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ORIGINAL PAPER

Chlorella pyrenoidosa Supplementation Reduces the Risk of Anemia, Proteinuria and Edema in Pregnant Women

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Abstract Pregnancy anemia and pregnancy-induced hypertension (PIH) are common and potentially dangerous disorder in human pregnancy, and nutritional status of pregnant women is one of the leading causes. Chlorella contains large quantities of folate, vitamin B-12 and iron, and can help improve anemia and hypertensive disorder. Our objective was to investigate the preventive effects of Chlorella supplement on pregnancy anemia and PIH in Japanese pregnant women. A total of 70 pregnant women were placed into the control group (n=38) or the Chlorella group (n=32). The subjects in the Chlorella group were supplemented daily from 12th–18th wk of gestation until delivery with 6 g of Chlorella supplement. The proportion of anemic (hemoglobin level <11 g/dL) subjects in the Chlorella group were significantly lower compared with the control group at the second and third trimesters. Additionally, in the Chlorella group, the incidences of proteinuria and edema, signs of PIH, were significantly lower during the third trimester. These results suggest that Chlorella supplementation significantly reduces the risk of pregnancy associated anemia, proteinuria and edema. Chlorella supplement may be useful as a resource of natural folate, vitamin B-12 and iron for pregnant women.

Keywords Anemia · *Chlorella pyrenoidosa* · Folate · Iron · Pregnancy-induced hypertension · Vitamin B-12

Abbreviations

IL	interleukin
iNOs	inducible nitric oxide synthetase
NF-κB	nuclear factor of κB
NO	nitric oxide
PIH	pregnancy-induced hypertension
RDA	recommended daily allowance
Th1	T-helper-1
Th2	T-helper-2

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Introduction

The requirements for energy and micronutrients in pregnant women are particularly high because of increased maternal metabolic rate and fetal demands. Deficiency of these nutrients causes various disorders in pregnant women and their fetuses. Pregnancy anemia is a major public health problem, affecting 35–75% of pregnant women in developing countries and about 18% of pregnant women in industrialized countries [1]. In pregnant women, the major factor for anemia is deficiency in nutrients such as iron, vitamin B-12 and folate [2, 3]. Iron and folate requirements during pregnancy are not easily fulfilled through diet alone, thus, many countries recommend that pregnant women consume iron and folic acid supplements [4]. In Japanese women, the frequency of anemia has been estimated at 17–22% [5]. However, in Japan, iron supplementation is not yet a national public health policy. As for folate, perinatal folate status is important for preventing neural tube defects [6]. Although the Japanese Ministry of Health, Labour and Welfare recommend that all women of childbearing age take 400 μg/day of folate through diet and nutritional supplements in 2000, visible achievements have not yet been made [7]. Pregnancy-induced hypertension (PIH) is a significant cause of low birth weight and maternal and fetal/infant morbidity and mortality in many countries. Prediction of PIH was made from signs, such as elevation of blood pressure and incidence of proteinuria or edema. In Japanese pregnant women, the frequency of PIH has been estimated at 4–8% [8]. With regard to prevention of PIH, Hernandez-Diaz et al. [9] reported that folic acid supplementation reduces the risk of gestational hypertension.

Chlorella is a freshwater unicellular green alga. Chlorella contains abundant protein and chlorophyll compared with other plants, and also large quantities of minerals, such as iron and magnesium, and vitamins, such as folate, vitamin B-6 and vitamin B-12. The proteins of Chlorella contain all the essential amino acids required for human growth and health [10]. Chlorella and Chlorella extracts have been reported to exert a variety of effects, including lowering of serum cholesterol [11] and antitumor activity [12]. In addition, several studies have demonstrated that Chlorella intake improved iron deficiency anemia in rats [13], and suppressed elevation of blood pressure in rat models or human subjects [14]. However, there has been, as of yet, few report available on the effects of Chlorella for pregnant women.

Thus, the aim of this study is to evaluate the preventive effects of Chlorella supplement, which is rich in vitamins and minerals, on pregnancy anemia and PIH in pregnant women.

