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Effect Of Is Spirulina Supplementation on Blood Haemoglobin (Hb) And Plasma Protein Level of HIV Infected Patients

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ABSTRACT

Drop in blood hemoglobin (Hb) and plasma protein level in HIV infected patient is commonly seen due to various reasons like ineffective functioning of body organs, diminished immune function, increased catabolism, low food intake, poor digestion and adverse effect of antiretroviral therapy. Study was conducted to examine the effect of spirulina supplementation on blood hemoglobin (Hb) and plasma protein level of HIV infected patient. Study included 62 HIV infected patients, T0 (control)- 29 patients (20 male, 9 female), T1 (Treatment) - 32 patient (23 male, 9 female). T1 (Treatment) were provided with 5 g/day of Spirulina supplementation in capsule form along with therapeutic diet for period of six months with their regular medical prescription. Data was collected at beginning and end of study and was statistically analyzed. Results reveled non-significant decrease in blood hemoglobin by -0.07 mg/dl (male) and -0.60 mg/dl (female) in control group. In T1 (Treatment) group blood hemoglobin increased by 0.067 mg/dl (female) and 0.83 mg/dl (male) which was significant. Plasma protein level found to decrease in by -0.185 mg/dl (male) and -0.34 mg/dl (female) in control group. In T1 (Treatment) group plasma protein level though nonsignificant increased by 0.28 mg/dl (male) and 0.19 mg/dl (female). Result revealed positive effect of spirulina supplementation on Blood hemoglobin (Hb) and plasma protein level of HIV infected patient

Keywords: *Blood hemoglobin, Plasma protein, HIV, Spirulina*.

1. INTRODUCTION

The term Acquired Immunodeficiency Syndrome (AIDS) was used to describe the condition like *Pneumocystis carinii* pneumonia and unexplained persistent lymphadenopathy in previously healthy homosexual men who had rare diseases such as Kaposi sarcoma. An infectious microorganism responsible for causing the condition was transmittable through sexual contact and/or through blood transfusion **MMWR, (1981), Fauci, (2003) and Hoffman, (2004).** Similar symptoms called OJAKSHAYA are found in Sushruta Samhita in ayurveda **Kulkarni (1999).** Total number of people living with HIV world wide is 39.9 million where adults (15+ years) 39.6 million, women (15+ years) 20.5 million, men (15+ years) 18.1 million and children (< 15 years) 1.4 million by end of Year 2023, **WHO (2024)**. World population review states that by end of 2023, India ranks second in world for having 2.5 million of patients living with HIV. Out of total 1.1 million (15+ years) are women and 0.6 million children (< 15 years) **UNAIDS (2024).** HIV-infected patients are at nutritional risk at any point in their illness. Severe malnutrition and weight loss, particularly loss of lean tissue, and delayed weight gain and height velocity in children, can affect morbidity and mortality. **Judith Nerad *et al.*, (2003)**

Consumption of a balanced healthy diet, is vital for health and survival for all individuals regardless of HIV status. Energy requirements in HIV are likely to increase by 10% to 30 % and protein content by 12% to 15% of total energy intake to maintain body weight and physical activity in asymptomatic HIV-infected adults. **Coors (2001).** Intake of micronutrients at RDA levels may not be sufficient to correct nutritional deficiencies in HIV-infected individual so micronutrient supplements, e.g. vitamin A, zinc and iron need to be provided. **Fawzi *et al.*, (2003).**

Spirulina (Cyanobacterium) is single cell blue green alga, first living organism on earth that converts sunlight into food or life energy by process of photosynthesis in presence of sunlight and water having evolution history of more than 3.6 billion years **Odgerel Bumandalai *et al.*, (2024).** Various species of *Spirulina* grow in different type of water viz. fresh water, tropical springs, salt water and salt pans under saline condition of pH between 9 to ll. Temperature does not matters more as growth is not affected up to 40°C **Jahan *et al.*,** (2023)

Haemoglodin classified as metalloprotein, chromoprotein, globulin protein present in red blood cells (RBCs) that transports oxygen in body through blood. Abnormal haematology is considered second leading cause of morbidity and mortality

in HIV-positive patients where prevalence of Anemia (haematological abnormality) is common and reduction in HB level is is considered as predictor of HIV disease progression in HIV patients. Presence of anaemia in HIV positive patients is estimated tobe 63% to 95%. **Owiredu *et al.,*** (2011), **Jam S *et al.,*** (2009) and **Sullivan PS *et al.,*** (1998)

Mostafa H. Ahmed *et al.,* 2020.Sylvia A *et al.,* 2023 conducted research on e-waste recyclers (n=142) and a reference population (n=65) and evaluated their blood profile. Results concluded that dietary intake of proteins and iron (Fe) was associated with concomitant increase in Hb levels of both groups. Hence consumption of protein along with iron (Fe) supplement is equally important.

