RESEARCH

24(103), May - June, 2020

ISSN 2321–7359 EISSN 2321–7367

Medical Science

Spirulina ameliorates immunity and reduces viral load in beta-thalassemia major children comorbid with hepatitis virus C: A single-arm clinical trial

Mohamed Ramadan Elshanshory[1], Mohamed Labib Salem[2], Mohamed Attia Saad Attia[3], Rasha Mohamed Gamal[1], Mostafa Mohamed El-Sheekh[4], Asmaa Abobakr Elshahat[5], Arwa MohammedShukri Turkistani[6], Hala Abd El-Rahman Khattab[6,7], Basma Ghazi Eid[8], Nagla Abd El-Aziz El-Shitany[8,9]

[1]Department of Pediatric, Faculty of Medicine, Tanta University, Tanta, Egypt
[2]Department of Immunology, Faculty of Science, Tanta University Tanta, Egypt
[3]Department of Clinical Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt
[4]Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt
[5]Department of Pediatric, El Mahalla El Kobra Hospital, El Mahalla El Kobra, Egypt
[6]Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah, Saudi Arabia
[7]Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt
[8]Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia
[9]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tanta University, Tanta, Egypt

Article History
Received: 28 December 2019
Reviewed: 29/December/2019 *to* 14/February/2020
Accepted: 15 February 2020
E-publication: 20 February 2020
P-Publication: May - June 2020

Citation
Mohamed Ramadan Elshanshory, Mohamed Labib Salem, Mohamed Attia Saad Attia, Rasha Mohamed Gamal, Mostafa Mohamed El-Sheekh, Asmaa Abobakr Elshahat, Arwa MohammedShukri Turkistani, Hala Abd El-Rahman Khattab, Basma Ghazi Eid, Nagla Abd El-Aziz El-Shitany. Spirulina ameliorates immunity and reduces viral load in beta-thalassemia major children comorbid with hepatitis virus C: A single-arm clinical trial. *Medical Science*, 2020, 24(103), 1142-1151

General Note

Article is recommended to print as color digital version in recycled paper.

ABSTRACT

Introduction: Patients with β-thalassemia major suffer from impaired function and components of the immune system. Hepatitis C virus infection exacerbates the inherited and acquired immunity of β-thalassemia patients. *Aim:* This study aimed to investigate the effect of spirulina administration on the indicators of immunity, serum liver function, ferritin and viral load in children of β-thalassemia major comorbid with the hepatitis C virus. *Methods:* This study was a single-arm clinical trial carried on 60 children (30 healthy and 30 with β-thalassemia major and hepatitis C). Spirulina was administered daily for 3 months to patients only. *Results:* The results showed amelioration of the blood picture (HB, RBCs, and WBCs), ferritin level, blood transfusion intervals, liver enzymes (ALT and AST), viral load, and many of the immune markers (IFN-gamma, CD4+, and CD4+CD25+) after spirulina therapy. *Conclusion:* The results of this study showed that spirulina ameliorated hepatitis caused by virus C in children comorbid with β-thalassemia major disease. The mechanism may be via lowering serum ferritin levels and improved immunity.

Keywords: Spirulina, β-thalassemia, children, hepatitis virus C, clinical trial, immunity, ferritin

1. INTRODUCTION

About 1.5% of the world's population has been diagnosed with β-thalassemia, a large proportion of whom are in the Mediterranean region (up to 10%), India (2.78-4%), Southeast Asia (1-10%) and Egypt (4-10%). Children recognized with beta-thalassemia major are diagnosed during the first two years of life and they need a regular blood transfusion in order to survive (Fahim *et al.,* 2013; Mohanty *et al.,* 2013; Uludağ *et al.,* 2016; Hossain *et al.,* 2017; Sanctis *et al.,* 2017). The hepatitis C virus is the leading cause of hepatitis acquired through blood transfusion. The virus causes acute inflammation in the liver cells and produces many chronic long-term complications, such as cirrhosis and hepatocellular carcinoma (Ataei *et al.,* 2012). Patients with β-thalassemia major of the most vulnerable to infection with the hepatitis C virus, transmitted to them through an infected blood transfusion (Aminianfar *et al.,* 2017). Infectious issues signify the 2nd most common reason for mortality and the principal motive of morbidity in β-thalassemia major, with a prevalence of 12-13%. In addition to the excessive threat of blood-borne infections accompanying with continuous transfusions, the elevated vulnerability of these patients to infectious illnesses has been assigned to the aberration of the immune system, which is apparent through systemic inflammation and immune insufficiency (Chirico *et al.,* 2015). Previous studies have shown that patients with β-thalassemia major suffer from impaired function and components of the immune system, especially β and T lymphocytes, immunoglobulins, and the complement system (Alidoost *et al.,* 2006; Morabito *et al.,* 2007). In an earlier study, the researchers reported that the infection by the hepatitis C virus in β-thalassemia patients exacerbates the inherited and acquired immunity (Al-Ofairi *et al.,* 2011).

