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Research Article

Effect of Chlorella vulgaris in Improving the Vitamin B12 Status among Vegetarian Obese and Overweight Young Adult Women

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Abstract

Background: Vegetarians are deficient in vitamin B_{12} and the complication increases with the obesity and overweight. *Chlorella vulgaris* a single cellular algae was found to contain the active vitamin B_{12} in it.

Objective: is to supplement *Chlorella vulgaris* as a source of vitamin B_{12} to vegetarian obese and overweight young adult women and to evaluate the effect of supplementation of *Chlorella vulgaris* in improving the vitamin B_{12} status among the vegetarian obese and overweight young adult women.

Methods: The study was conducted in Saibaba colony, Coimbatore. Study includes experimental and placebo group having a participant number of 20 each who are vegetarian young adult women either obese or overweight. The experimental group is supplemented with *Chlorella vulgaris juice* (3g/day as 200 ml juice) and placebo group with curd (200 ml/day). The anthropometrical measurements like height, weight, BMI, WHR and WtHR and biochemical parameters namely vitamin B_{12} and heamoglobin were assessed at baseline and after 50 days. The collected data were consolidated, tabulated and analysed stastically to assess the effectiveness in supplementing *Chlorella vulgaris*.

Results: Anthropometric measurements of the experimental group showed difference after supplementation but there was no difference in the placebo group. The vitamin B_{12} and haemoglobin status of the experimental group was statistically significant at 1% level and that of the placebo group was not significant.

Conclusion: the active vitamin B_{12} in *Chlorella vulgaris* were useful in improving the vitamin B_{12} status of the vegetarian obese and overweight young adult women. *Chlorella vulgaris* will be an alternative source for improving the vitamin B_{12} status of vegetarians

Introduction

An estimated 31 per cent of Indian population is aged between 10-24 years (Office of the Registrar General and Census Commissioner of India, [1]. Overall obesity rates have been increased through adulthood, rising from 23 per cent in those aged 20-24; to 35 per cent among those in their late 20s, 30s and early 40s; and to more than 40 per cent at older ages [2]. Some countries have strong cultural or religious traditions that promote vegetarianism, such as in India, while in other countries secular ethical concerns dominate, including animal rights and environmental protection, along with health concerns. India has more vegetarians than the rest of the world put together [3]. Vitamin B_{12} is absolutely critical for good mental and physical health. It is essential for the DNA (cell's genetic material) and works with other B vitamins to form all types of blood cells. It helps nerve fibre form and function in our brain, spinal cord and peripheral nerves [4]. Compared with lacto ovo vegetarians and omnivores, vegans typically have lower plasma vitamin B_{12} concentrations, higher prevalence of vitamin B_{12} deficiency, and higher concentrations of plasma homocysteine [5].

In vitamin B_{12} deficiency, high serum folate is associated with increased homocysteine and methylmalonic acid concentrations [6]. Absolute vitamin B_{12} deficiency was found in 6% patients in India, borderline vitamin B_{12} deficiency in 9%, absolute folic acid deficiency in 26% and combined vitamin B_{12} and folic acid deficiency in 10% patients. Values below 200 pg/ml indicate absolute vitamin B_{12} deficiency and the value between 200-250 pg/ml is said to be border line vitamin B12 deficiency and the values between 250-900 pg/ml is considered to be the normal vitamin B_{12} status [7]. Vitamin B_{12} is naturally found in animal products, including fish, meat, poultry, eggs, milk, and milk products. Vitamin B_{12} is generally not present in plant foods, but fortified breakfast cereals are readily available source of vitamin B_{12} with high bioavailability for vegetarians [8]. Nutritional needs should be met primarily from foods. Foods in nutrient-dense forms contain essential vitamins and minerals and also dietary fiber and other naturally occurring substances that may have positive health effects [9]. An alternative to meat source for vitamin B_{12} is algae. *Chlorella vulgaris* contain 10-50 microgram of vitamin B*12* per 100 g of dry weight [10]. Vitamin B_{12} in *Chlorella* is bioavailable and such dietary supplementation is a natural way for vegetarians and vegans to get the vitamin B_{12} they need [11].