Mao *et al.,* (2005) analysed nutritional composition of *Spirulina* and found moisture content of *Spirulina* to be 7 %, carbohydrate 15.7 %, protein to be 72 %, vitamin 0.75 %, ash 9 %. Iron present in dry *Spirulina* is 45 mg /100g. Iron uptake by body from *Spirulina* is high (high bioavailability) compared to other food products due to absence of iron absorption inhibitors **Uliyaramain *et al.,* (2000)**. Microorganisms used as protein sources eg. yeast (*Chlorella, Scenedesmu*s) have cellulose wall which is absent in *spirulina* cells but have a relatively fragile envelope wall of murein **Bujard E *et al.,* (1970)**. Presence of fragile envelope of murein is considered as main reason for very high digestibility of proteins present in spirulina (83-90% of digestibility against 95.1% for pure casein).

Gustafson K and Cardellina (1989) isolated Calcium *Spirulina* (Ca-Sp) a sulfated polysaccharide isolated from blue green alga *Spirulina* which found to be potent antiviral inhibitor of the replication of enveloped viruses such as herps simplex virus type-1 (HSV-1). **Hayashi *et al.,* (1993)** isolated sulfated-polysaccharide, Calcium Spirulan (Ca-SP), that inhibits the replication *in vitro* of several enveloped viruses including Herpes simplex type I (HSV-1), human cytomegalovirus (HVMV), measles virus, mumps virus, influenza A virus, and HIV-1 virus. Dextran sulfate (DS) is known potent anti-HIV-1 agent. **Hayashi *et al.,* (1998)** compared anti-HSV-1 activity of Dextran sulfate (DS) with Calcium Spirulan (Ca-SP) and revealed that anti-HIV-1 activity of Calcium Spirulan (Ca-SP) was higher than that of Dextran sulfate (DS).

Considering nutritional benefits of Spirulina research study was designed where spirulina powder supplementation in form of capsule was given to HIV infected patients

2. MATERIAL AND METHODS

Sample selection and sample size:

Concern research study totally focused on HIV patient, Survey was conducted on 146 family in Kolhapur, Sangli and Satara district where one of the person was suffering with HIV. 76 HIV infections patients between age of < 18 to 60 years were selected by purposive sampling method for study. Out of total 76 HIV infected, 15 patients got dropped out during study and final number of HIV infected patient by end of study was 61.

Study design:

Table 1: Samples selection for the experiment

Sr. No	**Treatment**	**Male**	**Female**	**Total**
1	T0	**20**	**9**	**29**
2	T1	**23**	**9**	**32**
Total		**43**	**18**	**61**

T0- Control - In this treatment only primary data was collected through questionnaire. Data related to Bio chemical analysis of blood was collected both at beginning and end of study.

T1- Treatment - In this treatment patient were given knowledge regarding nutrition and provided with *Spirulina* nutraceutical supplementation. Continuous follow up was taken for assure the regular consumption of *Spirulina* nutraceutical by the treatment group. Data related to Bio chemical analysis of blood was collected both at beginning and end of study.

Dosage - Being rich source of protein, nucleic acid content of spirulina is also more hence average recommended dose of dry spirulina is between 2 and 8.5 g in 24 hours. **FAO 2008.** Dry powder of *Spirulina Platensis* was procured from Antenna Trust, Madurai, Tamil Nadu and encapsulated in empty gelatin capsules of '0' size weighing 500 mg each. 10 capsule (500mg each) per day (5 g of dry spirulina powder per day) was supplemented to (T1)treatment group throughout the period of study.

Hemoglobin (Hb) analysis:

Cyanme themoglobin method was used to determine Hemoglobin (Hb) analysis Blood hemoglobin (Hb%) levels. Potassium

ferricyanide, potassium cyanide, and sodium bicarbonate was used to prepare Drabkin's solution. 5.0 ml of Drab kin's reagent and 20 microliters (µL) of blood mixed together and hold for 5 min to develop color **Pinta virooj *et al.*, 2021**. Samples were analyzed for results at 546 nm **Bashir and Derar 2019**. An average of three Hb readings was taken for each blood sample analysed. Average levels of Hb in each sample were then calculated as [A546unk × Hb Standard concentration (g/dl)]/A546std. The World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels < 13.0 g/dL was used to diagnose anemia in male e-waste recyclers and reference groups in this study.