Spirulina, the micro blue-green algae (Cyanophyceae), is considered one of the most important nutritional supplements of the century according to the Food and Agriculture Organization of the United Nations (García *et al.,* 2017). Spirulina has many pharmacological effects, including: anticancer, antiviral, antioxidant as well as an immune stimulating effect (Karkos *et al.,* 2011; Finamore *et al.,* 2017). The theory of this research is based on the hypothesis that the simultaneous occurrence of hepatitis C and β-thalassemia major in children negatively affects the immunity and that spirulina will improve the immunity. This study aimed to estimate the different immunological indicators in children of β-thalassemia major and hepatitis virus C infection. Besides, the effect of spirulina administration on the indicators of immunity, liver function, and hepatitis virus C load will be studied.

2. METHODOLOGY

Study subjects

A single arm clinical trial was conducted on 30 patients with β-thalassemia also infected with hepatitis C virus attending the Hematology Unit of Pediatric Department, Tanta University Hospital for medical check- up over one year. Another 30 healthy children of matched age and sex with proven negative hepatitis C virus infection were enrolled as a control group. This study was done after ethical approval of the ethical committee of Faculty of medicine, Tanta University, Tanta, Egypt. An informed written

parental consent was obtained for every child enrolled in this study. The study was registered on https://clinicaltrials.gov/ (NCT02674607).

Inclusion and exclusion criteria

Children over 3 years old, suffering from β-thalassemia and hepatitis C virus were diagnosed by serological detection of hepatitis C virus -Ab and confirmed by detection of serum hepatitis C virus RNA by polymerase chain reaction (PCR). However, children having other types of haemolytic anemia or below 3 years old were excluded.

Demographic and clinical characteristics

Demographic characteristics: age, gender, and anthropometric measures (weight, height, and head circumference) were recorded for the healthy (control) and patients' group. *Clinical data:* age at the first blood transfusion, disease duration, clinical picture, and type of chelating agents were also reported.

Study protocol

In addition to classic transfusion and chelation therapy, spirulina capsules (500 mg spirulina; GNC, USA) were consumed daily (250 mg/Kg, orally) by the thalassaemic group only (Gomaa *et al.,* 2017). Follow up of these patients was performed for 3 months. Re-evaluation of these children after 3 months of spirulina treatment was performed.

Sample collection

Whole venous peripheral blood (10 ml) was collected from each participant under complete aseptic conditions and divided into two tubes. In-patients' blood was obtained just before a scheduled transfusion of packed red blood cells. The first tube contained EDTA as anticoagulant for hematology, liver functions, and flow cytometry measures. The second tube was devoid of EDTA and allowed for complete clotting. Centrifugation was carried out at 3000 rpm for 10 min. Separated plasma and serum were collected and stored at -20 ^{o}C until used for different biochemical assays.

Biochemical assays

Complete blood count (CBC) was assessed using an automated instrument (ABX Micros 60 hematology analyzer, Horiba medical, USA) and hemoglobin (HB) by electrophoresis.

Liver enzymes were measured using enzymatic colorimetric kits, Centronic Chemicals Co, Germany

Hepatitis C virus antibody was assayed using ELISA kits (BioSource, San Diego, California, USA) and Hepatitis C virus RNA was assayed using PCR.

Serum ferritin levels were determined using ELISA kits (BioSource, San Diego, California, USA).

Determination of immunological parameters

Measurement of CD4+, CD4+/CD25+ and MDSCswas performed using anti-CD 4, anti-CD 25, anti-CD 127, Anti HLA-DR, anti-anti-CD33), and anti-CD11b (BD Biosciences, Germany) in automatic immunologic analyzer (AXSYM-ABBOTT, Germany).

Serum immunoglobulins (IgG, IgM and IgA) were estimated by SPINREACT ELISA kits (Santa Coloma, Spain).

Serum interferon gamma (IFN-gamma) level was estimated by RayBio® ELISA kits (RayBiotech, Norcross, GA30092).

Statistical analysis

All data were analyzed by SPSS version 24. Data were presented as mean ± SD for numerical data and number (n) or percentages (%) for categorical data. Paired t-test, unpaired t-test, and chi-square test were applied as necessary and the significance was settled at $p \leq 0.05$.

