Hematological Parameters, Ferritin and Vitamin B_{12} in Vegetarians

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Abstract

Hematological parameters and serum ferritin were compared between 179 vegetarians and 58 control subjects using Hematology analyzer H_3 and microparticle enzyme immunoassay, respectively. Serum Vitamin B_{12} was also compared between 68 vegetarians and 30 control subjects using microparticle enzyme immunoassay. It was found that hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemogolbin concentration, white blood cells, neutrophils, serum ferritin and serum vitamin B_{12} in vegetarian were significantly lower than control subjects ($P<0.05$). In addition, red cell distribution width and lymphocytes in vegetarians were significantly higher than control subjects ($P<0.05$).

There were 34 cases of iron deficiency in 179 vegetarians (19.%) which can be classified to iron depletion (4 cases), iron deficient erythropoiesis (12 cases) and iron deficiency anemia (18 cases). Vitamin B_{12} deficiency was found in 27 cases of 68 vegetarians (40%).

Key word : Hematological Parameters, Ferritin, Vitamin B_{12}, Vegetarians, Iron Deficiency, B_{12} Deficiency

Iron is an essential micronutrient which has several functions in the body. The main part of the iron in the body is present in the red cells as hemoglobin. In addition, iron is also an important component of myoglobin, heme enzymes, and non heme enzymes[1,2].

Iron deficiency is probably the most frequent nutritional deficiency disorder in the world. Iron deficiency can be classified in three stages as follows ;

First, iron depletion which is diagnosed by the absence of iron stores in reticuloendothelial cells in bone marrow and the low level of serum ferritin.

Second, iron deficient erythropoiesis which is diagnosed by a low level of serum ferritin, serum iron (SI) and a high level of total iron binding capacity (TIBC)

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The last stage is iron deficiency anemia which is diagnosed not only the same as the second stage but also the low level of hemoglobin because of anemia.

Vitamin B_{12} is essential for the conversion of inactive folate to active folate for the production of nucleic acid(3).

It is already known that iron deficiency and B_{12} deficiency can be found in some vegetarians with poor nutrition(4-6).

In Thailand, several studies have been done about iron deficiency and B_{12} deficiency in vegetarians(8-12). However, in the present study, the behavior pattern of eating among vegetarians may change with more knowledge about nutrition.

The purpose of the research is to compare hematological parameters, serum ferritin and serum vitamin B_{12} between vegetarians and control subjects. In addition, correlations of hematological parameters, serum ferritin and serum vitamin B_{12} with duration of practising vegetarianism were also studied.

MATERIAL AND METHOD

Subjects

For the study of hematological parameters and serum ferritin, the subjects were classified as 179 vegeterians (62 males, 117 females) and 58 control subjects (30 males, 28 females).

For the study of serum vitamin B_{12}, the subjects were classified as 68 vegetarians (35 males, 33 females) and 30 control subjects (4 males, 26 females).

All of the control subjects were screened for α - thalassemia by PCR and ß- thalassemia by HPLC. α or ß- thalassemia cases, either diseases or traits were excluded as control subjects. Moreover, those who had anemia and / or a history of hematological diseases were excluded as control subjects. All of the control subjects were healthy and consumed an ordinary Thai diet. The screening of control subjects was performed at the blood disease diagnosis center, Sirikit Research Center, Ramathibodi Hospital.

Questionnaires of demographic data of vegetarians were also studied.

Specimens

All the specimens were taken after 12 hours fasting. The specimens were used as follows;

1. Two ml of EDTA blood was used for complete blood count (CBC).

2. Five ml of clotted blood was used for collection of serum to investigate for serum iron (SI), total iron binding capacity (TIBC), serum ferritin and serum vitamin B_{12}.

Laboratory Techniques

Complete blood count was investigated by Hematology analyzer H_3. (Bayer, Technicon, Tarry Town Newyork) The hematological parameters were hemoglobin (Hb), hematocrit (Hct), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW).

Serum ferritin and serum vitamin B_{12} were investigated by microparticle enzyme immunoassay (MEIA) using an automated IMX and a reagent kit from Abbott Laboratories.

Serum iron (SI) and total iron binding capacity (TIBC) were also investigated if serum ferritin was less than 12 ng/ml to classify the stage of iron deficiency.

Statistical analysis

Non-parametric tests were applied for those parameters which dispersed from normal distribution or whose sample size was less than 30. Differences of medians and ranges of individual parameters of more than two groups were statistically analysed by Kruskal-Wallis test. The Mann-Whitney U- Wilcoxon Rank Sum W test (two tailed) was used to investigate the differences between the two groups. Spherman's rank correlation was calculated for bivarants of hematological parameters. All statistical analysis were computed by Epi 6. Multiple comparison was also analysed if Kruskal-Wallis is significant(7).