Since obesity, vitamin B_{12} deficiency and cardiovascular diseases are in emergence particularly among vegetarians there is urgency in controlling these conditions. Keeping the above mentioned facts and the benefits of *Chlorella vulgaris*, the purpose of the present study is to test the effect of *Chlorella vulgaris* which is an underutilized alga, would be of great help in order to improve the vitamin B_{12} status among the obese/overweight young adult vegetarian women.

Hence the null hypothesis of the study is "There is no effect on supplementing *Chlorella vulgaris* in improving the vitamin B12 status among obese and overweight young adult women".

With this view, the study was conducted with the following objectives: To

- Supplement *Chlorella vulgaris* as a source of vitamin B_{12} to vegetarian obese and overweight young adult women
- Evaluate the effect of supplementation of *Chlorella vulgaris* in improving the vitamin B_{12} status among the vegetarian obese and overweight young adult women.

Materials and Methods

Selection and procurement of sample

Sample selected for the study is *Chlorella vulgaris*. The sample is selected based upon the previous research which has been done on the vitamin B_{12} content. No fungi, plants or animals (including humans) are capable of producing vitamin B_{12}. Only bacteria and archaea have the enzymes needed for its synthesis [12]. The daily requirement of vitamin B_{12} is 2.4 µg [13]. *Chlorella vulgaris* contain 29.87 ± 2µg/100g and 26.84±2µg/100 g dry weight of vitamin B12 in the form of methylcobalamin which is an active vitamin B_{12} [14]. *Chlorella vulgaris* was procured in the form of dry powder from Divy Agro Industries, New Delhi.

Selection of area and participants

The area selected for the study was Coimbatore city which is known as the education hub. Selected areas included are Chinthamani nagar, Raja Annamalai street of Saibaba Colony and Ramalingam colony. Chinthamani Nagar, is an area in Saibaba Colony, Coimbatore where majority of the population are vegetarians.

Participants selected for the study are young adult women of age group 18-24 years since vitamin B_{12} is particularly an important vitamin for women of child bearing age. The samples for the study are selected by purposive sampling method. A purposive sample is a non-probability sample that is selected based on characteristics of a population and the objective of the study [15].

A total of 40 vegetarian young adult women of age 18-24 years, who are either overweight or obese, were selected for the study. Hence, the investigator selected purposive sampling technique to select the samples for the study with the following inclusion and exclusion criteria:

Inclusion criteria: Young adult women

- Of age 18-24 years
- Who are vegetarian?
- Who are either overweight or obese
- Who are willing to co-operate in the study?

Exclusion criteria: Young adult women

- Less than 18 and more than 24 years
- Who are non-vegetarians
- Who are normal weight and underweight

Anthropometric assessment of participants

Anthropometric measurements are body measurements and provide information on body muscles mass and fat reserves. A variety of anthropometric measurements can be made either by covering the whole body or parts of the body [16]. The anthropometric measurements which are measured in the study are: weight, height, BMI, waist circumference, hip circumference, WHR and WtHR.

Conduct of supplementation study

The study is an experimental placebo study; and therefore, the subjects are divided into two groups, that is the experimental group and placebo group with the proportion of 1:1 ratio. A sample size of 40 respondents was included in this study. Thus, a total of 20 respondents were included each in the experimental group and the same number of participants in placebo group. The participants are grouped according to their willingness to take either *Chlorella vulgaris* juice or curd.

