s to mid-60s.

Methods: We included the data of 1,370 women who underwent at least six periodic health check-ups, including those for blood Hb level, mean corpuscular volume (MCV), and platelet count. The transitions in and mutual relationships of these data were investigated.

Results: In women who did not receive anemia treatment at any health check-up, blood Hb levels began to decrease when they were in their mid-30s, remained low during their 40s, and increased again in their 50s. This tendency was stronger in women with anemia history than in those without anemia history. MCV decreased and platelet count increased in women in their 40s, but returned to previous levels in their 50s. The average age of menopause during the study period was 50.9 ± 3.1 (SD) years. Platelet count exceeded the reference value in 17.1% of women with blood Hb levels ≤ 11.0 g/dL. A strong negative correlation was observed between individual blood Hb level and platelet count over time, especially with blood Hb level fluctuations ≥ 3 g/dL.

Conclusions: The blood Hb level of women decreased when the women were in their 40s and recovered during their 50s. These changes were small in those without previous anemia history. As the blood Hb level decreased, the proportion of women with platelet counts exceeding the reference value slightly increased.

Keywords female, hemoglobin, platelet, transition

The annual reports of the National Health and Nutrition Survey[1] from 1977 to 2017, and an epidemiological study on health check-up records of healthy women in different age groups[2] showed that blood hemoglobin (Hb) levels were low in Japanese women in their 30s and 40s. This was not observed in men in the same age groups. The mean corpuscular volume (MCV) was low in women in these age groups primarily because of iron deficiency due to menstruation and menorrhagia[2].

Individuals in their 40s and 50s account for a large number of the health check-up examinees in Ningen Dock[3], and it is often necessary to provide proper instruction on anemia prevention or give treatment[4,5]. It is also useful to know the transitions in anemia indicators in these age groups. In addition, since platelet counts increase in patients with iron deficiency anemia[6–8], the present study aimed to clarify the frequency and extent of platelet count increases.

We investigated the secular transitions in anemia-related indicators in the current generation of examinees using time-series data from individuals who continually underwent health check-ups.

Subjects and Methods

Subjects

The study initially enrolled 1,379 women who underwent health check-ups including measurement of Hb, MCV, and platelet count at Ningen Dock and Medical Examination Center, Matsunami Medical Clinic, between April 2008 and March 2019. We included subjects who underwent at least six health check-ups because only 241 subjects underwent all 11 check-ups. Nine women who had previously undergone gastrectomy were excluded from the analysis because it was not possible to confirm whether it was total gastrectomy or not. Those

Ningen Dock and Medical Examination Center, Matsunami Health Promotion Clinic
Contact : Atsushi Hioki, Ningen Dock and Medical Examination Center, Matsunami Health Promotion Clinic, 10 Izumi-machi, Kasamatsu-cho, Gifu 501–6061, Japan. Tel : +81–58-387–2128 ; Fax : +81–58–387–8578 ; E-mail : hioki.a3@mghg.jp

Advance Publication
Ningen Dock International
Accepted for publication: October 29, 2019
Published online: December 20, 2019

undergoing dialysis for chronic renal failure, those receiving anticancer treatment and patients with severe infections or thalassemia had also been excluded. Therefore, the final number of women enrolled was 1,370 and the average number of health check-ups undergone was 8.7 ± 1.7 (SD). The initial mean age of these women at the first check-up was 47.6 ± 6.9 (SD) years.

Methods

Blood Hb level, MCV, platelet count, menstrual condition, and current history of anemia treatment at each check-up were recorded along with the age of each subject and medical history of anemia, uterine leiomyomas (fibroids), endometriosis, and other gynecological diseases. The medical history taken during the interview did not distinguish between renal anemia and other anemia and thus renal anemia may have been included. While most examinees underwent health check-ups at regular intervals, the check-up date sometimes varied between before and after the subject's birthday. Therefore, we defined the age at the first health check-up as the initial age and considered each subject to be 1 year older at the check-up in each year after that. The age-based transitions in blood test values for each subject were determined. Women receiving treatment for anemia during the health check-ups were analyzed separately.