RESULTS

Demographic data of vegetarians and control subjects are shown in Table 1. There were 129 lactovegetarians, 25 ovovegetarians and 22 lactoovovegetarians. The duration of practising vegetarianism ranged from one year to forty years.

For comparison of hematological para-meters, serum ferritin and serum viamin B_{12} between vegetarians and control subjects, it was found that Hb, Hct, MCH, MCHC, WBC, neutrophils, serum ferritin and serum vitamin B_{12}, in vegetarians were statisitically lower than in the control subjects (P

Table 1. Demographic data of vegetarians and control subjects.

Demographic data		Vegetarians (n=179)		Control subjects (n=58)	
Sex	male	62	(34.6%)	30	(51.7%)
	female	117	(65.4%)	28	(48.3%)
Ages (year)	18-30	35	(19.5%)	30	(51.7%)
	30-40	59	(33%)	19	(32.8%)
	> 40	85	(47.5%)	9	(15.5%)
Types of vegetarians					
	Lactovegtarians	132	(73.7%)		
	Ovovegetarians	25	(14%)		
	Lactoovovegtarians	22	(12.3%)		
Duration of practising vegetarianism		13*	(1-40)**		
	1-5 years	32	(18.5%)		
	6-10 years	32	(18.5%)		
	11-15 years	56	(32.4%)		
	16-20 years	36	(20.8%)		
	21-40 years	17	(9.8%)		

* mean
** ranges

Table 2. Median and ranges of hematological parameters serum ferriritin and serum vitamin B_{12} in vegetarians.

Hematological parameters	Vegetarians (n=179)	Control subjects (n=68)	P value
Hb (g/dl)	12.7* (7.1-16.7)	14 (10.5-16.8)	<0.001
Hct (%)	40.6* (21.5-51.4)	42.7 (31.8-50.8)	0.007
MCV (fl)	92.3 (58-107.4)	92.25 (51.7-103.8)	0.83
MCH (pg)	28.8* (18.4-33.3)	30.15 (22.3-33.9)	<0.001
MCHC (g/dl)	31.2* (29.4-34.3)	32.2 (28.9-36.1)	<0.001
RDW (%)	14.4* (12.7-23.7)	13.5 (12.3-20.1)	<0.001
RBC ($x10^{12}$/l)	4.46 (3.3-6.68)	4.6 (3.53-6.54)	0.24
WBC ($x10^9$/l)	5.57* (2.49-11.53)	7.41 (6.25-11.1)	<0.001
Plt ($x10^9$/l)	251.5 (102-367)	245 (28-567)	0.702
Neutrophil (%)	53.4* (27.2-78.5)	58.2 (41.4-77.9)	<0.001
Lymphocytes (%)	33.8* (14.2-55.2)	27.8 (12.7-46.9)	<0.001
Ferritin (ng/ml)	24.3* (3.41-563.8)	77.08 (12.68-457.35)	<0.001
Vitamin B_{12} (pg/ml)**	202* (75-2000)	470.5 (183-1432)	<0.001

* statistically significant differences from control subjects
Mann-Whitney U Wilkoxon Rank Sum W test (two tailed)
** for vitamin B_{12}, there were 68 cases of vegetarians and 30 cases of control subjects

<0.05). In addition, RDW and lymphocytes in vegetarians were statistically higher than the control subjects (P<0.05) as shown in Table 2.

RBC, and platelets were not statistically significantly different between vegetarians and control subjects (P>0.05), as shown in Table 2. In addition, monocytes and basophils in vegetarians were not statistically significantly different from the control subjects. (p>0.05) (data not shown)

Hematolgical parameters, serum ferritin and serum vitamin B_{12} were also compared in vegetarians with different years of practising vegetarianism as shown in Table 3. It was found that there were no statistically significant differences in all hematological parameters, serum ferritin and serum vitamin B_{12} among various durations of practising vegetarianism. However, many parameters were statistically significantly different between each group of different duration years of practising vegetarianism and the control subjects, except the group with 21-40 years of practising vegetarianism, as shown in Table 3. It may be the reason that the group with 21-40 years of practising vegetarianism was too small for statistical comparison.

Hematological parameters, serum ferritin and serum B_{12} were not statistically significantly correlated with duration of practising vegetrianism, as shown in Table 4.