Materials and Methods

Subjects and Protocol

The subjects for the present study were recruited from pregnant women who visited Saiseikai Nara Hospital (Nara, Japan) for prenatal care from January 2001 until April 2002. A total of 70 primiparous women (age: 18–38 years) in their first trimester of pregnancy participated voluntarily. None were taking Chlorella and other supplements for at least 1 year before beginning the study. The purposes and procedures of the study were explained to each subject, and all subjects provided written informed consent. Of these, 32 pregnant women agreed to take Sun Chlorella A tablets (Chlorella group). The subjects in the Chlorella group received 6 g (30 tablets)/day of Chlorella tablets from the 12th–18th wk of gestation (mean ± SD: 13.23±1.61 wk) until delivery. The subjects in the Chlorella group were instructed to ingest 10 tablets three times a day after each meal. Sun Chlorella A tablets (Sun Chlorella Corp., Kyoto, Japan) containing dry *Chlorella pyrenoidosa* powder as the active ingredient were used. The content of Chlorella tablets was as the following (/100 g): water 4.7 g, dietary fiber 9.8 g, protein 57.8 g, lipid 10.4 g, iron 120 mg, magnesium 330 mg, zinc 1.0 mg, vitamin B-6 1.81 mg, vitamin B-12 0.43 mg, folate 2.9 mg and chlorophyll 2.4 g. No restrictions were imposed on the 38 subjects of control

Table 1 General characteristics of the subjects

Characteristics	Control group (n=38)	Chlorella group (n=32)
Age (years)	28.1±4.7[a]	27.9±4.0
Height (cm)	159.3±5.3	158.8±5.5
Pre-pregnancy weight (kg)	52.5±7.3	51.0±7.6
Weight gain[b] (kg)	8.7±3.0	8.6±4.4

[a] Means ± SD

[b] Weight gain until the third trimester

There were no significant differences in age, height, pre-pregnancy weight and weight gain between the control and Chlorella groups, P<0.05 (Mann-Whitney U test)

Table 2 Effects of *Chlorella pyrenoidosa* supplementation on red blood cell count, hemoglobin concentration and hematocrit value in pregnant women

Variable	Control group (n=38)	Chlorella group (n=32)
Red blood cell (×10^4/μL)		
1st trimester	412.8±35.3[a]	410.3±26.3
2nd trimester	352.1±27.3	357.8±23.2
3rd trimester	363.6±31.9	380.6±22.4*
Hemoglobin (g/dL)		
1st trimester	12.8±1.0	12.9±0.7
2nd trimester	11.2±0.8	11.5±0.6
3rd trimester	10.7±1.1	11.3±0.8*
Hematocrit (%)		
1st trimester	38.0±6.3	37.6±2.3
2nd trimester	32.9±2.3	33.6±1.9
3rd trimester	31.7±2.8	33.7±2.2*

All of the data for the first trimester for the Chlorella group were obtained before the subjects had undergone treatment

[a] Means ± SD

*P<0.05 (Mann-Whitney U test)

Table 3 Comparison of hemoglobin concentration between the control and Chlorella groups

Hemoglobin	Control group (*n*=38)			Chlorella group (*n*=32)		
	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
≥ 11 g/dL	36 (94.7%)[b]	24 (63.2%)	15 (39.5%)	32 (100%)	28 (87.5%)	22 (68.8%)
< 11 g/dL[a]	2 (5.3%)	14 (36.8%)	23 (60.5%)	0 (0.0%)	4 (12.5%)*	10 (31.2%)*

All of the data for the first trimester for the Chlorella group were obtained before the subjects had undergone treatment

[a] Based on the World Health Organization cut-off value for hemoglobin of anemia in pregnant women

[b] Number of subjects, with % in parentheses

*P<0.05 (χ^2 test)

group, with the exception that they were asked not to take Chlorella and other supplements for the study period. The present study was conducted in accordance with the basic principle of the Declaration of Helsinki, and the study protocol was reviewed and approved by the Institutional Review Board of Saiseikai Nara Hospital.

Clinical Examinations

Clinical examinations of subjects were done once at the end of each trimester (mean ± SD: 10.15±1.10 wk, 24.12±1.09 wk and 36.06±0.79 wk, respectively) as part of routine antenatal care. Six indexes of anemia were evaluated in the blood samples: red blood cell count (RBC), hemoglobin concentration (HB), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined with an automated hematology analyzer. Four indexes were evaluated in the urinalysis: urine protein (U-P), urine glucose (U-GLU) and pH with test paper method, specific gravity (SG) was measured with the use of refractometry. U-P and U-GLU were qualitatively assessed in the following three grades: negative (−), questionable (±) and positive (+). The hematological examination and the urinalysis were performed in the laboratory of Saiseikai Nara Hospital. In addition, the attending obstetricians spoke to the subjects in order to assess subjective symptoms and side effects, and also measured blood pressure using the sphygmomanometer and diagnosed leg edema. Diagnosis of leg edema was made on the basis of a dimple in the skin that leave after acupressure on the swollen area for a few seconds.