Since serum iron or unsaturated iron-binding capacity (UIBC) measurements had been made for few subjects, we calculated the average values by age group for all examinees in whom they had been measured at our facility between April 2014 and March 2019 to assess the nature of anemia.

We first calculated the mean values of blood Hb level, MCV, and platelet count and the proportion of women with an Hb level < 10 g/dL for each age group of the respective subjects. Among subjects who reported menopause during the study period, we compared blood Hb levels, MCV, and platelet counts before and after menopause. We next investigated relationships among medical history of anemia, uterine leiomyomas, endometriosis, and other gynecological diseases. Among women who had not received anemia therapy at any health check-up, we compared mean blood Hb level and proportion of women with blood Hb level < 10 g/dL at least once, by with or without history of anemia or gynecological diseases. The ages at the first health check-ups were used to compare age differences by medical history among study subjects.

We calculated the mean, fluctuation range, SD, and coefficient of variation for Hb level, MCV, and platelet count for each subject and each age group. We determined the correlation between Hb levels and MCV to assess the involvement of iron deficiency and also that between Hb level and platelet count. These calculations were performed in Microsoft Office Excel 2007 SP3 (Microsoft, Redmond, WA, USA). We obtained intra-individual correlation coefficients between the time courses of Hb, MCV, and platelet count for each subject, as well as the inter-individual correlation coefficients between the mean Hb level, MCV, and platelet count for all subjects. Categorical variables were compared using chi-square or Fisher's exact tests. Comparisons between two groups were performed using paired or unpaired t-tests, and comparisons among more than two groups were performed using analysis of variance with multiple comparisons using the Tukey test. These analyses were conducted using IBM SPSS Statistics, version 24 (IBM Japan, Tokyo).

Before performing this study, the research methods and ethical considerations were approved by the Matsunami General Hospital Medical Ethics Committee, which is under the same medical corporation as our facility. The study was conducted according to the ethical guidelines for medical and health research involving human subjects in Japan.

Results

Of 1,370 women, 100 (7.3%) had received anemia treatment at a health check-up, 330 (24.1%) had not received treatment for anemia but had a history of anemia, and 940 (68.6%) had no history of anemia. The overall averages (± SD) of individual mean blood Hb level, mean MCV, and mean platelet count during the study period were 12.5 (± 1.0) g/dL, 88.2 (± 5.4) fl, and 24.2 (± 5.4) × 10^4 /μL, respectively. Information on the time of the initial anemia diagnosis among women with a history of anemia was limited.

Fig. 1 shows the transitions in blood Hb levels, MCV, and platelet counts from 32 to 65 years of age. In women not receiving anemia treatment during any health check-up, blood Hb levels began to decrease from the mid-30s, decreased to < 12.4 g/dL during their mid-40s and then increased to 13.0 g/dL in their 50s. The tendencies in these changes were more marked in women with a history of anemia and the magnitude of change was very small in women without a history of anemia. The blood Hb levels of women receiving anemia treatment remained < 11.0 g/dL until they reached their late 40s. In women without anemia treatment, MCV decreased and platelet count increased when they were in their 40s and returned to previous levels in their 50s, in parallel with fluctuations in blood Hb levels. In women without anemia history, the variations in MCV and platelet count were small. The average age at anemia onset was 29.2 ± 11.1 (SD) years in 161 subjects with a history of anemia but not currently treated for anemia and was younger than that of 30.6 ± 12.3 (SD) years in 70 subjects currently treated for anemia

Advance Publication
Ningen Dock International
Accepted for publication: October 29, 2019
Published online: December 20, 2019