Biochemical assessment

Biochemical assay was done for all the participants of the study to test the heamoglobin status and vitamin B_{12} status. For women, 12.0 to 15.5 grams per deciliter is the normal range of blood heamoglobin [17] and the vitamin B_{12} reference range is 211-911 pg/ml. The biochemical assay of blood heamoglobin and vitamin B_{12} was taken twice namely before supplementation and after supplementation of days to see the effect of supplementation. Two ml of blood was collected from the participants before and after supplementation for the biochemical assay. The blood sample was collected from the participants with the help of a trained lab technician. The hemoglobin was detected with SLS- Hemoglobin method. Serum Vitamin B_{12} was detected using fully automated bidirectionally interfaced chemi luminescent immunoassay.

Supplementation to the selected participants

A supplementation study was conducted for the period of 50 days. The supplementation given for experimental group and placebo group were *Chlorella vulgaris* juice and curd respectively. *Chlorella vulgaris* juice was prepared by adding three grams *Chlorella* in 200 ml of water containing 25 g of palm jaggery/person. Three grams of *Chlorella* contains about 120% of the recommended daily amount of vitamin B_{12} [18]. The *Chlorella vulgaris* juice was given to experimental group (N = 20) for a period of 50 days.

The *Chlorella vulgaris* was given in the form of juice as it was easy to prepare and consumption was also easy as it is sweet in taste. Since the participants are overweight and obese table sugar was not used due to the empty calories in sugar. Jaggery has proved itself better as compared to white sugar [19].

To the placebo group 200 ml of curd was given. The curd was given for placebo group as curd is also rich in vitamin B_{12} and milk product seem to be the only source of vitamin B_{12} for vegetarians. Two hundred ml of curd contain 0.6 µg of vitamin B_{12} [20]. Curd was supplemented to the placebo group for the duration of 50 days as that of the experimental group

Statistical analysis and interpretation of data

The collected data were consolidated, tabulated and statistically analysed to assess the effectiveness of *Chlorella vulgaris* in improving the vitamin B_{12} among the vegetarian obese and overweight young adult women. The study was submitted in the Human Ethical Committee of the Institute and approval was got. The approval number is AUW/IHEC/FSN-17-18/XPD/03.

Results and Discussion

Anthropometric measurements of the young adult women

Anthropometric measurements of the participants of the supplementation study

Table I shows the of anthropometric measurements of the participants of the supplementation study experimental and placebo group.

Variables	Experimental group		Placebo group	
	Initial	Final	Initial	Final
Weight (kg)	64.75 ± 7.81	64.32 ± 7.81	62.75 ± 7.08	62.75 ± 7.05
Height (cm)	155.45 ± 5.79	155.45 ± 5.79	152.85 ± 4.22	152.85 ± 4.22
BMI (kg/m^2)	26.77 ± 2.43	26.58 ± 2.40	27.01 ± 1.87	27.01 ± 1.89
Waist circumference (cm)	93.40 ± 6.65	92.79 ± 6.85	87.75 ± 4.39	87.75 ± 4.44
Hip circumference (cm)	104.65 ± 5.25	104.19 ± 5.19	100.7 ± 4.06	100.6 ± 4.12

Table I: Anthropometric measurements of the participants of the supplementation study N = 40.

Indicate the statistical differences with superscripts

After the supplementation the mean weight of the experimental group was slightly reduced than the initial weight. The weight after supplementation period is 64.32 kg. This shows the mean decrease in difference of 0.43 kg after the supplementation. There is no change in the weight after the supplementation period in placebo group. Because of the change in body weight of the experimental group there is a slight reduction in the BMI. The BMI changed from 26.77 kg/m^2 to 26.58 kg/m^2 so the mean difference was 0.19. But there was no change in the BMI of the placebo group as there was no change in the weight of the placebo group after supplementation. The waist circumference of the experimental group was slightly decreased (0.61 cm). there is no change in the waist circumference of the placebo group after supplementation. There was a difference in the hip circumference of both experimental and placebo group after supplementation. The hip circumference was changed from 104.65 cm to 104.19 cm and in placebo group the value was decreased from 100.7 cm to 100.6 cm.

