

## Short Communication

# The effects of Spiruline on the immune functions of HIV-infected undernourished children

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### Abstract

**Background:** Malnutrition is a public health problem in the entire world, particularly in the developing countries. The effect of Spiruline supplement in the weight recovery of HIV-negative and HIV-infected undernourished children has been largely demonstrated. The aim of this study is to determine the effect of Spiruline on the immune status of 46 HIV-infected undernourished children, aged 15 ± 5 months, and of 23 undernourished HIV-negative children, aged 14 ± 6 months.

**Methodology:** The duration of this study was eight weeks. To assess immune functions a count of CD4 lymphocyte subpopulation was performed before and after introducing the Spiruline supplement.

**Results:** The degree of anemia improved in all the children, but the effect was less evident among HIV-infected children; in fact, 81% of HIV-negative versus 62% of HIV-infected children showed signs of improvement. The mean values of CD4 lymphocyte subpopulation showed a consistent increment in HIV-negative children [from 1257 (range 531-2301) to 1562 (range 798-2552) mm<sup>3</sup>] while they showed an irregular increase in HIV-infected children [from 1339 (152-4000) to 2088 (244-4214) mm<sup>3</sup>].

**Conclusions:** The improvement of the immunological status due to Spiruline treatment might represent an effective barrier against infectious diseases, which both cause and result from malnutrition in underdeveloped countries.

**Key Words:** Spiruline, CD4 lymphocytes, undernourished children, HIV.

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### Introduction

Malnutrition is a global public health problem, particularly in developing countries [1]. It is presently recognized that malnutrition, coupled with vitamin and mineral deficiency [2,3], ineluctably precipitates a malnutrition-infectious pathology cycle [4]. HIV infection is one of the more frequently encountered infections in severely undernourished children in Burkina Faso where a high mother-to-child HIV transmission rate has been documented [5]. An HIV prevalence greater than 40% has been encountered in severely undernourished children and this worsens the prognosis for such children [6].

The Centre of Education and Nutritional Rehabilitation (CREN) at the Centre Medical Saint Camille (CMSC), Ouagadougou (Burkina Faso), has been administering Spiruline (*Spirulina platensis*) to hospitalized undernourished children since January 2000 with the objective of improving their status. As shown by our randomized study,

based on anthropometric and haematological parameters, Spiruline plus traditional meals or Spiruline plus Misola proves to be an efficient way to rehabilitate undernourished HIV-negative children [7]. Spiruline showed an analogous effect when added to traditional meals provided to HIV-infected children [8].

The aim of this study is to determine through subpopulation CD4 lymphocyte count whether Spiruline affects the nutritional status only or also influences the immune function as well. The improvement of the immunological status following Spiruline treatment may represent an effective barrier against infectious diseases, which both cause and result from malnutrition in underdeveloped countries.

### Materials and Methods

#### Patients

This study was carried out at the CMSC of Ouagadougou during 2005-2006. Out of 550

undernourished children followed at the CREN of the CMSC, informed consent was granted for 46 HIV-infected children, aged  $15 \pm 5$  months, and 23 HIV-negative children, aged  $14 \pm 6$  months, to receive Spiruline plus traditional meals (millet, vegetable, fruit). (The number of HIV-infected children recruited in this study is double that of HIV-negative due to the higher propensity of mothers of HIV-infected children to give consent). To confirm HIV serostatus, a serological screening for HIV was carried out by sequentially using the two rapid tests (i.e. Determinate® and Genie-II® HIV). No HIV-infected children received anti-retroviral drugs. This study was performed for eight weeks.

#### *Anthropometric and laboratory parameters*

All recruited children were undernourished according to the z-score criteria, recommended by WHO and UNICEF.

The nutritional status, evaluated via brachial perimeters, was compared to the Jelliffe's classification (9).

HAZ (Height for age z-score), WAZ (Weight for age z-score) and WHZ (Weight for height z-score) parameters were calculated according to the National Centre for Health Statistics (NCHS) protocols (10).