Hematological parameters were not stasistically correlated with serum ferritin in 34 cases of iron deficiency. However, high percentages of microcyte and hypochromia were found in some cases of iron deficiency anemia who had a low level of ferritin and hemoglobin. (data not shown)

In addition, hematological parameters were not statistcally correlated with serum vitamin B_{12} in 27 cases of B_{12} deficiency. (data not shown)

In conclusion, there were 34 cases of iron deficiency from 179 vegetarians (19.%) which can be classified into 3 stages of iron deficiency. In addition, there were 27 cases of vitamin B_{12} deficiency from 68 vegetarians (40 %), The classifica-

Table 3. Median and ranges of hematological parameters in control and vegetarians according to the duration of vegetarianism.

Hematological parameters	Control	Duration of practising vegetarianism (years)				
	(n=58)	1-5 (n=32)	6-10 (n=32)	11-15 (n=56)	16-20 (n=36)	21-40 (n=17)
Hemoglobin (g/dl)	14	13	12.6*	12.8*	12.8*	12.7
	(12.8-16.8)	(7.1-16)	(10.1-16.1)	(9.5-16.1)	(10.5-15.3)	(10.2-16.7)
Hct (%)	42.7	41.35	40.65	40.5	40.75	40.1
	(31.8-50.8)	(23.4-50.4)	(31.3-48)	(31.5-51.4)	(34.7-50.1)	(30.7-48.7)
MCV (fl)	92.25	88.4	90.75	92.95	95.3	92.6
	(51.7-103.8)	(62.3-107.4)	(63.7-104.1)	(58-104.3)	(77.4-106.9)	(64.6-103.8)
MCH (pg)	30.15	27.45*	28.25*	28.8*	29.5	29.4
	(22.3-33.9)	(18.9-33.1)	(21-32.4)	(18.4-33.1)	(23.8-33.3)	(21.5-31.7)
MCHC (g/dl)	32.2	31.3	31.25	31.3*	31*	31.3
	(28.9-36.1)	(30.1-32.9)	(29.8-33.5)	(29.8-32.7)	(29.4-33.8)	(29.6-34.3)
RDW (%)	13.5	14.85*	14.65*	14.5*	14.45*	13.9
	(12.3-20.1)	(13.4-23.7)	(13.1-17.1)	(13.0-18.3)	(12.8-17.5)	(13.3-15.6)
RBC ($x10^{12}/l$)	4.595	4.75	4.42	4.47	4.44	4.45
	(3.53-6.54)	(3.3-6.47)	(3.83-5.81)	(3.47-6.68)	(3.85-5.65)	(3.38-5.26)
WBC ($x10^{9}/l$)	7.41	5.74*	5.89*	5.56*	5.64*	5.33
	(2.9-11.13)	(2.85-11.53)	(3.22-9.75)	(2.87-8.32)	(2.49-10.42)	(2.77-7.41)
Plt ($x10^{9}/l$)	251.5	239	257	250	249	256
	(102-367)	(71-432)	(169-382)	(53-567)	(28-358)	(110-306)
Ferritin (ng/ml)	77.08	20.74*	25*	22.67*	26.32*	35.3
	(12.6-457.35)	(3.41-194.8)	(4.7-94.71)	(4.3-563.8)	(5.5-206.4)	(18.8-203.21)
Vitamin B_{12} (pg/ml)	470.5 (n=28)	202* (n=21)	173* (n=8)	181* (n=19)	305.5 (n=12)	233 (n=5)
	(183-1432)	(75-20000)	(128-391)	(109-1163)	(99-1213)	(170-1610)

* statistically significant different from control subject. (p<0.05)
Kruskal Wallis test and multiple comparison.

Table 4. The correlation of hematological parameters, serum ferritin and serum vitamin B_{12} with duration of practicing vegetarianism.

Hematological Parameter		γ
Hb	(g/dl)	-0.04
Hct	(%)	-0.03
ferritin	(ng/ml)	0.10
MCV	(fL)	0.15
MCH	(pg)	0.14
MCHC	(g/dl)	-0.03
RBC	($x10^{12}$/l)	-0.18
WBC	($x10^{9}$/l)	-0.13
Plt	($x10^{9}$/l)	-0.02
RDW	(%)	-0.22
Vitamin B_{12}	(pg/ml)	0.22

tion of these cases is shown in Table 5.

DISCUSSION

From this study, almost all the cases were derived from Santi-Asoke (166 cases). The remainded were derived from Pho Man Arama which is Chinese vegetarians and all of them were male (13 cases). The duration of practising vegetarianism from Santi-Asoke was longer than Pho Man Arama. However, all cases were true vegetarians.