Derived body composition variables

Table 2 reveals the derived body composition variables of the experimental and placebo group.

Variable	Cutoff value	Experimental group		Placebo group	
		Initial	Final	Initial	Final
WHR	0.81	0.88 ± 0.04	0.88 ± 0.04	0.86 ± 0.02	0.86 ± 0.03
WtHR	0.5	0.60 ± 0.04	0.59 ± 0.05	0.57 ± 0.02	0.57 ± 0.02

Table 2: Derived body composition variables.

N = 40

Indicate statistical differences with superscripts

The mean waist hip ratio before supplementation for the experimental group was 0.88 and that for placebo group was 0.86. The values were almost similar. This observation shows that both experimental and placebo group were above their normal cut off value for WHR. In the present study the WtHR of experimental group was 0.60 and the placebo group was 0.57. This clearly states that experimental group is in higher risk when compared to the placebo group.

After the supplementation there was no change in the waist hip ratio of both experimental and placebo group. In the case of waist to height ratio there was a slight reduction in the experimental group after the supplementation and there was no change in WtHR among the placebo group.

Effect of supplementation of Chlorella vulgaris on the vitamin B12 status of the young adult women

Supplementation for experimental group with *Chlorella vulgaris* juice and placebo group with curd was given for a period of 50 days. Table 3 and Figure 1 presents the effect of supplementation of *Chlorella vulgaris* on the vitamin B12 status of the young adult women.

Variables	Experimental group		Mean difference	t value	Placebo group		Mean difference	t value
	Initial	Final			Initial	Final		
Vitamin B_{12}	227.50 ± 76.16	338.60 ± 75.26	+111.1	9.268**	238.90 ± 52.71	241.60 ± 51.71	+2.70	1.4^{NS}
Heamoglobin	11.61 ± 0.77	12.24 ± 0.87	+0.63	4.709**	11.98 ± 0.74	12.04± 0.74	+0.60	7.48^{NS}

Table 3: Effect of supplementation of *chlorella vulgaris* on the vitamin b_{12} status of the young adult women.

**- significant at 1% level, $^{NS-}$ not significant. Add superscripts

Figure 1:

The mean value of vitamin B12 experimental group before supplementation was 227.50 pg/ml and after the supplementation the mean value was increased to 338.60 pg/ml. Hence the mean increase in difference was 111.1 pg/ml. At the end of 50 days of supplementation the mean value of vitamin B_{12} among the experimental group showed a statistically significant difference at the $p < 0.01$ level. The mean value of placebo group before supplementation was 238.90 pg/ml and after supplementation the value was increased to 241.60 pg/ml, the mean increase in difference was 2.70. The difference between initial and final were found to be not statistically significant.

The mean hemoglobin value of experimental group before supplementation was 11.61 g/dl and the value found to be significant at the $p < 0.01$ level after the supplementation (t value = 4.709). Mean difference after the supplementation is 0.63 g/dl. The hemoglobin before supplementation in the placebo group is 11.98 g/dl and the mean value after supplementation is 12.04 g/dl. And the value showed no significant difference (t value=0.60, not significant).

The formulated supplement was found to have an impact in the vitamin B12 status of vegetarian obese and overweight young adult women and hence the objective was fulfilled.

Conclusion

In conclusion, the study revealed that the there was an effect in improving the vitamin B_{12} status among the obese and overweight young adult women. The *Chlorella vulgaris* juice can be easily prepared by just mixing with water and if necessary the sweetener like jaggery could be added. And this will be an effective way for preventing the vitamin B_{12} deficiency especially among the child bearing age group. A normal vitamin B_{12} status prevented high circulating homocysteine in the blood due to elevated methylmalonic acid and reduced the cardiovascular diseases especially among the obese. Hence, *Chlorella vulgaris* may be an alternative source of vitamin B_{12} among the vegetarians.

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