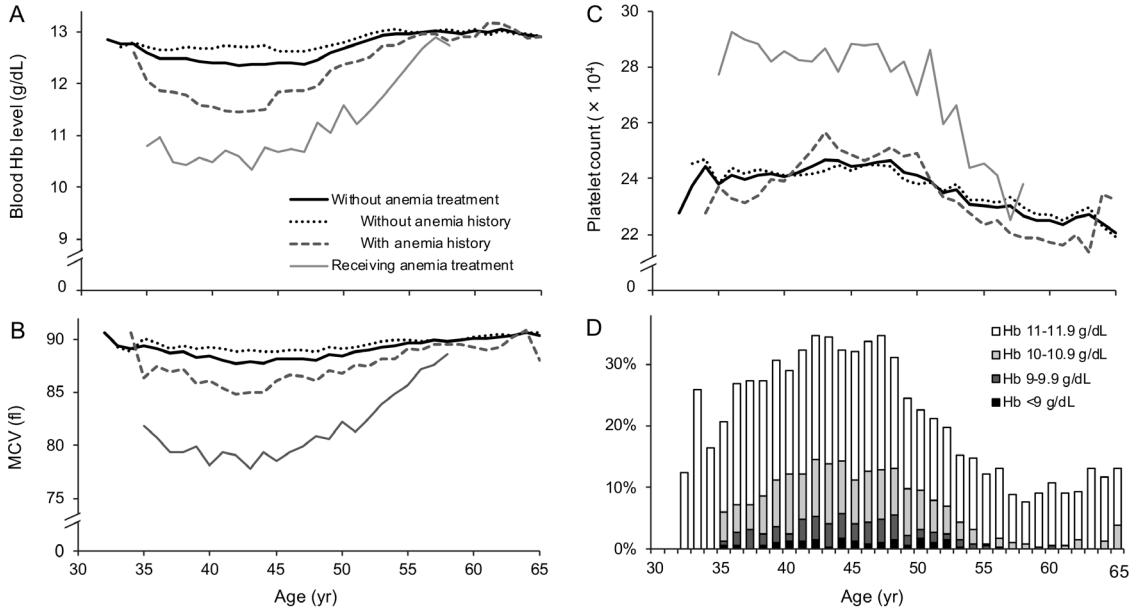

Fig.1. Transitions in blood hemoglobin (Hb) level, mean corpuscular volume (MCV), and platelet count among subjects without anemia treatment at any health check-up (total number = 1,270) and those receiving anemia treatment (total number = 100)
The former was divided into 2 subgroups: those responding that they had no history of anemia (n = 940) and those who had a history of anemia (n = 330). A, blood Hb level; B, MCV; C, platelet count; and D, percentage of subjects with low blood Hb levels among those without anemia treatment.

Table 1. Relationships between Medical Histories of Gynecological Diseases and Anemia

	Number and percentage of subjects without anemia treatment at any health check-up		Number and percentage of subjects with anemia treatment at any health check-up	Total	Chi-square test among groups
	Without medical history of anemia	With medical history of anemia			
Total	940 (68.6%)	330 (24.1%)	100 (7.3%)	1370	
Without history of uterine leiomyoma	750 (71.6%)	244 (23.3%)	54 (5.2%)	1048	$p < 0.001$
With history of uterine leiomyoma	145 (62.0%)	57 (24.4%)	32 (13.7%**)	234	
With history of uterine leiomyoma surgery	45 (51.1%)	29 (33.0%)	14 (15.9%**)	88	
Without history of endometriosis	891 (69.3%)	308 (24.0%)	86 (6.7%)	1285	$p < 0.001$
With history of endometriosis	42 (63.6%)	16 (24.2%)	8 (12.1%)	66	
With history of endometriosis surgery	7 (36.8%)	6 (31.6%)	6 (31.6%**)	19	
Without history of other gynecological diseases	816 (70.4%)	270 (23.3%)	73 (6.3%)	1159	$p = 0.003$
With history of other gynecological diseases	53 (59.6%)	26 (29.2%)	10 (11.2%)	89	
With history of other gynecological surgery	71 (58.2%)	34 (27.9%)	17 (13.9%*)	122	

*$p < 0.01$, **$p < 0.001$ vs. subjects without respective diseases by Fisher's exact test