Hematological (Coulter counter) parameters were evaluated in blood collected from a peripheral vein.

The immune status of both HIV-infected and HIV-negative children was evaluated before and after Spiruline supplementation by direct immunofluorescence lymphocyte CD4 count. The viral loads of infected children were not determined, since viral load and immune deficiency are known to be linearly correlated in HIV-infected children.

The Ethical Committee of CMSC granted permission for this study and all parents signed a written consent for their children to participate.

#### *Preparation and administration of Spiruline*

The mothers of undernourished children received 70 grams of Spiruline in a bag per week; a total of 10 grams of Spiruline was added twice a day (5 grams/dose) to traditional meals in a marked container. The choice of this amount was dictated by the poor results obtained by Brancer et al., 2003 [11] who administered 5 g/day. The

competence of mothers in preparing the mixes and feeding their children was monitored and they were allowed to keep administering the mixture at home. Weight and other anthropometric parameters of the children were checked daily at CREN.

#### *Statistical analysis*

Anthropologic and haematologic/immunologic parameters are reported as mean  $\pm$  SD and median (range). Significance of differences before and after Spiruline was obtained by Student's T test for One-Sample and Matched-Pairs tests and by Wilcoxon Matched-Pairs Signed-Ranks Test.

#### **Results**

Anthropometric parameters are reported in Table I. The analysis of these data shows that the most significant improvement was obtained for HIV-negative children. On average a weight gain of 15.5 grams and 24.5 grams per day was reached in HIV-infected and in HIV-negative children respectively.

The haematological/immunological parameters are reported in Table II. HIV-infected children were more anaemic ( $3.30 \times 10^6/\text{mm}^3$  and 8.0 g/dl of haemoglobin) than HIV-negative ( $3.60 \times 10^6/\text{mm}^3$  and 8.50 g/dl of haemoglobin). The severity of anaemia decreased in all the children during the study; however, such a reduction was less evident among HIV-infected children. At the end of the eight weeks, the anaemia was slightly corrected for both HIV-infected and HIV-negative children, who still remained slightly anaemic ( $3.92 \times 10^6/\text{mm}^3$  and 9.70 g/dl of haemoglobin). Following Spiruline administration, the leukocyte count showed a significant decrease in HIV-infected ( $13.0 \pm 3.0 \times 10^3$  versus  $10.0 \pm 4.03 \times 10^3$ ,  $P = 0.0001$ ), which was not significant in HIV-negative children. The lymphocyte CD4 count in HIV-negative showed a significant and consistent increase after eight weeks (Figure 1); in contrast, the lymphocyte CD4 count in HIV-infected children did not show a regular pattern of response (Figure 2). The mean values of CD4 lymphocytes of HIV-infected children were significantly different before and after Spiruline administration [from 1339 (152-4000) to 2088 (244-4214)  $\text{mm}^3$ ,  $P = 0.0005$ ], whilst they only slightly differed in HIV-negative children [from 1257 (531-2301) to 1562 (798-2552)  $\text{mm}^3$ ,  $P = 0.0195$ ]. Disaggregating HIV-infected and HIV-

negative children for age classes, no significant change (data not shown) was observed, probably due to the limited number of studied children.

The compliance to treatment was excellent and no children dropped out. The mothers reported that they rarely had difficulties in feeding their children. No unwanted effect of Spiruline was noticed among the recruited subjects.

**Table 1.** Anthropologic parameters collected at the beginning (1) and at the end of the study with Spiruline (2).

	23 children HIV-negative treated with Spiruline		46 children HIV-infected treated with Spiruline	
	Mean	SD	Mean	SD
WHZ <sub>1</sub>	-2.90	0.71	-2.85	1.00
WHZ <sub>2</sub>	-1.72	1.60	-2.21	1.00
WHZ <sub>1</sub> → WHZ <sub>2</sub>	P = 0.0023*		P = 0.0028*	
WAZ <sub>1</sub>	-3.81	0.93	-4.09	0.82
WAZ <sub>2</sub>	-2.96	1.14	-3.57	0.90
WAZ <sub>1</sub> → WAZ <sub>2</sub>	P = 0.0082*		P = 0.0047*	

WAZ<sub>1</sub> = Weight for age z-score at the beginning of study; WAZ<sub>2</sub> = Weight for age z-score at the end of the study. \*significant at Student's T test.