It was found, in this study, that Hb and Hct in vegetarians were statistically lower than the control subjects which is not in accordance with Tungtrongchitr et al[8]. In cases of iron deficiency, the lower Hb concentration may be due to the imbalance of equilibrium between inhibitor and enhancer of iron absorbtion. In Santi-Asoke a high consumption of citrus fruits leads to an excess intake of vitamin C[8]. So, the reason for the imbalance of iron may be due to the high intake of fibre, tannate as well as phytate which inhibits iron absorbtion. Moreover, the lower Hb concentration was seen in some cases of B_{12} deficiency. In these cases, it may be due to the impairment of Hb synthesis because the deficiency in specific enzyme is due to inadequate protein intake of vegetarians[12].

MCH and MCHC were statistically lower than the control subjects because Hb which is the content of red blood cells was low in the vegetarians.

RDW in the vegetarians was statistically higher than the control subjects because marked anisocytosis can be found in both iron deficiency and B_{12} deficiency.

Serum ferritin levels in the vegetarians were statistically lower than the control subjects which is in accordance with previous studies[8-11], it may be due to high intakes of inhibitor of iron absorbtion.

Surprisingly, WBC in the vegetarians was statistically lower than the control subjects, which is not in accordance with previous studies[8-11]. Neutrophils in the vegetarians were statistically lower than the controls but lymphocytes were statistically higher than the controls. These results are not in accordance with the previous study[8] which found that lymphocytes in vegetarians were statistically lower than the control subjects. The result of neutropenia may be the sign of ineffective granulopoiesis in bone marrow which may be due to megaloblastic changes in cases of B_{12} deficiency.

Serum vitamin B_{12} levels in the vegetarians were statistically lower than the control subjects which is in accordance with previous studies [8-12]. There were fewer males than females as control subjects of serum B_{12} in this study (4 males and 26 females). However, the serum vitamin B_{12} levels were compared in the total controls, not seperated by sex. In addition, it was found from previous studies that serum B_{12} levels in male and female controls were not significantly different[8,12].

Tungtrongchitr et al[12] reported that basopilic stippling can be found in most cases of B_{12}

Table 5. The classification of vegetarians in various stages of iron deficiency and B_{12} deficiency.

State of deficiency	Lactovegetarians	Lactoovovegetarians	Vegan
1. Iron depletion (male 1, female 3)	3	-	1
2. Iron deficient erythropoiesis (male 3, female 9)	7	2	3
3. Iron deficiency anemia (male 1, female 17)	10	6	2
4. B_{12} deficiency (male 14, female 13)	19	4	4

deficiency. Unfortunately, we couldn't find any basophilic stippling in B_{12} deficiency, because we used dip-quick which is the rapid staining method instead of the standard method of Wright's stain.

For comparison of hematological parameters, serum ferritin and serum vitamin B_{12} in various groups with different years of practising vegetarianism, no significant differences of all parameters among various groups with different years of practising vegetarianism were found. This result is in accordance with the correlation study, of hematological parameters, serum ferritin and serum vitamin B_{12} with duration of practising vegetarianism, in which no correlation was found.

However, significant differences were found in many parameters between each group of duration years of practising vegetarianism and control subjects. First, Hb levels in groups of more than five years were statistically lower than the control subjects ($p<0.05$). The comparison of MCH was not in accordance with Hb. It may be due to the imprecision of MCH in Technicon H_3 which calculates MCH from Hb and RBC count. MCHC levels after more than 10 years of practising vegetarianism were statistically lower than the control subjects. MCHC results correlated with the Hb level because Technicon H_3 can measure Hb content in RBC directly. MCHC levels were significantly decreased after more than 10 years, while Hb levels were significantly decreased after more than 5 years. This means that Hb is a more sensitve index than MCHC.

Serum ferritin levels in all duration years of practising vegetarianism were lower than the control subjects because ferritin is the most sensitive index for iron deficiency before anemia can be present[2].

RDW levels in vegetarians were statistically higher than the control subjects in all durations of practising vegetarianism because anisocytosis can be found in both B_{12} and iron deficiency.

