Multiple Micronutrients (vitamins E, C, beta-carotene) Intervention to Immunomicronutrients deficient Drug Addicts

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Citation

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Abstract

Background: The study has conducted on immunodeficient drug addicts. They were supplemented with immnunoenhancer antioxidant micronutrients aiming to improve the immunity. Method: Fifty immunodeficient drug addicts (IgG-deficient with impaired immune cells) were supplemented with vitamins E, C and -carotene for 60 days. Twentyfive immunodeficient addicts treated as placebo control. Immunoglobulins and immune cells profile were analyzed before and on completion of micronutrients intervention. BMI of the study subjects were also assessed. Results: Supplementation of vitamin E, C and beta-carotene to the immnunodeficient drug addicts had significantly ($p<0.05$) increased BMI (18.3±2.4 vs 19.5±1.9), serum IgG (4.85±0.46g/L vs 6.06±0.65g/L), lymphocytes (60.2±19.5 vs 68.4±13.6), eosinophils (6.6±3.7 vs 7.8±5.9), but had decreased IgM (1.33±0.38 g/L vs 1.18±0.27g/L), monocytes (6.5±4.1 vs 5.2±2.3) and neutrophils (126.4±19.8 vs 118.5±16.2). BMI or immune components did not apparently alter in the placebo group during this 60 days period. However, compared to the placebo control group, supplementation of the micronutrients resulted in a significant ($p<0.05$) increase of IgG (6.06±0.65 g/L vs 5.04±0.40g/L), lymphocytes (68.4±13.6 vs 57.3±9.8), eosinophils (7.8±5.9 vs 6.1±2.3), but a decrease of IgM (1.18±0.27g/L vs 1.40±0.27g/L in the interventional group. Conclusion: Supplementation of immunoenhancing antioxidant vitamin E, C and -carotene to immunodeficient subject upregulates immunity.

INTRODUCTION

Illicit drug use induces immunmicronutrients deficiencies (Islam et al, 2001; 2004: Thomas et al, 1995) that contribute to the development of immunodeficiency (Chandra, 1997; Marcos et al, 1997; Varela et al, 1997a; 1997b). This leads the drug addicts to be susceptible to infectious agents including HIV (Islam et al, 2003; Chandra, 1997; Varela et al, 1997a; 1997b). It is well documented that natural antioxidant nutrients like vitamin E, C and beta-carotene are potent immunoenhancers[678]; Blackburn, 2001) and they play an important role in the maintenance of immunity (Chandra, 2001) Like deficiency, overload of certain micronutrients is immunotoxic (Chandra, 2001; Shankar and Prasad, 1998). It has recently been documented that multiple micronutrient therapy has become a leader over the single micronutrient therapy to improve immunity in deficient subjects (Bachou, 2001; Hurrell, 2001;Allard et al, 1998; Scrimshaw and SanGiovanni, 1997; Jeng et al, 1996; Shankar and Prasad, 1998). Therefore, in the present study, multiple natural antioxidant vitamins have supplemented to multiple immunomicronutrient deficient drug addicts to improve their immunity.

MATERIALS AND METHODS

Immunomicronutrient deficient drug addicts, who had vitamin E, C, A, and particular IgG deficiencies and impaired peripheral immune cell status, were recruited for therapeutic supplementation with antioxidant vitamin E, C and -carotene. A total of seventy-five IgG-deficient (IgG ≤5.50g/L) with impaired immune cells was singled out by screening 253 antioxidant vitamins deficient drug addicts. Fifty of them were grouped as supplemental group and twenty-five as placebo group.

They were admitted into the hospital. After briefing the perspective of the study and having a consent, antropometric data (height and weight) of each of the deficient subjects were measured. A volume of 5ml venous blood specimen was collected aseptically from antecubital vein of each of the deficient addicts. An antioxidant vitamin preparation containing -tocopherol (50mg), ascorbic acid (200mg) and -

carotene (6mg) each, branded as 'Carocet®' of Beximco Pharma Ltd, Dhaka, Bangladesh, was supplemented to the immunonutrient deficient drug addicts. A single dose of 'Carocet®' was given daily for 60 days. After 15 days of intervention in the hospital, they were discharged with prescription to continue this medication for another 45 days, and to attend the follow up clinic every week. Carocet® dose for 7 days was given when they attended at the follow up clinic. The placebo group received placebo tablet. Of the study subjects, finally 44 drug addicts had completed the intervention therapy and 20 immunodeficient addicts remained as placebo control. On the 60th day, weight of each addict was measured and 5ml venous blood sample was again collected. No dietary advice was given to either the case or placebo subjects, but they were followed up strictly to abstain from addictive drugs during the period of intervention therapy.