(p =0.037). The number and mean age of subjects who reported menopause during the study period were 405 and 50.9 ± 3.1 (SD) years, respectively. The mean blood Hb levels increased from 12.7 ± 1.3 (SD) g/dL to 13.0 ± 0.9 (SD) g/dL ($p < 0.001$), while MCV increased from 88.9 ± 5.9 fl to 89.4 ± 4.9 fl ($p < 0.003$) and the platelet count decreased from 23.9 ± 5.5 × 10^4 /μL to 23.1 ± 4.8 × 10^4 /μL ($p < 0.001$) in these women between before and after menopause. The proportion of women with blood Hb levels < 10 g/dL began to increase when the women were in their late 30s, remained high in the 40s and this was followed by a decrease in the early 50s. The highest percentage of women with blood Hb levels < 10 g/dL was 6.1%, at 44 years of age. The highest percentage of women with platelet counts ≥ 40 × 10^4 /μL was 2.0%, at 44 years of age.

The mean (±SD) values of serum iron for all examinees were 111 ± 39, 105 ± 38, 98 ± 47, 105 ± 37, and 108 ± 31 μg/dL for women in their 20s (n = 59), 30s (n = 39), 40s (n = 135), 50s (n = 161), and 60s (n = 156), respectively (not significant). The mean (±SD) UIBC values were 220 ± 24, 219 ± 66, 236 ± 83, 216 ± 63, and 189 ± 49 μg/dL, respectively ($p < 0.001$).

Medical histories of uterine leiomyoma, endometriosis and other gynecological diseases were reported by 23.5%, 6.2%, and 15.4% of the women, respectively. The proportions of women receiving anemia treatment during a health check-up were higher in those with a

Advance Publication
Ningen Dock International
Accepted for publication: October 29, 2019
Published online: December 20, 2019

medical history of uterine leiomyoma, uterine leiomyoma surgery, endometriosis surgery, or other gynecological surgery (**Table 1**).

In women who had not received anemia treatment, the average individual mean Hb level over time was higher in women without anemia history than in women with anemia history (**Table 2**). The Hb level was higher in women who had undergone uterine leiomyoma surgery. Among them, the mean Hb level of 44 women with confirmed total hysterectomy was 13.1 ± 0.9 (SD) g/dL. There was no difference in the average Hb level between those with and without a medical history of endometriosis or other gynecological diseases. The average Hb level was lower and percentage of subjects with blood Hb levels <10.0 g/dL was higher in women with a history of anemia treatment at a health check-up than in women without treatment history.

The individual mean Hb and MCV values over time were positively correlated in subjects without anemia treatment and overall ($r = 0.544$, $p < 0.001$) (**Fig.2**). Individual mean platelet counts tended to be high when the mean blood Hb level was low, but overall, there was a weak, negative correlation between the mean blood Hb level and mean platelet count ($r = -0.235$, $p < 0.001$). The percentage of women with a mean platelet count ≥ 33×10^4 /μL was 4.4% overall compared to 17.1% of women with Hb levels ≤ 11.0 g/dL. The number and percentage of women overall with platelet counts exceeding 40×10^4 /μL were 12 and 0.9%, respectively. In women with blood Hb levels ≤ 11 g/dL, the maximum mean platelet count was close to 50×10^4 /μL. The inter-individual coefficient of variation for each mean Hb

Table 2. Relationships between Medical History of Anemia or Gynecological Diseases and Blood Hemoglobin (Hb) Level

	Number of subjects	Average starting age (y.o.)	SD	Average mean Hb level (g/dL)	SD	Number and percentage of women with Hb level <10 g/dL
Total	1370	43.3	6.9	12.5	1.0	207 (15.1%)
Without anemia treatment	1270	43.4	7.0	12.6	0.9	128 (10.1%)
Without medical history of anemia	940	43.5	7.2	12.8	0.8	34 (3.6%)
With medical history of anemia	330	43.2	6.3	12.1**	1.2	94 (28.5%)
Without history of uterine leiomyoma	994	43.1	7.1	12.6	0.9	99 (10.0%)
With history of uterine leiomyoma	202	44.6*	6.3	12.7**	0.9	23 (11.4%)
With history of uterine leiomyoma surgery	74	44.8	5.8	13.0	0.9	6 (8.1%)
Without history of endometriosis	1199	43.5	7.0	12.6	0.9	120 (10.0%)
With history of endometriosis	58	41.6	6.4	12.7	0.9	6 (10.3%)
With history of endometriosis surgery	13	45.2	6.1	12.7	0.9	2 (15.4%)
Without history of other gynecological disease	1086	43.4	7.0	12.6	0.9	103 (9.5%)
With history of other gynecological disease	79	42.7	6.8	12.5	0.9	11 (13.9%)
With history of other gynecological surgery	105	44.1	5.7	12.7	0.9	14 (13.3%)
With anemia treatment	100	41.1**	5.1	11.0**	1.1	79 (79.0%)