Statistical Analysis

Continuous data are presented as means ± SD, whereas categorical data are presented as frequencies. Below normal HB concentration was defined as <11 g/dL, which is based on World Health Organization recommendation for pregnant women [1]. Comparisons between the groups when using continuous variables were made by use of the Mann-Whitney U tests. The χ^2 test was used to compare categorical variables such as anemic status (HB level< 11 g/dL), proteinuria, glycosuria and leg edema between the groups. P values<0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL).

Table 4 Effects of *Chlorella pyrenoidosa* supplementation on indexes of urinalysis in pregnant women

Variable	Control group (*n*=38)			Chlorella group (*n*=32)		
	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
U-P	2 (5.3%)[a]	4 (10.5%)	9 (23.7%)	0 (0.0%)	2 (6.3%)	2 (6.3%)*
U-GLU	2 (5.3%)[a]	3 (7.9%)	5 (13.2%)	2 (6.3%)	3 (9.4%)	4 (12.5%)
pH	6.3±0.7[b]	6.6±0.8	6.4±0.7	6.3±0.7	6.3±0.5	6.2±0.6
SG	1.021±0.007[b]	1.018±0.007	1.018±0.009	1.021±0.008	1.016±0.007	1.017±0.008

All of the data for the first trimester for the Chlorella group were obtained before the subjects had undergone treatment

U-P urine protein, U-GLU urine glucose, SG specific gravity

U-P and U-GLU were qualitatively assessed in the three grades of negative (−), questionable (±) and positive (+) by an urine test paper

[a] Number of subjects with questionable (±) or positive (+), with % in parentheses

[b] Means ± SD

*P<0.05 (χ^2 test)

Table 5 Effects of *Chlorella pyrenoidosa* supplementation on blood pressure in pregnant women

	Control group (n=38)			Chlorella group (n=32)		
	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
Systole (mmHg)	104.2±11.1[a]	102.5±9.7	108.4±10.9	103.4±8.4	104.2±8.1	106.1±6.3
Diastole (mmHg)	49.4±6.9	51.8±6.1	52.6±8.7	51.6±7.1	51.1±6.8	52.3±6.2

All of the data for the first trimester for the Chlorella group were obtained before the subjects had undergone treatment

[a] Means ± SD. There were no significant differences in systolic and diastolic pressures between the control and Chlorella groups, $P<0.05$ (Mann-Whitney U test)

Results

General characteristics of the subjects are shown in Table 1. No significant differences in age, height, pre-pregnancy body weight and weight gain until the third trimester were observed between the Chlorella group and the control group. The stools of subjects in the Chlorella group displayed green discoloration, but this was due to the excretion of chlorophyll in *C. pyrenoidosa*. No other adverse reactions were observed.

As presented in Table 2, although the initial RBC, HB and HT before starting supplementation were not significantly different between the groups, these anemia indexes in the Chlorella group were significantly higher than in the control group at the third trimester (P=0.015; P=0.008; P= 0.001, respectively). At the first trimester, there was no significant difference in the proportion of anemic (HB level<11 g/dL) subjects between the groups. However, at the second and third trimesters, the Chlorella group had a significant lower proportion of anemic subjects compared with the control group (P=0.020; P=0.015, respectively; Table 3). No significant differences were observed between the groups in MCV, MCH and MCHC.

Table 4 presents the incidence of subjects with positive or questionable results on urine protein test, in the Chlorella group it was significantly lower than in the control group at the third trimester (P=0.046). No significant differences in other indexes of urinalysis were observed between the groups.

As presented in Table 5, no significant differences in systolic and diastolic blood pressure were observed between the Chlorella and control groups on all three occasions.

With regard to leg edema, the incidence at the third trimester was 44.7% among subjects in the control group and 9.4% among subjects in the Chlorella group. The proportion of subjects with leg edema in the Chlorella group was significantly lower than in the control group (P=0.001; Table 6).

Discussion

The results of this study suggested that Chlorella supplementation significantly reduces the risk of pregnancy associated anemia, proteinuria and edema. Furthermore, Chlorella supplement was well tolerated and no side effect was observed. However, we failed to find the effect of Chlorella supplement on blood pressure in pregnant women, because there was no subject with abnormal elevation of blood pressure in both groups. There is a need for further investigation with a larger sample size to aid in evaluating the effects of Chlorella supplement on hypertensive disorder.