3. RESULTS

Demographic and clinical characteristics of the HCV infected β thalassemia major patients

The data indicated that there was no significant difference between the patients' group and the control group in regard to age and gender. The patients suffered from a significant decrease in body weight and height, while they had a significantly increased head circumference compared to healthy children. The history of patients showed that the onset of β thalassemia major had an average of 9 years and that the start of blood transfusion ranged from 3 to 48 months of age. Concerning chelation therapy, 63% of patients were on deferasirox and the rest were on deferoxamine. The clinical picture showed that all patients had a pale appearance and

suffered from jaundice, while 77% suffered from an enlarged liver, 20% from an enlarged spleen and 80% had a splenectomy (Table 1).

Table 1 Demographic and clinical characteristics of the HCV infected β thalassemia major patients and control subjects included in the study. Data are expressed as either mean ± SD or number (n). The p-values are calculated either by chi square or unpaired student t test and settled significance at 0.05.

Character	Patients (n = 30)	Control (n = 30)
Age (Y)	10.22 ± 3.01	9.53 ± 2.64
Gender (Boy/Girl) (n)	15/15	13/17
Weight (kg)	29.10 ± 10.02[a]	37.17 ± 11.27
Height (cm)	110.90 ± 17.80[a]	122.10 ± 17.98
Head Circumference (cm)	53.27 ± 1.46[a]	49.73 ± 1.84
Age at the First Blood Transfusion (month)	13.80 ± 12.73	
Disease Duration (year)	9.03 ± 2.85	
Clinical Picture Pallor (n) Jaundice (n) Hepatomegaly (n) Splenomegaly (n) Splenectomy (n)	 30 30 23 6 24	
Type of Chelation (n) Deferasirox Deferoxamine	 19 11	

Effects of spirulina treatment on the complete blood picture of HCV infected β thalassemia major patients

The results showed that prior to spirulina treatment, patients were suffering from significantly decreased blood HB, RBCs count, and neutrophils % compared to the control group. While the results showed that patients had a significant increase in the number of WBCs and the lymphocytes % compared to the control group. Treatment of patients with spirulina resulted in a significant increase in HB and RBCs count compared to the patients' group before treatment. In addition, treatment with spirulina significantly decreased the number of WBCs compared to the patients' group before treatment. It was noted that treatment with Spirulina resulted in WBCs returning to normal levels similar to the control group. However, there remained a difference in the rest of the blood picture components after treatment with spirulina when compared to the control group (Table 2).

Table 2 Effect of spirulina treatment on the complete blood picture of HCV infected β thalassemia major patients

	Control (n = 30)	Patients (n = 30)	
		Before	After
HB (g/dl)	12.01 ± 0.91	8.27 ± 1.32 [a]	9.02 ± 1.69 [a, b]
RBCs (million/mm^3)	4.40 ± 0.64	3.16 ± 0.61 [a]	3.47 ± 0.62 [a, b]
PLT (thousand/mm^3)	318 ± 41	381 ± 249	335 ± 197
WBCs (million/mm^3)	9.57 ± 1.06	16.44 ± 15.27 [a]	11.24 ± 7.80 [b]
Neutrophils %	53.03 ± 4.94	46.01 ± 12.52 [a]	45.33 ±8.78 [a]
Lymphocytes (%)	34.43 ± 2.75	44.47±12.17 [a]	46.13 ±8.53 [a]

Data are presented as mean SD.

[a] significant difference compared to the non-treated control group ($p \leq 0.05$) (unpaired student t test).

[b] significant difference between before and after spirulina therapy in the patient group ($p \leq 0.05$) (paired student t test).

Effect of spirulina treatment on serum ferritin and blood transfusion intervals measured in HCV infected β thalassemia major patients

There was a significant rise in the level of ferritin in the serum of the patient group compared to the control group. Treatment of patients with spirulina resulted in a significant decrease in the level of ferritin in the serum compared to the group of patients before treatment. Furthermore, the treatment of patients with spirulina resulted in a significant increase in the percentage of patients (66%) who underwent blood transfusion for an extended period (more than 15 days) compared to their percentage (33%) in the before treatment group (Table 3).

Table 3 Effect of spirulina treatment on serum ferritin and blood transfusion intervals measured in HCV infected β thalassemia major patients

	Control (n = 30)	Patients (n = 30)	
		Before	After
Ferritin (ng/ml)	32.47 ± 7.13	2745 ± 1491 [a]	2134 ± 1462 [a, b]
Blood Transfusion Intervals ≤ 15 Day > 15 Day		n = 20 n = 10	n = 12 [c] n = 18 [c]

Data are presented as mean ± SD or number (n).