*$p < 0.05$, **$p < 0.001$ vs. subjects without respective disease history by t-test or Tukey test

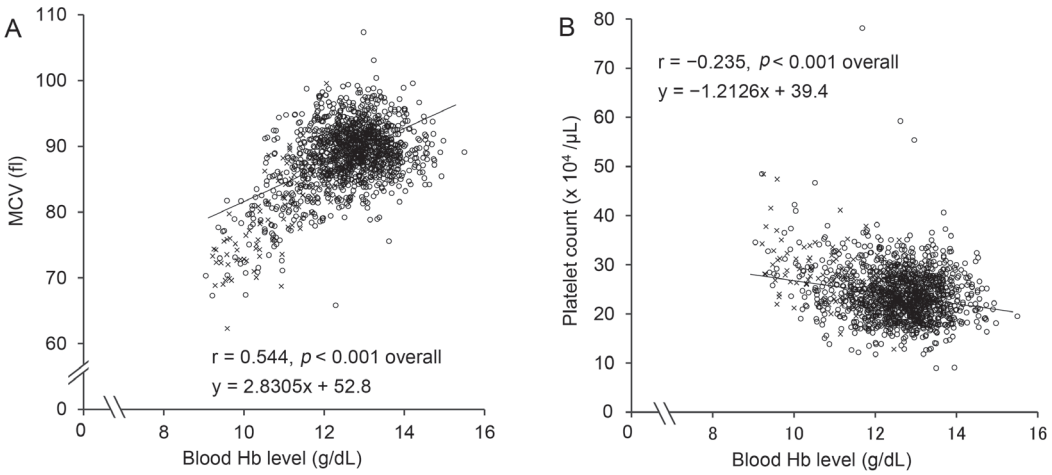

Fig.2. Relationships of individual mean MCV (panel A) or mean platelet count (panel B) with blood Hb level during health check-up period
Circles: subjects without anemia treatment at any health check-up; and crosses: subjects treated for anemia at any health check-up

Advance Publication
Ningen Dock International
Accepted for publication: October 29, 2019
Published online: December 20, 2019

Table 3. Distribution of Correlation Coefficients between Blood Hb Level and MCV or Platelet Count for Each Subject Over Time by Blood Hb Level Fluctuation Ranges in Women without Anemia Treatment

Fluctuation range of Hb	<2 (*n* = 956)	2 – 3 (*n* = 180)	≥ 3 (*n* = 134)	Total (*n* = 1270)
Correlation coefficient between blood Hb level and MCV				
-1.00 – -0.70	42 (4.4%)	4 (2.2%)	0 (0.0%)	46 (3.6%)
-0.69 – -0.40	155 (16.2%)	15 (8.3%)	1 (0.7%)	171 (13.5%)
-0.39 – 0.39	537 (56.2%)	49 (27.2%)	3 (2.2%)	589 (46.4%)
0.40 – 0.69	156 (16.3%)	31 (17.2%)	7 (5.2%)	194 (15.3%)
0.70 – 1.00	66 (6.9%)	81 (45.0%)	123 (91.8%)	270 (21.3%)
Correlation coefficient between blood Hb level and platelet count				
-1.00 – -0.70	32 (3.3%)	32 (17.8%)	102 (76.1%)	166 (13.1%)
-0.69 – -0.40	116 (12.1%)	52 (28.9%)	29 (21.6%)	197 (15.5%)
-0.39 – 0.39	570 (59.6%)	69 (38.3%)	3 (2.2%)	642 (50.6%)
0.40 – 0.69	181 (18.9%)	20 (11.1%)	0 (0.0%)	201 (15.8%)
0.70 – 1.00	57 (6.0%)	7 (3.9%)	0 (0.0%)	64 (5.0%)

