

Impact of Spirulina on Nutritional Status, Haematological Profile and Anaemia Status in Malnourished Children in the Gaza Strip: Randomized Clinical Trial

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Abstract

Background: Spirulina is a cyanobacterium blue-green alga rich in a wide range of nutrients include; protein, lipids, high level of various B vitamins, and minerals including calcium, iron, magnesium, manganese, potassium and zinc.

Aims: The objective of this study is to assess the impact of nutritional rehabilitation using *Spirulina platensis* versus vitamins and minerals supplementation on the nutritional status, hematological profile, and anemia status of malnourished children less than 5 years of age.

Method: The study utilized the experimental design in which 87 malnourished children aged less than 5 years attending an AEI rehabilitation program were enrolled. Children were stratified and simply randomized into two groups.

Groups: Vitamin and Mineral group (A) 30 children treated daily with selected vitamins and minerals; and Spirulina group (B) 30 children of the same age range who were given 3 grams of Spirulina. Anthropometric measurements and blood samples were collected at baseline and after 3 months of the trial.

Results: Baseline anthropometric indices of all the children were; Weight for Age Z (WAZ) score was – 2.42, Height for Age Z (HAZ) score was – 2.13, and Weight for Height Z (WHZ) score was – 2.09, which indicated that the children had poor anthropometric characteristics.

After 12 weeks of intervention, Spirulina supplementation showed more significant improving effect on weight ($p < 0.011$) and height ($p < 0.016$) when compared with Vitamin and Mineral group. Moreover, Spirulina supplementation was more effective treatment for increasing the ferritin (5.97 ng/ml-38.71 ng/ml) and iron levels (66.09 µg/dL to 95.52 µg/dL) in subjects with moderate malnutrition before intervention. The results of conducting one-way repeated measure ANOVA indicated that Spirulina supplementation has significant improvement in Haemoglobin ($p < 0.001$), Mean Corpuscle Volume ($p < 0.001$) and Mean Corpuscular Haemoglobin ($p < 0.023$) before and after the intervention between the two groups.

Conclusions: The results of the study reflected that Spirulina was a valuable and more effective in treating malnutrition and anemia and could be used in a wide range of settings since it is cheap compared to other conventional pharmaceutical preparations and formulas.

Trial registration: Current Controlled Trials PALMOH132234

Keywords: Spirulina; Malnutrition; Anaemia; Gaza strip

Introduction

Malnutrition is a global health problem that mainly affects children in developing countries with high poverty rates. According to Food and Agriculture Organization (FAO), one in eight people, was suffering from chronic under nutrition in 2010-2012 [1]. An estimated 230 million children under-five were chronically malnourished in developing countries [2]. Nearly 20 million children under-five suffer from severe acute malnutrition which is a life threatening condition requiring urgent treatment. In fact, it is estimated that it contributes to 1 million child deaths every year [3]. According to local reports, 11 out of 100 children under-five suffer from chronic malnutrition; 11.3% in West Bank (WB) and 9.9% in Gaza Strip [4]. Malnutrition is physiologically associated with a variable decline in several changes of the hematopoietic system, including anaemia and immunosenescence. An adequate nutritional intake is physiologically necessary for normal red cell production and even in the absence of clinically significant anaemia, red cell defects may reflect a nutritionally at risk host related to poor nutritional decisions [5]. Iron deficiency is the most common

nutritional deficiency in the world, with over 2 billion people worldwide affected [6,7]. Malnourished children need to be treated with proper diet complements containing protein, high calories, vitamins and minerals to overcome deficiency in these elements.

The Palestinian Bureau of Statistics reported that 10% of children under-five years of age in the Gaza Strip (GS) suffer from chronic malnutrition and 3.7% of children reported to be underweight in 2010. In GS, the rate of underweight increased from 2006 to 2010 (2.4 vs. 3.5

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respectively), which is worrying especially when linking this elevation of underweight rates with the current circumstances in GS of siege imposed on GS and closure of borders and restriction of movement with external world. An alarmingly high prevalence of iron deficiency anemia (33.5%) among young children in the Gaza Strip justifies the necessity for national intervention programs to improve the health status [8].