[a] significant difference compared to the non-treated control group ($p \leq 0.05$) (unpaired student t test).

[b] significant difference between before and after spirulina therapy in the patient group ($p \leq 0.05$) (paired student t test).

[c] significant difference between before and after spirulina therapy in the patient group ($p \leq 0.05$) (Chi square test).

Effect of spirulina treatment on plasma liver enzymes and hepatitis virus C load measured in HCV infected β thalassemia major patients

There was a significant increase in the level of plasma ALT, AST, and load of hepatitis C virus in the patient group compared to the control group. Treatment of patients with spirulina resulted in a significant decrease in the level of liver enzymes (ALT and AST), as well as the load of hepatitis C virus in the plasma of patients compared to the value of the before treatment group. The liver function markers and the load of hepatitis C virus measurements in the patients' group after spirulina treatment remained statistically high compared to control group (Fig. 1, 2, and 3).

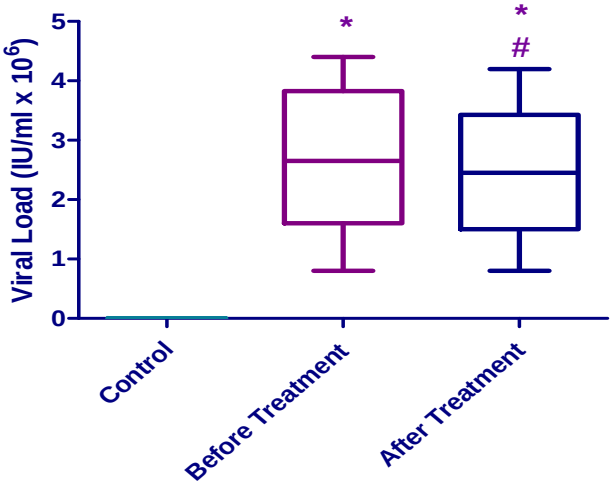

Figure 1 Effect of spirulina treatment on hepatitis virus C load measured in control and HCV infected β thalassemia major patients before and after treatment. Results are presented as Whisker: Min to Max plot. Each box included the mean of all results falling between the 25th and 75th percentiles, and the median value was represented as a horizontal line inside each box. *significant difference compared to the non-treated control group ($p \leq 0.05$) (unpaired student t test). #significant difference between before and after spirulina therapy in the patient group ($p \leq 0.05$) (paired student t test).

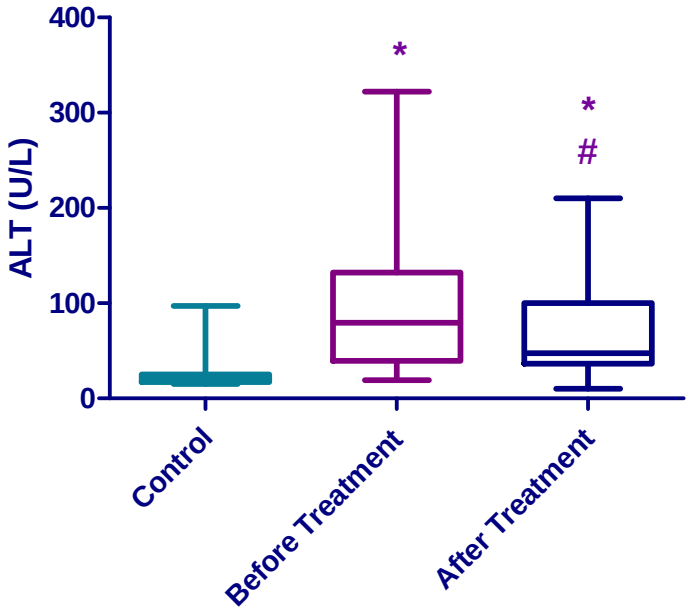

Figure 2 Effect of spirulina treatment on ALT measured in control and HCV infected β thalassemia major patients before and after treatment. Results are presented as Whisker: Min to Max plot. Each box included the mean of all results falling between the 25th and 75th percentiles, and the median value was represented as a horizontal line inside each box. *significant difference compared to the non-treated control group ($p \leq 0.05$) (unpaired student t test). #significant difference between before and after spirulina therapy in the patient group ($p \leq 0.05$) (paired student t test).