Serum vitamin B_{12} levels were statistically lower than the controls in those who practised vegetarianism for less than 15 years. This result supports the findings of previous studies that vitamin B_{12} deficiency was mostly found in persons practising vegetarianism for 6-10 years[8,12]. However, we found that a slight increase in serum vitamin B_{12} was observed in vegetarians practising vegetarianism for more than 15 years, rather than 10 years for the previous study. It may be due to the bigger sample size and details of classification in this study. Although dietary intakes of vitamin B_{12} were low, the vegetarians can compensate for this by utilizing vitamin B_{12} produced by their normal gut flora. Adults who choose a strict vegetarianism diet lacking in vitamin B_{12} generally have enough vitamin B_{12} stored in their liver to supply vitamin B_{12} for 5-6 years before deficiency symptoms set in[10]. A high serum vitamin B_{12} deficiency can therefore be observed only in vegetarians who have been practising vegetarianism for >6 years.

WBC in all duration years of practising vegetarianism was statistically lower than the control subjects. The explanation for this in vegetarians is unclear.

From this study, cases of iron deficiency, may be due to the intake of food that suppresses iron absorption (such as tannate or phytate). In cases of B_{12} deficiency, it is probable that they intake insufficient soy bean or soy bean products which are the only major source of vitamin B_{12} in vegetarians.

No correlation was found between hematological parameters and ferritin in 34 cases of iron deficiency because stage 1 (4 cases) and stage 2 (12 cases), with normal red blood cells, were included in these cases. There were only 18 cases of iron deficiency anemia but the number of cases was too small for correlation study. However, high percentages of microcyte and hypochromia were found in some cases with low serum ferritin and hemoglobin.

In cases of vitamin B_{12} deficiency, there was no correlation between hematological parameters and vitamin B_{12} level. It may be that these cases had a mild form of B_{12} deficiency because hemoglobin and per cent macrocyte were normal

From this study we can conclude that the incidence of iron deficiency and B_{12} deficiency was much lower than a previous study[12] because of more advanced knowledge about nutrition in Santi-Asoke. Nevertheless, iron deficiency and B_{12} deficiency was found in some cases.

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พารามิเตอร์ทางโลหิตวิทยา, ระดับของเฟอริติน และวิตะมินบีสิบสองในซีรัมของผู้ที่ทานมังสวิรัติ

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ได้มีการเปรียบเทียบพารามิเตอร์ทางโลหิตวิทยา และระดับของเฟอริตินในซีรัมของผู้ที่ทานมังสวิรัติ 179 ราย เทียบกับคนปกติ 58 ราย โดยใช้เครื่อง Technicon H_3 ในการหาพารามิเตอร์ทางโลหิตวิทยา และใช้วิธี Microparticle enzyme immunoassay ในการหาระดับของเฟอริตินในซีรัม พบว่า ระดับของฮีโมโกลบิน, ฮีมาโตคริต, MCH, MCHC, ค่าเม็ดเลือดขาว, เม็ดเลือดขาวชนิดนิวโตรฟิล, ระดับของเฟอริติน ในผู้ที่ทานมังสวิรัติมีค่าต่ำกว่าคนปกติอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) แต่ RDW และเม็ดเลือดขาวชนิดลิมโฟซัยต์ มีค่าสูงกว่าคนปกติอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) นอกจากนี้ ได้มีการหาระดับของวิตะมินบีสิบสองในซีรัมของผู้ที่ทานมังสวิรัติ 68 ราย เทียบกับคนปกติ 30 ราย โดยวิธี Microparticle enzyme immunoassay พบว่า ระดับของวิตะมินบีสิบสองในผู้ที่ทานมังสวิรัติมีค่าต่ำกว่าคนปกติอย่างมีนัยสำคัญทางสถิติ ($P<0.05$)

จากการศึกษาวิจัยครั้งนี้พบว่าผู้ที่ทานมังสวิรัติที่อยู่ในภาวะขาดเหล็กมีจำนวนทั้งสิ้น 34 ใน 179 ราย (19.%) แบ่งเป็นระยะของการพร่องเหล็กเก็บสะสม 4 ราย, ระยะของการพร่องเหล็กที่จะนำไปใช้ในการสร้างเม็ดเลือดแดง 12 ราย และระยะที่เป็นโลหิตจางเนื่องจากการขาดเหล็ก 18 ราย และพบว่าผู้ที่ทานมังสวิรัติที่อยู่ในภาวะขาดวิตะมินบีสิบสอง มีจำนวนทั้งสิ้น 27 ใน 68 ราย (40%)

คำสำคัญ : พารามิเตอร์ทางโลหิตวิทยา, เฟอริติน, วิตะมินบีสิบสอง, ผู้ที่ทานมังสวิรัติ, ภาวะขาดเหล็ก, ภาวะขาดวิตะมินบีสิบสอง

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