Spirulina, a cyanobacterium blue-green alga, has been reported to be a nutritional source since the 16th century, and the United Nations, in its world conference held during 1974, hailed Spirulina as the possible "best food for the future" [9]. Spirulina is rich in a wide range of nutrients; protein accounted for about 70% of its contents, 5-8% lipids, and 2-5% is sulpholipids, in addition it contains high level of various B vitamins, and minerals including calcium, iron, magnesium, manganese, potassium and zinc [10-13]. Spirulina products contain bioactive proteins with the ability to stimulate the intestinal immune system [11] to enhance the responsiveness to vaccines and improve allergic rhinitis [14,15]. It is the only food source other than breast milk containing substantial amounts of essential fatty acid, essential amino acids and Gamma linolenic Acid (GLA) that helps to regulate the entire hormone system [16].

It is proposed that it can sustainably contribute to alleviating malnutrition because it is rich in various nutrients, is easy to produce, and can be added to many traditional foods [17]. In nutrition literature, several studies have provided the evidence of efficacy of Spirulina on the treatment of malnutrition. For example effectiveness of Spirulina on human growth, particularly on weight gain, and concluded that daily intake of Spirulina for 12 weeks significantly improved weight and BMI among HIV-infected patients who received Spirulina. Spirulina's effectiveness among HIV-infected was also tested and found that its daily intake for six months improved the weight and arm girth of the patients [18]. In addition to the effects on physical growth, beneficial effects on serum iron levels and blood haemoglobin levels were suggested in both animal testing and clinical studies [19-21]. Interestingly, even smaller amounts of Spirulina (1 g/day), led to positive results on the haematological status and intellectual performance of children [22].

The main objective of this study was to assess the impact of nutritional rehabilitation using *Spirulina platensis* versus vitamins and minerals supplementation on the nutritional status, haematological profile, and anaemia status in malnourished children less than 5 years of age.

Method and Materials

Study protocol

This research was conducted at Ard El Insan Palestinian Benevolent Association (AEI) during 2013-2014. AEI association, a Palestinian non-governmental organization (NGO), was established in 1984 as an affiliate of the Swiss agency International Federation Terre Des Hommes. In 1997 it was localized and became a Palestinian NGO. AEI association provides nutritional and health services to the most needy and marginalized children under-five years old, their mothers, and families.

The study utilized the experimental design in which 87 malnourished children aged <5 years attending AEI rehabilitation program were enrolled using the CONSORT criteria [23]. Children suffering from degenerative diseases or severely dehydrated children were excluded from this study. Each child admitted to the protocol

study was given a progressive number and at the end, each was selected with a casual number generator program as shown in Figure 1. Children were stratified and simply randomized into two groups: Vitamin and Mineral group (A), 30 children treated daily with selected vitamins and minerals and Spirulina group (B), 30 children of the same age range were given 3 grams of Spirulina. Anthropometric measurements and blood samples were collected at baseline and after 3 months of the trial to measure the differences in anthropometric measures and haematological profile between children who received vitamins and minerals and those who received Spirulina supplements.

Study patients

All the children studied were undernourished according to the z-score criteria (Weight-for-Age, Height-for-Age, or Weight-for-Height as Z-score were < -1 SD), recommended by the WHO and UNICEF. Average age was 21.55 months (range 12-45). The ages were confirmed by birth certificates. Many of them had diarrhoea, which was treated according to the AEI protocol [24], but the rehydration therapy treatment was interrupted before inclusion and commencing of the intervention procedures. An approval letter was obtained from the Helsinki Committee in GS. Also, approval letter to conduct the study was obtained from the Ministry of Health. Parents were informed of the study protocol, and each child was included after written consent was obtained from his/her proxy.

Sampling design

The targeted malnourish children were stratified according to sex (male/female), age (0 to ≤ 2.5 years/<2.5 to <5 years), and nutritional status (Stunting; Height, Underweight; Weight for Age Z score < -1 and wasting; Weight for Height Z score < -1), and all the targeted subjects were randomly allocated (simple random method) into two groups; (A) group and (B) group.