Calculation-

$$\text{Hemoglobin unknown g/dL} = \frac{\text{Unknown sample}}{\text{Standard known Hb content}} \times \text{Con. of Hb standard (g/dl)}$$

Plasma protein analysis:

Plasma protein was analyzed by using Biuret reagent. 0.1 ml aliquot of standard, test and blank (distilled water) were add 5 ml of biuret reagent. Mix well and keep for 30 min and readings of OD and standard were taken at 540 nm. **NIN (2023)**

Calculation:

$$\text{Total protein g/100ml} = \frac{\text{O.D of Test}}{\text{O.D of Standard}} \times \text{Conc. Of Std.}$$

Statistical analysis

Comparison of blood metal Hb and plasma protein overtime of HIV infected patient. As sample size in treatment was near to 30 't test' was used for statistical analysis. 't' test is used to measure difference between two treatments expressed in unit of standard error. 'p' value was used to measure the probability of findings provided at extreme 't' level. **Takyi *et al.*, (2021), SA Takyi *et al.*, (2020), SA Takyi *et al.*, (2020).** Data was analyzed by using SPSS statistics software.

3. RESULTS

Table 2 and Figure 1 shows the Effect of special nutritional support on Blood Hb of HIV infected patients during course of research work. Conducted study stated that mean blood Hb level of men in control (TO) group dropped from 12.30 (g/dl) to 11.60(g/dl). Reduction in HB was 0.7 mg/dl showing 't value' of 0.065 which was non-significant. Females (Control TO group) already had low Hb compared to that of males. Hb level decreased from 10.50 mg/dl to 9.90 mg/dl which was by 0.6 mg/dl during research period. Dropdown in Hb level was non-significant having 'p value' of 0.415. Similar noting's were done by **Opie J. (2012)**

In Male (T1 group) blood Hb level increased by 0.90 mg/dl stating the importance of nutritional diet and Spirulina supplementation having 'p value' of 0.0465 which was significant at 5 % level. In female (T1 group) Blood Hb level increased by 1.1 mg/dl in T1 group and 't value' of 0.022 which was significant at 5 % level. Simpler findings were noted by **Rajbir Sachdeva *et al.*, (2004)** where thirty under nourished girls when supplied with two grams of Spirulina for five days in weak for period of two months shower significant increase in HB level from 11.7 (g/dl) to 12.5 (g/dl)

Table 2: Effect of special nutritional support on Blood Hb of HIV infected patients

Gender	Treatment	Mean Before	Mean After	Means Difference	S.E.of Difference	't'cal	'p'value
Male	**TO**	12.35	11.60	-0.70	0.37	1.90	0.0652 NS
	T1	11.80	12.70	0.90	0.35	1.82	0.0465*
Female	**TO**	10.50	9.90	-0.60	0.72	0.84	0.415 NS
	T1	10.81	11.92	1.10	0.63	1.97	0.022*

* indicate significance of value at P=0.05, NS = Non-significant, The result is significant at $p < 0.05$, T0-Control, Tl- Spirulina supplementation.