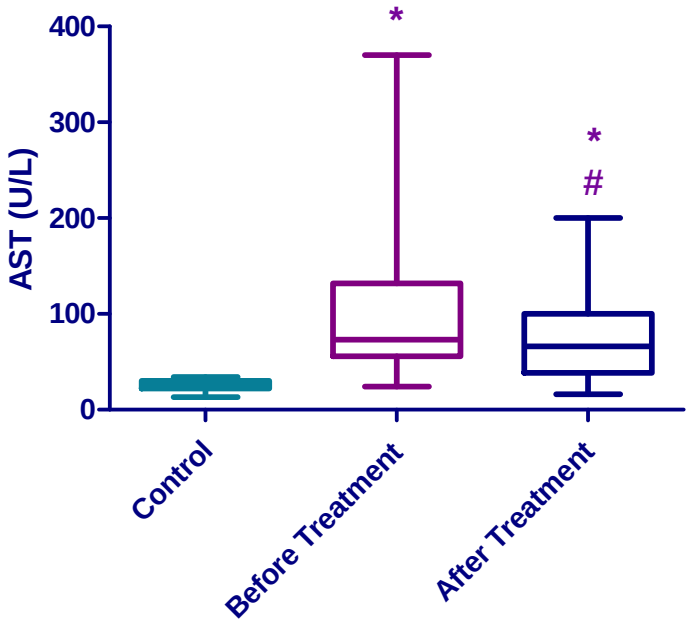

Figure 3 Effect of spirulina treatment on AST measured in control and HCV infected β thalassemia major patients before and after treatment. Results are presented as Whisker: Min to Max plot. Each box included the mean of all results falling between the 25th and 75th percentiles, and the median value was represented as a horizontal line inside each box. *significant difference compared to the non-treated control group ($p \leq 0.05$) (unpaired student t test). #significant difference between before and after spirulina therapy in the patient group ($p \leq 0.05$) (paired student t test).

Effect of spirulina treatment on some immunological parameters measured in HCV infected β thalassemia major patients

The study revealed a significant decrease in the level of IFN-gamma, IgG, CD4%, and CD4 absolute count in the patient group before treatment compared to the control group. Whereas, a significant increase in IgA, IgM, CD4+CD25+ %, CD4+CD25+ absolute count, MDSCs%, and MDSCs absolute count was observed in the patient group before treatment compared to the control group. Treating patients with spirulina, significantly increased the level of IFN-gamma, CD4%, and CD4 absolute count compared to their level before treatment. Moreover, treatment with spirulina resulted in a significant decrease in CD4+CD25+ % and CD4+CD25+ absolute count compared to the patient group before treatment. All immunological measurements in the patients' group after spirulina treatment remained statistically less than those of the control group, except for the return of the CD4+CD25+ absolute count to its level in the control group (Table 4).

Table 4 Effect of spirulina treatment on some immunological parameters measured in HCV infected β thalassemia major patients

	Control (n = 30)	Patients (n = 30)	
		Before	After
IFN-gamma (pg/dl)	4.51 ± 1.98	0.34 ± 0.16 [a]	1.32 ± 0.87 [a, b]
IgG (mg/dl)	132.70 ± 62.02	58.37 ± 29.99 [a]	58.80 ± 29.96 [a]
IgA (mg/dl)	1066 ± 174	1445 ± 296 [a]	1497 ± 242 [a]
IgM (mg/dl)	171 ± 59	294 ± 78 [a]	276 ± 89 [a]
CD4 %	41.07 ± 4.05	26.60 ± 3.60 [a]	32.00±5.15 [a, b]
CD4 Absolute Count (cell/mm^3)	1351 ± 212	782 ± 319 [a]	1313 ±524 [b]
CD4+CD25+ %	4.81 ± 1.20	11.03 ± 2.36 [a]	8.40 ± 2.08 [a, b]
CD4+CD25+ Absolute Count (cell/mm^3)	67 ± 23	103 ± 68 [a]	81 ± 35 [b]
MDSCs %	1.44 ± 0.76	12.60 ± 7.76 [a]	10.86 ± 6.70 [a]
MDSCs Absolute Count (cell/mm^3)	127 ± 75	2828 ± 4371 [a]	1592 ± 1991 [a]

Data are presented as mean SD.

[a] significant difference compared to the non-treated control group ($p \leq 0.05$) (unpaired student t test).

[b] significant difference between before and after spirulina therapy in the patient group ($p \leq 0.05$) (paired student t test).