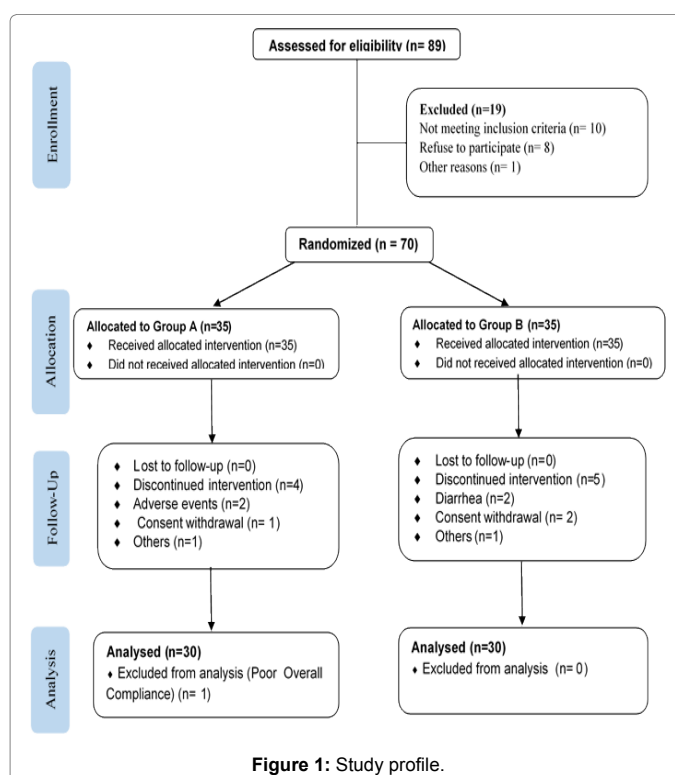


Figure 1: Study profile.

Intervention protocol

The 60 malnourished children followed one of two rehabilitation protocols: (A) 30 malnourished children were given one spoon of dates jam daily like the children in group (B), they also received carefully selected vitamins, minerals and essential nutrients including; B1, B2, B3, B6, vitamin E, Colecalciferol, Dexpanthenol, lysine hydrochloride, calcium and Phosphorus and sodium (Pharmaton Kiddi syrup manufactured by Ginsana SA Bioggio-Switzerland) 5 ml/day, and vitamins A and D (Adol drops, manufactured by Birzeit Pharmaceutical company) 2 drops/day, and Iron oral drops (Iron Baby, manufactured by Meditec company) 8 drops/day and (B) 30 malnourished children of the same age range were given three grams of Spirulina powder mixed with dates molasses to make its taste acceptable for children. The mixture of Spirulina and dates jam was given to the children every morning, half an hour before breakfast and on a daily bases for three months (October to December 2013). The enrolment of a control group might appear unethical among these very young and severely malnourished children and it would have been hard for the mothers to accept the study protocol in which no supplements will be given to their children.

Monitoring tools

The monitoring procedure for the intervention program was throughout the intervention program and its function was to ensure that targeted children received the supplements at the correct level (safe and compliant with programs guidelines) over an appropriate period of time and also to ensure coverage of those at highest risk.

Record keeping

We developed different records for monitoring purposes:

- Lists of subjects with all information such as name address and contact number.
- Compliance records were provided to the mothers (proxies) of the targeted children in order to record the daily compliance toward the supplements in both groups.
- Weekly health recalls were developed to monitor any changes which could have been encountered during the intervention and hindered the growth of the child such as diarrhoea, vomiting, and other serious complications.
- Anthropometric, biochemical, and haematological measuring reports to record all the measurements at the two levels of assessments: (PRE, POST).

Anthropometry measurements

The anthropometry data gathered from participants included height and weight. Subject's height was measured using a portable height rod with a horizontal head board attachment- SECA body meter. Participants removed their shoes, stood as tall and straight as possible with their head level and their shoulders and upper arms relaxed. The vertical distance between the standing surface and the top of their head was measured at the maximum point of inhalation. The measurement was repeated two times to a precision of 0.1 cm and a mean value calculated. For younger children (1-2 years old), recumbent length was measured using an infantometer with a fixed head piece and horizontal backboard and an adjustable foot piece [25].

Weight was obtained using a SECA digital weighing scale (to the nearest 0.1 kg). Minimal clothing and no shoes or socks were to

be worn by the child during the weighing. The age of the child was calculated in months from their birth date to the day of data collection. The age, weight and height of the children were translated into three indices Height - Age, (HAZ), Weight - Age (WAZ) and Weight - Height (WHZ) using the Epi. Info software.

Biological and biochemical analyses

The Haemoglobin level (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were measured before the study and after three months duration. Hb levels were measured in order to determine the anaemic status. Blood numeration was done using a Coulter Counter T540. Serum iron and ferritin levels were measured by biochemical and quantitative luminance methods, respectively.

Data Analysis

All analyses were conducted using the Statistical Package for the Social Sciences statistical software package version 16.0 (SPSS Inc., Chicago, IL, USA). Repeated measures analysis of variance (one-way repeated measures ANOVA) was used to evaluate the changes in all the continuous variables over the study period. Repeated measures ANOVA measured the changes between the groups, within the group, and time and group interaction. All analyses will be conducted in SPSS 18.0 for Windows. Pearson Chi-Square was also used to compare the proportions in both the groups before and after the intervention. Statistical significance was accepted at $p < 0.05$.