BLOOD ANALYSIS

A blood film was prepared on a microscopic slide for immune cell estimation (Islam et al, 2004). Blood sample was then processed for serum extraction, which was aliquoted into eppendorf tubes and stored at –20°C for analysis of immunoglobulins.

Serum concentrations of mmunoglobulins were estimated by solid phase indirect ELISA as described by Islam et al (2004). Microtiter ELISA plate (NUNC Immuno plate, Denmark) was coated with 100µl respective diluted (1:1000 with PBS) anti-human IgG, IgA, IgM (Sigma Chemicals Co, USA), incubated for overnight at 4°C, washed (x3) with PBS (containing 0.5% Tween20) and dried by gentle striking the plate face down on wads of paper towels. The wells were blocked with 100µl sheep serum solution (1%v/v in washing buffer), incubated for 1h at 37°C and treated as above. Then 100µl diluted test sera and serially diluted standard immunoglobulins (Sera-Pak®, Immuno, Bayer, USA) were pipetted into the pre-marked wells and incubated at 37°C for 2h. After aspirating excess antibodies, plates were washed and dried similarly. Next 100µl diluted (1:500) peroxidase conjugated respective anti-human IgG, IgA, IgM (Sigma Chemicals Co, USA) was pipetted into each well, and incubated and treated as above. Finally, 100µl of substrate solution (0.001% TMB in 0.1M sodium acetate buffer containing H_2O_2) was added to the every well and incubated in dark at room temperature for 50min. Then 50µl of 10% sulphuric acid was added to each well to stop enzyme reaction. The plates were read at 450nm in an ELISA plate reader (Labsystems, MultiskanEX, Finland).

RESULTS

Supplementation of vitamin E, C and β-carotene to the immnunodeficient drug addicts had significantly ($p<0.05$) increased BMI (18.3±2.4 vs 19.5±1.9), serum IgG (4.85±0.46g/L vs 6.06±0.65g/L), lymphocytes (60.2±19.5 vs 68.4±13.6), eosinophils (6.6±3.7 vs 7.8±5.9), but had decreased IgM (1.33±0.38 g/L vs 1.18±0.27g/L), monocytes (6.5±4.1 vs 5.2±2.3) and neutrophils (126.4±19.8 vs 118.5±16.2) (table 1). BMI or immune components did not apparently alter in the placebo group during this 60 days period (table 2). However, compared to the placebo control group, supplementation of the micronutrients resulted in a significant ($p<0.05$) increase of IgG (6.06±0.65 g/L vs 5.04±0.40g/L), lymphocytes (68.4±13.6 vs 57.3±9.8), eosinophils (7.8±5.9 vs 6.1±2.3), but a decrease of IgM (1.18±0.27g/Lvs 1.40±0.27g/L in the interventional group (table 3).

Figure 1

Table 1: Immunonutritional profile of vitamins receiving drug addicts (n=44)

Parameter	0 Days		60 Days		P-value*
	% (n)	Mean±SD	% (n)	Mean±SD	
BMI 14.0- 16.0	6.8(3)		0		
16.1- 16.9	22.7(10)	18.3±2.4	0	19.5±1.9	t=2.47
17.0- 18.4	38.6(17)		36.4(16)		p=0.02
18.5- 25.0	27.4(12)		59.1(26)		
25.1- 28.0	4.5(2)		4.5(2)		
Immunoglobulin (g/L)			0		
IgG 3.50- 4.50	25.0(11)		20.5 (9)		
4.51- 5.50	75.0(33)	4.85±0.46	54.5 (24)	6.06±0.65	t=10.09
5.51- 6.50	0		25.0 (11)		p=0.00
6.51- 7.50	0				
IgA 1.50- 2.50	61.4(27)	2.45.±0.65	22.7(10)	2.29±0.50	t=1.27
2.51 – 3.50	29.5(13)		77.3(34)		p=0.21
3.51- 4.50	9.1 (4)		0		
IgM 0.50- 1.00	29.5 (13)	1.33±0.38	22.7(10)	1.18±0.27	t=2.14
1.01- 2.30	70.5 (31)		77.3(34)		p=0.04
2.31- 3.00	0		0		
Immune cells					
Lymphocytes					
30- 50	40.9 (18)	60.2±19.5	6.8 (3)	68.4±13.6	t=2.29
51- 80	36.4 (16)		79.5 (35)		p=0.03
81- 100	22.7 (10)		13.6 (6)		
Monocytes					
2- 5	59.1 (26)	6.5±4.1	68.9(29)	5.2±2.3	t=1.83
6- 15	36.4(16)		31.8 (14)		p=0.07
16- 25	4.5 (2)		2.3 (1)		
Neutrophils					
60- 80	0	126.4±19.8	4.5 (2)	118.5±16.2	t=2.01
81- 120	17(38.6)		52.3 (23)		p=0.05
121- 160	27(61.4)		43.2 (19)		
Eosinophils					
4- 10	79.5(35)	6.6±3.7	86.4(38)	7.8±5.9	t=1.10
11- 15	18.2(8)		4.5 (2)		P=0.27
16- 32	2.3(1)		9.1(4)		