4. DISCUSSION

The results of this study showed a change in the blood picture in children with β-thalassemia major infected with viral hepatitis C, as they were diagnosed with anemia, while the number of WBCs and lymphocytes was increased. The present study also showed a high level of liver enzymes in the injured children. Consistent with these results, a previous study conducted on male and female adults with β-thalassemia major reported similar changes (Ayyash and Sirdah, 2018). The high level of liver enzymes is due to the marked increase in the concentration of ferritin resulting from frequent blood transfusions in these patients (Omar *et al.,* 2011; Origa and Comitini, 2019). The marked increase in liver function may also be attributed to the infection of children with viral hepatitis C and the high viral load in these patients (Li *et al.,* 2002; Salama *et al.,* 2015). Previous studies have discussed the harmful irreversible effects of increasing the concentration of iron in the blood on the body organs, especially the heart and liver (Suman *et al.,* 2016). Cardiac toxicity due to iron causes death in these patients, however iron toxicity to the liver is dangerous also, as it leads to cirrhosis and ascites (Origa and Comitini, 2019).

Treatment of patients with spirulina led to an improvement in the indicators of anemia in the blood picture, the return of the number of WBCs to their normal level, regardless of the lack of improvement in the % of neutrophils and lymphocytes. The study attributed the continued increase in the percentage of lymphocytes to the infection of these children with hepatitis C virus, as lymphocytes increase during viral infection (Zhu *et al.,* 2013). The viral infection leads to lymphocyte activation and the secretion of antibodies, although the immune system depends mainly on T lymphocytes during its defence against viruses (Hislop *et al.,* 2007). In line with our current study, it was reported that anemia improved in the elderly when spirulina was given. The improvement of blood markers in these patients may be due to the high protein content of spirulina (60-70% of all essential amino acids), carotenes, vitamins (B1, 2, 3 ,6, 9, and 12) and salts, all of which help correct anemia (Babadzhanov *et al.,* 2004; Khan *et al*., 2005; Selmi *et al.,* 2011). The results of this study showed that treatment with spirulina led to improved liver function, as well as decreased hepatitis virus C load in patients. This may be due to the effectiveness of spirulina in reducing the level of ferritin and reducing the blood

transfusion frequency in these children (Gomaa *et al.,* 2017). Supporting the results of this study, the low level of liver enzymes in rats and humans associated with high blood lipid profile under treatment with Spirulina (El-Sheekh *et al.*, 2014).

The results of this study indicate that children of major thalassemia with hepatitis C infection suffer from a lack of different immune indicators, where a significant decrease appeared in the level of IFN-gamma, IgG, number and % of CD4. Moreover, the number and % of CD4+CD25+ and MDSCs, the known immunity inhibitors T cells, were significantly increased in these patients. Likewise, the results of a previous study reported an increase in the number of Treg cells (CD4+CD25+FoxP3+) with thalassemia major, which inhibits the immunity in these patients. The study also attributed this to the antigenic reaction, which occurs as a result of repeated blood transfusions (Bozdogan *et al.,* 2010). In another study, the researchers found a marked increase in CD4+, CD4+CD25+, and CD4+CD25+FoxP3+ Treg cells in the spleen of mice induced by the thalassemia model (Bao *et al.*, 2014). The increase in Treg cells during thalassemia may be attributed to a compensatory-defensive mechanism from the body to suppress the chronic inflammation caused by stress erythropoiesis and the increased RBCs breakdown at the same time (Belkaid *et al.,* 2002; Bao *et al.*, 2014). In addition, previous studies reported an increased number of immune-suppressing MDSCs during chronic infection with hepatitis C virus that was attributed to the immune reaction against the virus core protein. Immune inhibition function of MDSCs cells is due to an increase in the activity of indoleamine 2,3-dioxygenase (IDO) enzyme. In the same context, it was reported that the gene expression of IDO protein is increased in the liver of chronic hepatitis C infected patients (Zhai *et al.,* 2017).

The results of this study showed that treatment of patients with spirulina led to an increase in the level of IFN-gamma, number and % of CD4+, and % and percentage of CD4+CD25+. The increase in the immunity stimulation CD4+ cells and IFN-gamma concomitant with the decrease in the immunity suppressant CD4+CD25+ cells may underlie the mechanism of amelioration of liver function and decrease the hepatitis virus C load observed in this study (Yakoot and Salem, 2012). In addition to the immune stimulant function of spirulina, some of the compounds extracted from this algae have shown an inhibitory function on hepatitis C virus replication (calcium spirulan) and other that inhibit the interaction of the viral core protein with target cell receptors (cyanovirin-N and microvirin) (Esser *et al.,* 1999; Huskens *et al.,* 2010).