In pregnant women, the major factor of anemia is deficiency in nutrients such as iron, folate and vitamin B-12 [2, 3]. In Japan, the recommended daily allowance (RDA) of iron for menstruating women is 10.5 mg/day and that for pregnant women is 19.5 mg/day. However, according to the National Health and Nutrition Examination Survey conducted in 2004, the average iron intake in

Table 6 Effects of *Chlorella pyrenoidosa* supplementation on the occurrence of leg edema in pregnant women

	Control group (n=38)			Chlorella group (n=32)		
	1st trimester	2nd trimester	3rd trimester	1st trimester	2nd trimester	3rd trimester
Leg edema	0 (0.0%)[a]	7 (18.4%)	17 (44.7%)	0 (0.0%)	2 (6.3%)	3 (9.4%)*

All of the data for the first trimester for the Chlorella group were obtained before the subjects had undergone treatment

[a] Number of subjects with leg edema, with % in parentheses

*$P<0.05$ (χ^2 test)

Japanese women was 7.7 mg [15]. The Chlorella tablets used in this study contains 120 mg/100 g of iron, and thus the present dose of 6 g/day provides 7.2 mg of iron. Matsuura et al. [13] reported that the efficacy of a diet containing 5% Chlorella powder (iron contents: 7.4 mg/100 g) was comparable to a commercial complete diet (iron contents: 32.5 mg/100 g) in rats with iron deficiency anemia. Thus, it appears that the bioavailability of iron in Chlorella is very high and ingredients of Chlorella other than iron may play an important role in improving anemia. In the treatment of anemia during pregnancy, Juarez-Vazquez et al. [16] reported that supplementation of iron plus folic acid is more effective than iron supplementation alone. Folate functions in combination with vitamin B-12 in the hematopoietic system, and folate deficiency can lead to anemia [17]. According to the National Health and Nutrition Examination Survey conducted in 2004, the average folate intake of Japanese women in ages from 15 to 49 years was about 258 μg, which is much lower than the RDA [15]. The Chlorella supplement used in the present study contains 2,900 μg/100 g of folate, indicating that the dose of 6 g provides 174 μg of folate, therefore, the use of Chlorella supplement would fulfill the RDA for folate when combined with diet during pregnancy. In particular, the significant point is that Chlorella supplement would be recommended as a resource of natural folate [18, 19].

In the present study, several subjects had mild proteinuria or edema. Chlorella supplementation significantly reduced the incidence of proteinuria and leg edema during the third trimester. Furthermore, in the control group, five of 38 subjects had both proteinuria and edema, in contrast, there was no subject with proteinuria plus edema in the Chlorella group. The mechanism by which *C. pyrenoidosa* has preventive effects on proteinuria and edema have not fully been clarified. However, there are several possible mechanisms. In proteinuria and edema, which are the signs of PIH, endothelial dysfunction is a known risk factor [20]. With regard to the cause of endothelial cell dysfunction in PIH, several studies have suggested the overproduction of nitric oxide (NO) due to the overexpression of inducible nitric oxide synthetase (iNOS) [21]. Park et al. [22] reported that, in RAW 264.7 macrophages stimulated with lipopolysaccharide, Chlorella extract suppressed iNOS expression and NO production through down-regulating NF-κB activity. Therefore, Chlorella supplementation appears to protect endothelial cells against oxidative stress through suppression of NO overproduction. Next, in pregnant women with PIH, the Th1/Th2 balance shifts to Th1 predominant state via the increase in IL-12 production by macrophages [23]. It has been reported that an aqueous extracts of Chlorella induced Th1 cytokine, while markedly increased the production of IL-10, a potent anti-inflammatory regulatory cytokine, in human peripheral blood mononuclear cells [24]. These findings suggest that Chlorella ameliorates Th1 predominant state, thereby prevents endothelial cell dysfunction.

In this study, the Chlorella supplementation was conducted based on the acceptance by pregnant women. This is a potential source of bias. As for this point, as HB levels, which were indexes for anemic status, were similar in both groups at the first trimester, this potential source of bias was not a major concern. Additionally, a potential weakness of this study is a lack of daily diet record to evaluate their intake of iron, folate and other nutrients for the study period. With regard to this point, all subjects received the same counseling about daily diet and risk behaviors in Saiseikai Nara Hospital, thus, it is likely that almost all participants had a normal diet pattern. However, our results should be considered based on these bias. Therefore, further randomized controlled studies are required.

In conclusion, the consumption of *C. pyrenoidosa* supplement was found to result in the significant reduction of the risk of anemia, proteinuria and edema in pregnant women. It appears that the beneficial effects of *C. pyrenoidosa* supplement for pregnant women are due to the synergistic contribution of micronutrients in *C. pyrenoidosa* and the influences on immune system. *C. pyrenoidosa* supplement is likely to be useful not only to pregnant women but to the large population with deficiency for iron or folate as well.