## Discussion

Anthropometric and haematological parameters allow us to appreciate both the nutritional and biological effects of Spiruline supplement to traditional meals. In particular the rehabilitation by Spiruline seems to correct the anaemia and the weight loss in HIV-infected patients, but more quickly in HIV-negative, undernourished children. The lymphocyte CD4 count increases after 8 weeks of Spiruline supplementation; however this increase is not consistent in HIV-infected children. In the context of low protein intake, 10 grams a day in Africa against 29 grams in Latin America and 63 grams in the industrialized countries, the integration of a traditional meal with Spiruline and Misola plus Spiruline (57% protein) improves the nutritional and micronutrient requirement for undernourished children [7]. The improvement of immune function in these undernourished HIV-infected children awaiting enrolment in antiretroviral treatment protocols could be a consequence of increased protein uptake and Zn deficiency correction. Undernourished children typically require a supplement of Zn before starting nutrition rehabilitation [12]. In this study the Zn plasma level

was not reported due to technical limitations. This determination is not regularly performed at CMSC.

The study of the immune status in children HIV positive, who do not respond to Spiruline supplement, should be extended, taking into account the metabolic and immune effects of this supplement and the possibility of an inhibition of HIV-1 replication by Spiruline [13].

At the beginning of this study the number of leukocytes was found elevated in the HIV-infected children and this number decreased after eight weeks of Spiruline supplement (Table II), against an increment of CD4 lymphocytes, supporting an immunomodulatory effect of Spiruline. However, a consistent response was documented only in HIV-negative children likely because the absence of HIV represents a significant advantage for the immune system [14]. The significant increase of CD 4 in coincidence with the weight gain suggests the importance of nutrition status in the evolution of HIV infection, especially in children, which could be independent from the viral load. It is clear that the determination of viral load synchronized with CD4 lymphocyte count could add more information on the mechanism of Spiruline. We do not know whether the restoration of the immune system is associated with a decrease of viral charge, but the results obtained by Ayehunie et al. (1998) [13], suggest that this is possible. Moreover, it is possible that some of the HIV-infected children probably did not receive adequate doses of Spiruline to modulate their immune functions and this may explain the non-uniformity of the increment in CD4 counts.

Another reason for the effects seen from the administration of Spiruline may be the intake of high amounts of  $\omega$ -6 lipid, specifically  $\gamma$ -linolenic acid [15]. The exclusive presence of  $\omega$ -6 represents a metabolic advantage, since desaturase could be deficient in the undernourished children [16]. In sub-Saharan Africa, maize and millet are the typical staples and therefore kwashiorkor and/or marasma is frequently found. The maize/millet diet is characterized by high intake of linoleic acid and is deficient in other polyunsaturated fatty acids and in riboflavin, which could result in high tissue production of prostaglandin E<sub>2</sub>, causing inhibition of the proliferation and cytokine production of Th1 cells, effectors of cellular immunity [17]. A diet-associated inhibition of the Th1 subset is a major

contributor to the high prevalence of infectious diseases in these areas. Spirulina, with its high amount of  $\omega$ -6 lipid fraction and iron, could support an efficient recovery of the precarious immune systems of these children [16]. Also the high content of Zn in Spiruline could correct the deficit of this mineral, which is important for the immune response in HIV-infected children [18].