5. CONCLUSION

The results of this study showed that T regulatory cells, MDSCs cells, serum IFN-γ, and immunoglobulin levels may be associated with subsequent down regulation of cellular adaptive immune status in children with β-thalassemia infected with HCV, reflecting the severity of liver inflammation in these patients. Spirulina ameliorated hepatitis caused by virus C in children comorbid with β-thalassemia major disease. The mechanism may be via lowering serum ferritin levels and improved immunity.

List of Abbreviations

HB: Hemoglobin; RBCs: Red blood cells; WBCs: White blood cells; Alanine transaminase: ALT; Aspartate transaminase AST; Interferon gamma: IFN-gamma.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Study limitations

The wide variability of the measure parameters, short follow up period, and the limited number of cases.

REFERENCE

1. Alidoost F, Gharagozloo M, Bagherpour B, Jafarian A, Sajjadi SE, Hourfar H, Moayedi B. Effects of silymarin on the proliferation and glutathione levels of peripheral blood mononuclear cells from β-thalassemia major patients. Int Immunopharmacol 2006; 6: 1305–1310.
2. Al-Ofairi BA, Barakat AB, Ghanim HEDA, Shehata IH, El-Sayed MH. A study of innate and adaptive immune responses in beta-thalassemic patients with chronic hepatitis C virus infection. Egypt J Immunol 2011; 18: 61–76.
3. Aminianfar M, Khani F, Ghasemzadeh I. Evaluation of hepatitis C, hepatitis B, and HIV virus Serology pandemic in thalassemia patients of Shahid Mohammadi Hospital of Bandar Abbas, Iran Electron Physician 2017; 9: 4014–4019.
4. Ataei B, Hashemipour M, Kassaian N, Hassannejad R, Nokhodian Z, Adibi P. Prevalence of anti HCV infection in patients with Beta-thalassemia in isfahan-iran. Int J Prev Med 2012; 3: S118-S123.
5. Ayyash H, Sirdah M. Hematological and biochemical evaluation of β-thalassemia major (βTM) patients in Gaza