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References

1. World Health Organization (1992) The prevalence of anemia in women: a tabulation of available information, 2nd edn. World Health Organization, Geneva
2. Hercberg S, Galan P (1992) Nutritional anaemias. Baillieres Clin Haematol 5:143–168
3. Van den Broek NR, Letsky EA (2000) Etiology of anemia in pregnancy in South Malawi. Am J Clin Nutr 72:247S–256S
4. Centers for Disease and Prevention (1992) Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR 41:1–8
5. Kusumi E, Matsumura T, Yuji K, Kami M, Shoji M, Endou S, Yoneyama A, Kishi Y, Shibata T, Murashige N, Hamaki T (2006) Prevalence of anemia among healthy women in 2 metropolitan areas of Japan. Int J Hematology 84:217–219
6. MRC Vitamin Study Research Group (1991) Prevention of neural tube defects: results of the medical research council vitamin study. Lancet 338:131–137
7. Sumiyoshi Y (2002) Folic acid and spondyloschisis. Spine Spinal Cord 15:825–832 (in Japanese)
8. Sumiyoshi K, Ikenoue T (2007) Our management of pregnancy induced hypertension. Obstetrical Gynecological Therapy 94:1104–1107 (in Japanese)

9. Hernandez-Diaz S, Werler MM, Louik C, Mitchell AA (2002) Risk of gestational hypertension in relation to folic acid supplementation during pregnancy. Am J Epidemiol 156:806–812
10. Tamura E, Nishihara A, Isobe S, Matsuno N (1959) Nutrition studies on Chlorella. Jpn J Nutr 17:19–20 (in Japanese)
11. Fujiwara Y, Hirakawa K, Sinpo K (1990) Effect of long-term administration of Chlorella tablets on hyperlipemia. J Jpn Nutr Food Sci 43:167–173 (in Japanese)
12. Tanaka K, Konishi K, Himeno K, Taniguchi K, Nomoto K (1984) Augmentation of antitumor resistance by a strain of unicellular green algae, *Chlorella vulgaris*. Cancer Immunol Immunother 17:90–94
13. Matsuura E, Nemoto T, Hozumi H, Izumi K, Saito Y, Ishida H, Fukimbara T, Kawahara H (1991) Effect of Chlorella on rats with iron deficient anemia. Kitasato Arch Exp Med 64:193–204
14. Inoue K, Mukaiyama Y, Oka H, Sansawa H (1995) Clinical effects of Chlorella in hypertensive men. J Jpn Nutr Food Sci 48:485–489 (in Japanese)
15. Ministry of Health, Labour and Welfare Japan (2007) National health and nutrition examination survey 2004. Dai-ichi Shuppan, Tokyo (in Japanese)
16. Juarez-Vazquez J, Bonizzoni E, Scotti A (2002) Iron plus folate is more effective than iron alone in the treatment of iron deficiency anemia in pregnancy: a randomized double blind clinical trial. BJOG 109:1009–1014
17. Herbert V (1967) Biochemical and hematological lesions in folic acid deficiency. Am J Clin Nutr 20:562–572
18. Hannon-Fletcher MP, Armstrong NC, Scott JM, Pentieva K, Bradbury I, Ward M, Strain JJ, Dunn AA, Molloy AM, Kerr MA, McNulty H (2004) Determining bioavailability of food folate in a controlled intervention study. Am J Clin Nutr 80:911–918
19. Gujska E, Majewska K (2005) Effect of baking process on added folic acid and endogenous folates stability in wheat and rye breads. Plant Foods Hum Nutr 60:37–42
20. Kobayashi T (2003) Study on pathogenesis of preeclampsia: Endothelial dysfunction related to hypercoagulation, hyperactivity of sympathetic nerves, vasospasms and hypercoagulative mice models. Acta Obst Gynaec Jpn 55:851–862 (in Japanese)
21. Roggensak AM, Zhang Y, Davidge ST (1999) Evidence for peroxynitrite formation in the vasculature of women with preeclampsia. Hypertension 33:83–89
22. Park JY, Cho HY, Kim JK, Noh KE, Yang JR, Ahn JM, Lee MO, Song YS (2005) Chlorella dichloromethane extract ameliorates NO production and iNOS expression through the down-regulation of NF kappa B activity mediated by suppressed oxidative stress in RAW 264.7 macrophages. Clin Chim Acta 351:185–196
23. Saito S, Sakai M (2003) Th1/Th2 balance in preeclampsia. J Reprod Immunol 59:161–173
24. Ewart HS, Bloch O, Girouard GS, Kralovec J, Barrow CJ, Ben-Yehudah G, Suárez ER, Rapoport MJ (2007) Stimulation of cytokine production in human peripheral blood mononuclear cells by aqueous Chlorella extract. Planta Med 73:762–768