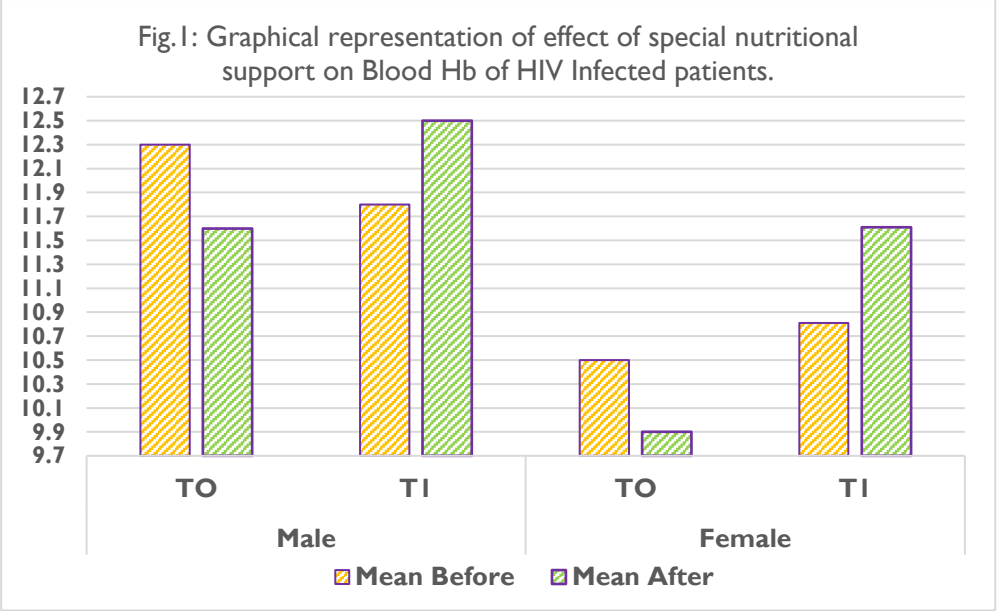
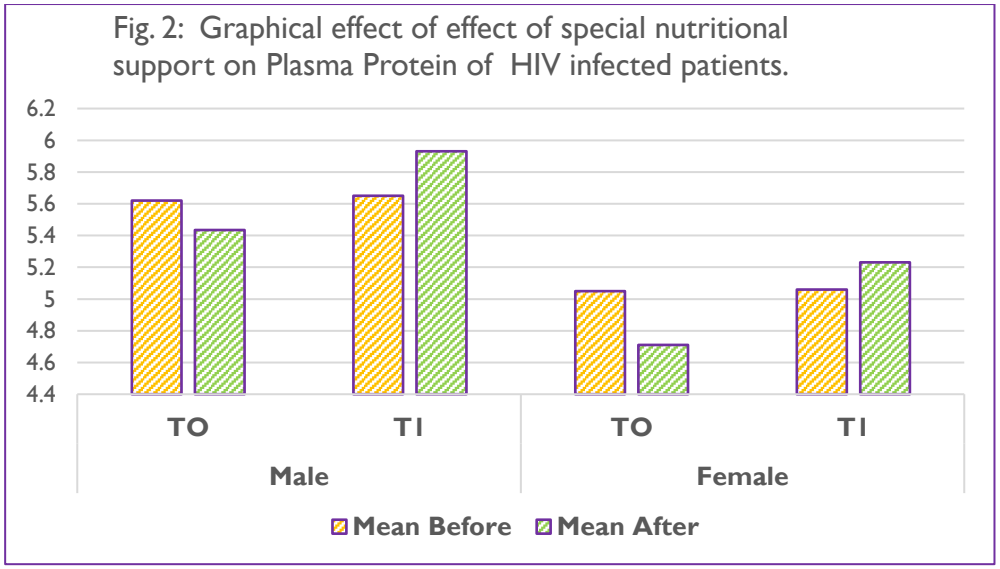


Table 3 and Figure 2 shows the Effect of special nutritional support on Plasma Protein of HIV infected patients during course of research work. Conducted study stated that mean plasma protein level of men in control (TO) group dropped from 5.62 (g/dl) to 5.43 (g/dl). Reduction in Plasma protein was -0.185 g/dl showing 't value' of 0.22 which was non-significant. Females (Control TO group) level decreased from 5.05 g/dl to 4.71 g/dl which was by -0.26 g/dl during research period. Dropdown was non-significant having 'p value' of 0.22. Similar finding noted by **Jamirah Nazziwa *et al.*, 2019** where lower protein balance was seen due to a lower dietary intake of energy and protein and a greater rate of protein oxidation. In treatment group Male (T1 group) Plasma Protein level increased by 0.28 g/dl having 'p value' of 0.488 which was not significant at 5 % level. In female (T1 group) Plasma Protein level increased by 0.17 g/dl and had 'p value' of 0.562 which was not significant rise at 5 % level.

Table 3: Effect of special nutritional support on Plasma Protein of HIV infected patients

Gender	Treatment	Mean Before	Mean After	Means Difference	S.E.of Difference	't'cal	'p'value
Male	**TO**	5.62	5.435	-0.185	0.15	1.25	0.22 NS
	T1	5.65	5.93	0.28	0.17	2.03	0.488 NS
Female	**TO**	5.05	4.71	-0.34	0.26	1.28	0.22 NS
	T1	5.06	5.23	0.17	0.29	0.59	0.562 NS

* indicate significance of value at P=0.05, NS = Non-significant, The result is significant at $p < 0.05$, T0-Control, Tl- Spirulina supplementation.



4. CONCLUSION

Research findings concluded that blood Hb level and plasma protein level reduce gradually in control group (TO). Drop down in blood Hb both in female and male was observed which was non-significant, but significant drop down could be observed if study could have been extended further period of next six month. Similar picture of non-significant drop down in control group was seen in case of plasma protein. In treatment group (T1) positive findings were seen in case of Blood Hb as rise seen was significant both in male and female. Positive change in plasma protein was seen in treatment group though statistical rise was non-significant. Thus it can be conclude that Spirulina supplementation had positive effect on blood HB level in HV infected patient

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