Results

Table 1 showed that at baseline the mean weight of children was 8.27 kg and mean height was 77.0 cm. The mean HAZ, WAZ, and WHZ were all negative, suggesting a generally poor nutritional status (moderate malnutrition) of all the children in the study. Among the children, 83% were stunted, 98.3% were underweight, and 91.7% were wasted (significant and mild). The present study did not report any significant difference in Weight for Age, Height for Age and Weight for Height z scores of the children between any of the groups; therefore no adjustment of the nutritional status was required while performing the outcome analyses.

Anthropometric changes

After 12 weeks of intervention, significant changes were observed in the weight and height of the malnourished children in either group as shown Table 2. In Spirulina group, the weight was significantly increased (from 7.90 kg to 8.59 kg, $p < 0.001$) and the height showed a tendency of an increase (from 75.11 cm to 78.01 cm, $p < 0.001$). Spirulina supplementation showed more significant improving effect on weight ($p < 0.011$) and height ($p < 0.016$) when compared with Vitamin and Mineral group by repeated test for treatment (time x treatment interaction).

Variables	Mean(SD)
Age (months)	21.55(1.22)
Weight (kg)	8.27(1.77)
Height (cm)	77.0(8.45)
Weight of birth (kg)	2.84(0.49)
Weight for age (Z score)	-2.42(1.08)
Height for age (Z score)	-2.13(1.19)
Weight for height (Z score)	-2.09(0.87)

Table 1: Anthropometric characteristics of study participants.

Ferritin and serum iron

Changes of ferritin and serum iron levels during 12 weeks of intervention period are shown in Table 3. After 12 weeks of intervention, reduction in the ferritin level in the Vitamin and Mineral group was observed. Conversely, the same group showed significant improvement in the level of serum iron (63.42 µg/dL-77.95 µg/dL). On the other hand, Spirulina supplementation showed a significant improving effect on ferritin (5.97 ng/ml-38.71 ng/ml) as well as on serum iron levels (66.09 µg/dL-95.52 µg/dL) within the group. Repeated test for treatment showed significant effects of Spirulina supplementation on improving ferritin level (time×treatment interaction, $p<0.001$) and on increasing serum iron concentration (time×treatment interaction, $p<0.043$). Therefore, Spirulina supplementation was probably more effective for increasing the ferritin and iron levels in subjects with moderate malnutrition before intervention.

Changes in the haematological profiles

The mean concentration of Hb, MCV, MCH and MCHC allowed us to diagnose anemia (10.3 g/dL of Hb, 75.2 fL of MCV, 23.4 pg/cell of MCH and 31.0 g/dL of MCHC) in all children. Table 4 showed that there were statistically significant improvements ($p<0.001$) in all the hematological parameters (Hb, MCH and MCHC) except for MCV between baseline and end of 12-week intervention period of receiving Spirulina. On the other hand, the results of the Vitamin and Mineral group showed significant improvements in the MCV and MCHC levels after three months of supplementation Table 5. The results of conducting one-way repeated measure ANOVA indicated that Spirulina supplementation has significant improvement in Haemoglobin ($p<0.001$), Mean Corpuscle Volume ($p<0.001$) and Mean Corpuscular Haemoglobin ($p<0.023$) before and after the intervention between the two groups. Consequently, nutritional rehabilitation using Spirulina platensis was probably more effective in correcting

Variables	Vitamins-Mineral (n=30)		Spirulina (n=30)		P value ^a
	Pre	Post	Pre	Post	
Weight (kg)	8.643 (2.10) ^b	9.24 (1.97) ^{***}	7.90 (1.30)	8.59 (1.30) ^{***}	0.011
Height (cm)	78.88 (9.30)	80.8 (8.97) ^{***}	75.11 (7.39)	78.01 (6.92) ^{***}	0.016

^aRepeated Measure ANOVA between Vitamins – mineral group and spirulina group

^bMean (Standard Deviation)

The level of significance is < 0.05

Asterisk = significantly different by paired t-test between baseline and 12th week in the same intervention group, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 2: Differences in weight and height in the vitamins and mineral group and the spirulina group.