This study demonstrates that the involvement of mothers, who must correctly follow instructions for the Spiruline administration, is essential to contain the malnutrition. Growth recovery is slower than the weight recovery, probably due to the diarrhoea which often is present at the beginning of treatment of these children [19]. It is evident from this study that the quantity of Spiruline must be at least 10 grams/day. A previous study, by Branger et al. [11] in Burkina Faso, did not show significant improvements when half the amount of Spirulina used in our study (5 grams vs. 10 grams) was added to traditional meals. Moreover, our study gave better results than one conducted in Dakar by Alling et al. [20], in which weight gain was inferior, probably due also in this case to a reduced quantity in Spiruline, and one in Zambia by Kelly et al. [21] in undernourished HIV-infected children with persistent diarrhoea. The conclusions of our study are clinically relevant and suggest that there may be value in extending the evaluation of immune status in HIV-infected children, who could receive the Spiruline supplement for longer periods, until they are candidates to start antiretroviral therapy.

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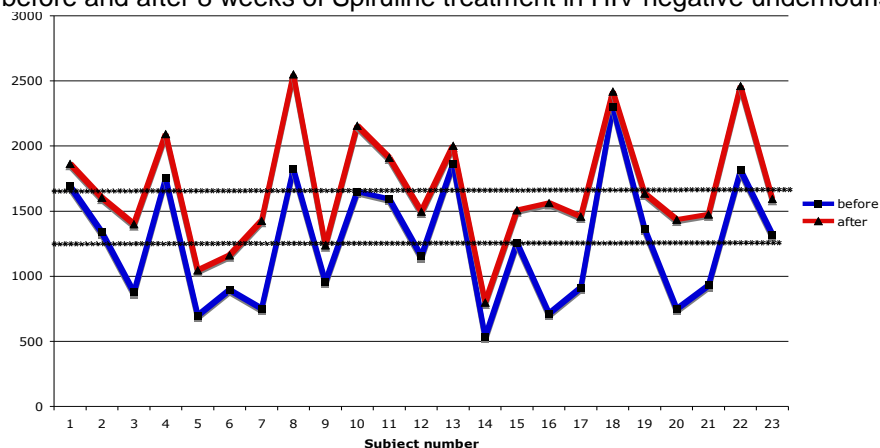
**Conflict of Interests:** The authors declare that they  
have no conflict of interests.

**Table 2.** Hematologic/immunologic parameters collected at the beginning (1) and at the end of the study with Spiruline (2).

	23 children HIV-negative treated with Spiruline		46 children HIV- infected treated with Spiruline	
	Mean	SD	Mean	SD
Red Cell x 10 <sup>6</sup> mm <sup>3</sup> (1)	3.60	0.81	3.30	0.63
Red Cell x 10 <sup>6</sup> mm <sup>3</sup> (2)	3.92	0.62	3.71	0.53
Red Cell (1)→Red Cell (2)	P = NS		P = 0.0014	
Hb gr/dl (1)	8.50	1.54	8.00	1.10
Hb gr/dl (2)	9.70	0.82	9.40	1.19
Hb gr/dl (1)→ Hb gr/dl (1)	P = 0.0018		P = 0.0001	
Leukocytes x10 <sup>3</sup> mm <sup>3</sup> (1)	11.73	3.02	13.00	3.00
Leukocytes x10 <sup>3</sup> mm <sup>3</sup> (2)	12.00	5.51	10.00	4.03
Leukocytes (1)→Leukocytes (2)	P = NS		P=0.0001	
CD4/mm <sup>3</sup> (1)	1257	531-2301	1339	152-4000
CD4/mm <sup>3</sup> (2)	1562	798-2552	2088	244-4214
CD4/mm <sup>3</sup> (1)→CD4/mm <sup>3</sup> (2)	P=2.891e-05 *		P =0.0005*	

Student's T test; NS = not significant; \* Wilcoxon test.

**Figure 1.** CD4 before and after 8 weeks of Spiruline treatment in HIV negative undernourished children.



**Figure 2.** CD4 before and after 8 weeks of Spiruline treatment in HIV positive children.