level, MCV, and platelet count in all women were 0.08, 0.06 and 0.22, respectively. In women without treatment for anemia, the blood Hb level and MCV during the health check-up were more positively correlated than in those with blood Hb level fluctuations ≥ 2 g/dL in (**Table 3**). In addition, there were stronger negative correlations between blood Hb level and platelet count in women with larger fluctuation ranges, especially for blood Hb fluctuations of ≥3 g/dL. These correlations became stronger after including women receiving anemia treatment.

Discussion

The blood Hb levels of female examinees who were not treated for anemia decreased most when the women were in their 40s and recovered when they were in their menopausal 50s. This tendency was more pronounced among women with a history of anemia. Over 5% of women in their 40s had Hb values <10 g/dL. The MCV was lower among women in their 40s, likely because of iron deficiency[2]. Our findings of tendencies for reduced serum iron levels and higher UIBC among women in their 40s suggest iron deficiency.

The National Health and Nutrition Survey reported low serum ferritin levels and high total iron-binding capacity among women in their 20s, 30s, and 40s[1]. In addition, Japanese women have high rates of iron deficiency anemia[4,9]. For women with low Hb levels, iron-rich food and supplements should be recommended early when they are in their 30s and 40s[4,10,11]. Furthermore, post-health check-up guidance should be carried out by Ningen Dock facilities. Since it is difficult for women to quantitatively evaluate the amount of bleeding during menstruation[12], it is important to consider this blood loss in subjects with low Hb levels and to perform gynecological examinations and give lifestyle guidance. However, 70% of subjects in their 40s had no history of anemia and small decreases in blood Hb levels.

The mean Hb level was low in women with a medical history of uterine leiomyoma and high in those who had undergone surgery for uterine leiomyoma. Among gynecological diseases, it has been suggested that uterine leiomyoma affects anemia. Abnormal uterine bleeding accounts for 20–30% of the etiology of iron deficiency anemia[13]. However, in the present study, we were unable to assess the frequencies and extent of the effects of gynecological diseases on blood Hb levels because this information was not available for each subject.

The mean platelet count during the health check-up period exceeded the reference range ($14.5–32.9 \times 10^4$ /μL) in 17.1% of women with blood Hb levels meeting the criteria for "medical care needed" of ≤ 11.0 g/dL[14]. In this regard, extreme thrombocytosis (> 100×10^4 /μL) has been reported in patients with iron deficiency anemia[7]. Also, reactive thrombocytosis in iron deficiency is caused by increased erythropoietin, while thrombocytosis in chronic inflammation and cancer is caused by other cytokines such as thrombopoietin and interleukin-6[15–17].

In the present study, the inter-individual correlation coefficients between blood Hb level and platelet count were lower than those between blood Hb level and MCV. The most important factor affecting platelet count increase is a decrease in iron saturation in women with iron deficiency anemia[8]. Also, the inter-individual coefficients of variation in mean platelet count for each subject were larger than those for mean Hb or MCV. This might be due to the effects of factors other than iron deficiency anemia on platelet count[6–8]. Platelet count increases in thrombocythemia and iron deficiency anemia and decreases in aplastic anemia, idiopathic thrombocytopenic purpura, and liver cirrhosis[14]. Among 12 subjects with mean platelet counts exceeding 40×10^4 /μL in the present study, one woman with a mean blood Hb level of 11.7 g/dL was diagnosed with essential thrombocythemia. The other 11 subjects

Advance Publication
Ningen Dock International
Accepted for publication: October 29, 2019
Published online: December 20, 2019

reported no medical history affecting the platelet count. We could not analyze the effects of factors other than blood Hb levels on platelet count in this study. Interventions to prevent and treat anemia might decrease the percentage of subjects with platelet counts outside of the reference range.