Variables	Vitamins-Mineral (n=30)		Spirulina (n=30)		P value ^a
	Pre	Post	Pre	Post	
Ferritin (ng/ml)	24.08 (18.50)	20.34 (18.46)	5.97 (1.94)	38.71 (9.23) ^{***}	0
Serum Iron (µg/dL)	63.42 (26.24)	77.95 (16.50) [*]	66.09 (21.35)	95.52 (25.77) ^{***}	0.043

^aRepeated Measure ANOVA between Vitamins – mineral group and spirulina group

^bMean (Standard Deviation)

The level of significance is < 0.05

Asterisk = significantly different by paired t-test between baseline and 12th week in the same intervention group, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 3: Ferritin and serum iron of the subjects during intervention period.

Variables	Vitamins-Mineral (n=30)		Spirulina (n=30)		P value ^a
	Pre	Post	Pre	Post	
Hb (gm)	10.28 (0.85)	10.45 (0.82)	10.38 (1.09)	11.42 (0.82) ^{***}	0
MCV (fL)	74.54 (7.53)	70.36 (7.99) ^{***}	75.96 (7.44)	76.93 (5.52)	0
MCH (pg/cell)	23.12 (2.98)	23.34 (3.26)	23.67 (3.00)	25.13 (2.33) ^{***}	0.023
MCHC (g/dL)	30.94 (1.86)	33.06 (1.39) ^{***}	31.13 (1.89)	32.78 (1.27) ^{***}	0.362

(Hb = Haemoglobin, MCV= Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration)

^aRepeated Measure ANOVA between Vitamins – mineral group and spirulina group

^bMean (Standard Deviation)

The level of significance is < 0.05

Asterisk = significantly different by paired t-test between baseline and 12th week in the same intervention group, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 4: Hematological profiles of the subjects during intervention.

Variables	Vitamins-Mineral (n=30)		Spirulina (n=30)	
	Pre	Post	Pre	Post
Non Anemia	8 (26.7) ^a	8 (26.7)	10 (33.3)	20 (66.7)
Mild Anemia	11 (36.7)	15 (50.0)	9 (30.0)	10 (33.3)
Moderate Anemia	11 (36.7)	7 (23.3)	11 (36.7)	0 (0)

^aFrequency (Percentage)

Table 5: The prevalence of anemia before and after the intervention in two groups.

the haematological parameters when compared with the conventional vitamins and minerals supplementation.

Anaemia status

According to the WHO classification of anaemia [26], no significance difference in the distribution of anaemia between both groups at the baseline level, but after three months of intervention period there was significant reduction in frequency of anaemia between the two groups after intervention in favour of the Spirulina group ($p < 0.001$). For anaemic children treated with Spirulina, mean Hb increase was 1.04 g/dL, with 66.7% reaching values > 11.0 g/dL. An improvement in Hb level was reported as there was increase in the number of subjects towards normal category. Conversely, the Vitamin and Mineral group did not report any increase in non-anaemic children as illustrated in Figure 2.

Discussion

The siege on GS and the blockade has resulted in thousands of workers lost their jobs, elevation of inflation to dangerous levels, and increase poverty, which led to inability of families to afford healthy, balanced food to their children and rely on aids from local and international organizations. These economic restrictions and the limited access to healthy and nutritious diet were reflected on the nutritional status of the children in term of poor anthropometric characteristics at the baseline level of the study.

After three months of study, malnourished children treated with Spirulina improved, their weight and height had increased and many of them appeared less anaemic. This improvement was significant in both study groups indicating that the integration of traditional meals with Spirulina improves the nutritional and micronutrient requirements of undernourished children [16,27,28]. Compared to the vitamins and minerals, Spirulina is richer in essential amino acids, important for anabolism and muscle mass reconstitution [12,28-30]. These nutrients could be of great value in overcoming malnutrition among vulnerable children. The result demonstrates the benefits of Spirulina in the treatment of child malnutrition, which was in line with the work carried out in Ouagadougou and Central African Republic, respectively and reported positive impact on the nutritional rehabilitation of malnourished and HIV-infected children [18,20,27,31]. The study also reported remarkable improvement in height (2.9 cm) which was consistent with previous trials [16,32]. The study reported a growth recovery which is slower than previous reports, and this might be

attributed to the diarrhoea which was present at the beginning of treatment of these children.

The serum ferritin level is the most specific biochemical test that correlates with relative total body iron stores. A low serum ferritin level reflects depleted iron stores and hence is a precondition for iron deficiency in the absence of infection. The results reported significant improvements in the ferritin as well as iron levels at the end of the study. Spirulina supplementation was probably more effective for increasing the ferritin and iron levels in subjects with moderate malnutrition before intervention. Spirulina is a rich source of iron with levels equivalent to that contained in beef [33]. The iron content in the Spirulina species has the ability to replenish the serum iron as well as the ferritin stores.