Strip: A cross-sectional study. Int J Health Sci (Qassim) 2018; 12: 18–24.
6. Babadzhanov AS, Abdusamatova N, Yusupova FM, Faizullaeva N, Mezhlumyan LG, Malikova MK. Chemical composition of Spirulina platensis cultivated in Uzbekistan. Chem Nat Compd 2004; 40: 276–279.
7. Bao W, Zhong H, Yazdanbakhsh K. Immunologic characterization suggests reduced alloimmunization in a murine model of thalassemia intermedia. Transfusion 2014; 54: 2880–2891.
8. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sack DL. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 2002; 420: 502–507.
9. Bozdogan G, Erdem E, Demirel GY, Yildirmak Y. The role of Treg cells and foxP3 expression in immunity of β-thalassemia major and β-thalassemia trait patients. Pediatr Hematol Oncol 2010; 27: 534–545.
10. Chirico V, Lacquaniti A, Piraino B, Cutrupi M, Cuppari C, Grasso L, Rigoli L, David A, Arrigo T, Salpietro C. Thalassaemia major and infectious risk: High Mobility Group Box-1 represents a novel diagnostic and prognostic biomarker. Br J Haematol 2015; 171, 130–136.
11. El-Sheekh MM, Hamad SM, Gomaa M. Protective effects of Spirulina on the liver function and hyperlipidemia of rats and human. Brazilian Arch Biol Technol 2014; 57:77–86.
12. Esser MT, Mori T, Mondor I, Sattentau QJ, Dey B, Berger EA, Boyd MR, Lifson JD. Cyanovirin-N binds to gp120 to interfere with CD4-dependent human immunodeficiency virus type 1 virion binding, fusion, and infectivity but does not affect the CD4 binding site on gp120 or soluble CD4-induced conformational changes in gp120. J Virol 1999; 73: 4360–4371.
13. Fahim FM, Saad K, Askar EA, Eldin EN, Thabet AF. Growth parameters and vitamin D status in children with thalassemia major in upper Egypt. Int J Hematol Stem Cell Res 2013; 7: 10–14.
14. Finamore A, Palmery M, Bensehaila S, Peluso I. Antioxidant, immunomodulating, and microbial-modulating activities of the sustainable and ecofriendly spirulina. Oxid Med Cell Longev 2017;
15. García JL, de Vicente M, Galán B. Microalgae, old sustainable food and fashion nutraceuticals. Microb Biotechnol 2017.
16. Gomaa M, El-Sheekh MM, El-shafey AS, Metwally MA, El-shanshory MR, Eid MA. Immunological Studies in Egyptian Thalassemic Children Infected with Hepatitis C Virus 2017; 4:20–26.
17. Hislop AD, Taylor GS, Sauce D, Rickinson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. Annu Rev Immunol 2007; 25: 587–617.
18. Hossain MS, Raheem E, Sultana TA, Ferdous S, Nahar N, Islam S, Arifuzzaman M, Razzaque MA, Alam R, Aziz S, Khatun H, Rahim A, Morshed M. Thalassemias in South Asia: clinical lessons learnt from Bangladesh. Orphanet J Rare Dis 2017.
19. Huskens D, Férir G, Vermeire K, Kehr JC, Balzarini J, Dittmann E, Schols D. Microvirin, a novel α(1,2)-mannose-specific lectin isolated from Microcystis aeruginosa, has anti-HIV-1 activity comparable with that of cyanovirin-N but a much higher safety profile. J Biol Chem 2010; 285: 24845–24854.
20. Karkos PD, Leong SC, Karkos CD, Sivaji N, Assimakopoulos DA. Spirulina in clinical practice: Evidence-based human applications. Evidence-based Complement Altern Med 2011.
21. Khan Z, Bhadouria P, Bisen P. Nutritional and therapeutic potential of spirulina. Curr Pharm Biotechnol 2005; 6: 373–379.
22. Li CK, Chik KW, Lam CWK, To KF, Yu SCH, LeeV, Shing MMK, Cheung AYK, Yuen PMP. Liver disease in transfusion dependent thalassaemia major. Arch DisChild 2002; 86, 344–347.
23. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, Sharma SK, Chaudhari U, Ghosh M, Das S, Britt RP, Singh S, Ross C, Jagannathan L, Kaul R, ShuklaDK, Muthuswamy V. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: A multicentre study. J Community Genet 2013; 4: 33–42.
24. Morabito N, Russo GT, Gaudio A, LascoA, Catalano A, Morini E, Franchina F, Maisano D, La Rosa M, Plota M, Crifò A, Meo A, Frisina N. The "lively" cytokines network in β-Thalassemia major-related osteoporosis. Bone 2007; 40; 1588–1594.
25. Omar N, Salama K, Adolf S, El-Saeed GSM, Abdel Ghaffar N, Ezzat N. Major risk of blood transfusion in hemolytic anemia patients. Blood Coagul Fibrinolysis 2011; 22: 280–284.
26. Origa R, Comitini F. Pregnancy in thalassemia. Mediterr J Hematol Infect Dis 2019; 11, e2019019.
27. Salama KM, Ibrahim OM, Kaddah AM, Boseila S, Ismail LA, Abdel Hamid MM. Liver enzymes in children with beta-thalassemia major: Correlation with iron overload and viral hepatitis. Maced J Med Sci 2015; 3: 287–292.
28. Sanctis VDe, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, Daar S, Wali Y, Yassin M, Soliman N, Sobti P, Jaouni S, Al Kholy ME, Fiscina B, Angastiniotis M. β-thalassemia distribution in the old world: An ancient disease seen from a historical standpoint. Mediterr J Hematol Infect Dis 2017; 9.
29. Selmi C, Leung PS, Fischer L, German B, Yang CY, Kenny TP, Cysewski GR, Gershwin ME. The effects of Spirulina on anemia and immune function in senior citizens. Cell. Mol. Immunol. 2011; 8: 248–254.
30. Suman R, Sanadhya A, Meena P, Goyal S. Correlation of liver enzymes with serum ferritin levels in beta-thalassemia major. Int J Res Med Sci 2016; 4: 3271–3274.

31. Uludağ A, Uysal A, Uludağ A, Ertekin YH, Tekin M, Kütük B, Silan FÖ. Prevalence and mutations of β-thalassemia trait and abnormal hemoglobins in premarital screening in Çanakkale province, Turkey Balk J Med Genet 2016; 19: 29–34.
32. Yakoot M, Salem A. Spirulina platensis versus silymarin in the treatment of chronic hepatitis C virus infection. A pilot randomized, comparative clinical trial. BMC Gastroenterol 2012; 12.
33. Zhai N, Li H, Song H, Yang Y, Cui A, Li T, Niu J, Crispe IN, Su L, Tu Z. Hepatitis C virus induces MDSCs-like monocytes through TLR2/PI3K/AKT/STAT3 signaling. PLoS One 2017; 12.
34. Zhu Y, Cao X, Tao G, Xie W, Hu Z, Xu D. The lymph index: A potential hematological parameter for viral infection. Int J Infect Dis 2013; 17