Limitations

Our study subjects were women who underwent detailed health check-ups so they did not represent the general population. Furthermore, the number of women aged < 35 years was insufficient, and it was not possible to determine transitions in Hb level from adolescence. Regarding changes in anemia indicators, the effects of dietary guidance cannot be ruled out for subjects who were identified as having anemia during the observation period. Finally, clinical history and treatment status were self-reported and might have been inaccurate due to recall bias, etc.

Conclusions

Hb levels in women aged 32–65 years decreased most in the 40s and recovered in the 50s. This tendency was small in those without past anemia history. The average age of menopause during the study period was 50.9 ± 3.1 (SD) years. As the blood Hb level decreased, the proportion of subjects in whom the platelet count exceeded the reference value slightly increased.

Conflicts of Interest

The authors have no conflict of interest to declare.

References

1. National Institute of Health and Nutrition, Japan: Physical status questionnaire: view by survey item, National Health and Nutrition Survey results. http://www.nibiohn.go.jp/eiken/kenkounippon21/en/eiyouchousa/kekka_shintai_chousa_koumoku.html (accessed September 21, 2019).
2. Kusumi E, Shoji M, Endou S, *et al.*: Prevalence of anemia among healthy women in 2 metropolitan areas of Japan. Int J Hematol 2006; 84: 217–219.
3. Committee for Statistical Survey of Ningen Dock Examination: Report on National Aggregate Results of Ningen Dock in 2014. Ningen Dock 2015; 30: 750–762. (in Japanese)
4. Uchida T: II. Iron Deficiency. 1. Prevalence and Pathogenesis of Iron Deficiency in Japan. Nihon Naika Gakkai Zasshi 2010; 99: 1194–1200. (in Japanese)
5. Okada S: II. Iron Deficiency. 5. Treatment of Iron Deficiency Anemia.. Nihon Naika Gakkai Zasshi 2010; 99: 1220–1225. (in Japanese)
6. Akan H, Güven N, Aydogdu I, *et al.*: Thrombopoietic cytokines in patients with iron deficiency anemia with or without thrombocytosis. Acta Haematol 2000; 103: 152–156.
7. Dan K: Thrombocytosis in iron deficiency anemia. Intern Med 2005; 44: 1025–1026.
8. Kadikoylu G, Yavasoglu I, Bolaman Z, *et al.*: Platelet parameters in women with iron deficiency anemia. J Natl Med Assoc 2006; 98: 398–402.
9. Kofune M: 3. Epidemiology and symptoms of iron deficiency, iron deficiency anemia. In: Japanese BioIron Society (ed), Anemia treatment guidelines revised by the proper use of iron. 3rd ed., Kyobunsha, Sapporo, 2015, 9–13. (in Japanese)
10. Cade JE, Moreton JA, O'Hara B, *et al.*: Diet and genetic factors associated with iron status in middle-aged women. Am J Clin Nutr 2005; 82: 813–820.
11. Zimmermann MB, Hurrell RF: Nutritional iron deficiency. Lancet 2007; 370: 511–520.
12. Quinn SD, Higham J: Outcome measures for heavy menstrual bleeding. Womens Health (Lond) 2016; 12: 21–26.
13. Short MW, Domagalski JE: Iron deficiency anemia: evaluation and management. Am Fam Physician 2013; 87: 98–104.
14. Japan Society of Ningen Dock: Blood tests. https://www.ningen-dock.jp/public/inspection/blood#a6 (accessed August 16, 2019) (in Japanese)
15. Akan H, Güven N, Aydogdu I, *et al.*: Thrombopoietic cytokines in patients with iron deficiency anemia with or without thrombocytosis. Acta Haematol 2000; 103: 152–156.
16. Ertenli I, Kiraz S, Oztürk MA, *et al.*: Pathologic thrombopoiesis of rheumatoid arthritis. Rheumatol Int 2003; 23: 49–60.
17. Kowata S, Ishida Y: II. Diagnostic Approach. 3. Thrombocytosis. Nihon Naika Gakkai Zasshi 2007; 96: 1363–1367. (in Japanese)

(Received August 21, 2019 ; Accepted October 29, 2019)