Spirulina supplementation has significant improvement in Haemoglobin ($p < 0.001$), Mean Corpuscle Volume ($p < 0.001$) and Mean Corpuscular Haemoglobin ($p < 0.023$) before and after the intervention between the two groups, which could be attributed to the following reasons. Firstly highly available form of iron present in Spirulina. An average increase of 1.1 g/dl in Hb level as a result of supplementation of 1 g of Spirulina/ day for three months was also reported by Kauser et al. [34]. Secondly; phycocyanin, a pigment of Spirulina was found to possess high erythropoietin (34), thirdly; phycocyanin also showed protection of human erythrocytes against lysis by peroxy radicals (34) and finally, the high content of vitamin B12 (110 to 400 $\mu\text{g}/100$ g Spirulina platensis) which essential for normal maturation and development of blood cells [35].

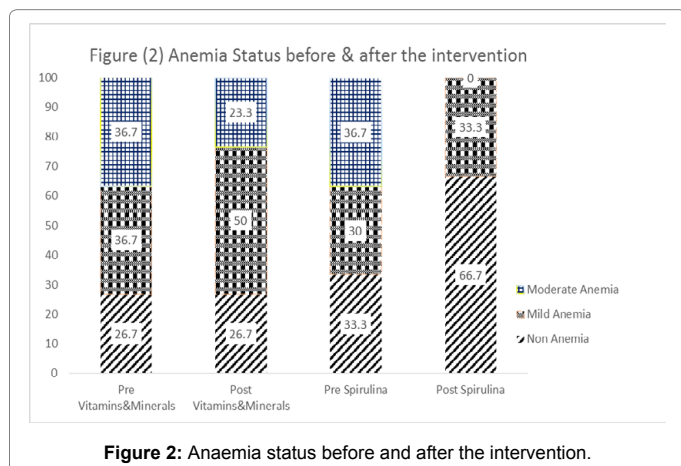
Children were classified according to WHO classification [26]. According to this classification, none of the respondents in both the groups fell in severe anemic category ($\text{Hb} < 7 \text{g/dl}$). An improvement in Hb level was reported as there was an increase in the number of subjects towards the "normal" category (20 anemic children to 10 anaemic children). The associated improvements in Hb, MCH and MCHC reflect the positive changes in the hypochromic anaemia towards the normal values [36]. The results reported parallel increment in the MCV level along with MCH and MCHC indicating positive changes in the microcytic anaemia, though these MCV changes was not significant in the Spirulina group. The nutritional benefits of the Spirulina over the vitamins and minerals supplementations might refer to the functional activities and the phytochemical contents of the Spirulina [36].

Conclusions and Recommendations

The results of this study are comparable to previous studies examining the growth and hematologic effect of Spirulina. The anthropometric, haematological and bio-chemical parameters have improved after the use of Spirulina thus making it an ideal nutritional supplement which is inexpensive and shows positive results within a short span of time.

Though the study discussed an innovative method of treating malnutrition and anaemia, the study findings need to be viewed within some contextual limitations. It was a controlled dose 'efficacy' trial and should not be viewed as an 'effectiveness' study. The limited fund of the study had compromised the number of measurement levels and the levels were restricted to two only. The absence of control group due to ethical consideration was another limitation. Despite these limitations, we find it safe to conclude that Spirulina supplementation is an effective procedure to treat malnutrition and anaemia.

It seems that Spirulina deserves attention as potential natural dietary supplements for use in nutritional rehabilitation of moderately



malnourished children. Health care providers in the Gaza Strip (Ministry of Health, United Nations, Local health organizations) need to formulate some policy of supplying Spirulina capsules free of cost to the vulnerable sections of the society especially children in poor communities to alleviate iron deficiency anemia and to improve nutritional status in the Gaza Strip.

Authors' Contributions

Abed El-Raziq Salama contributed to the data collection, data entry, data analysis and wrote the manuscript. Suliman Eljabour, responsible for the application of grant, budget and ethical approval. Ihab AN led the overall study, contributed to the design of the study, contacted the authorities involved in the study, supervised the data collection in the fieldwork and wrote the initial draft manuscript. Mahmoud A. El-Sheikh Ali totally involved in data collection and participated in the design of the study. All authors participated in the review of the manuscripts, read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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