*Significant P< 0.05.

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Human normal serum IgG, IgA and IgM ranges are 5.0-12.0 g/L, 0.5- 3.5 g/L and 0.3- 2.3g/L respectively (34,35). Human peripheral immune cell ranges are- lymphocytes: 40-90 cells, monocytes: 4- 20 cells, neutrophils: 80-150 cells and eosinophils: 2- 12 cells / 200 leukocytes (35).

Legend Replicate analysis was carried out for every sample.

Descriptive statistics: Frequencies, descriptive, crosstabes.

Compare means: Independent-sample t- test.

Figure 2

Table 2: Immunonutritional profile of placebo receiving drug addicts (n=20)

Parameter	0 Days		60 Days		P-value*
	% (n)	Mean±SD	% (n)	Mean±SD	
BMI 14.0- 16.0	10.0(2)		5.0(1)		
16.1- 16.9	15.0(3)	18.7±2.6	5.0(1)	19.0±2.4	t=0.42
17.0- 18.4	35.0(7)		40.0(8)		p=0.34
18.5- 25.0	35.0(7)		50.0(10)		
25.1- 28.0	5.0(1)		0		
Immunoglobulin (g/L)					
IgG 3.50- 4.50	30.0(6)		0		t=1.93
4.51- 5.50	70.0(14)	4.80±0.41	70.0 (14)	5.04±0.40	p=0.06
5.51- 6.50	0		30.0(6)		
6.51- 7.50	0		0		
IgA 1.50- 2.50	70.0(14)		70.0(14)		t=0.58
2.51 – 3.50	25.0(5)	2.32.±0.62	30.0(6)	2.22±0.42	p=0.56
3.51- 4.50	5.0(1)		0		
IgM 0.50- 1.00	30.0 (6)		10.0(2)		t=0.94
1.01- 2.30	70.0 (14)	1.31±0.35	90.0(18)	1.40±0.27	p=0.35
2.31- 3.00	0		0		
Immune cells					
Lymphocytes	55.0(11)		35.0(7)		t=0.19
30- 50	25.0(5)	58.3±20.5	65.0(13)	57.3±9.8	p=0.85
51- 80	20.0(4)		0		
81- 100					
Monocytes					
2- 5	55.0(11)	6.4±3.5	80.0(16)	4.7±1.5	t=2.06
6- 15	40.0(8)		20.0(4)		p=0.05
16- 25	5.0(1)		0		
Neutrophils					
60- 80	0	129.7±21.7	0	132.1±11.2	t=0.44
81- 120	35.0(7)		25.0(5)		p=0.66
121- 160	65.0(13)		75.0(15)		
Eosinophils					
4- 10	80.0(16)	6.2±3.3	100.0(20)	6.1±2.3	t=0.11
11- 15	20.0(4)		0		P=0.91
16- 32	0		0		

*Significant P< 0.05

Legend Replicate analysis was carried out for every sample.

Descriptive statistics: Frequencies, descriptive, crosstabes.

Compare means: Independent-sample t- test.

Figure 3

Table 3: Comparison between supplement group (n=44) and placebo group (n=20)

Parameter	Supplement group		Placebo group		P-value*
	% (n)	Mean±SD	% (n)	Mean±SD	
BMI 14.0- 16.0	0		5.0(1)		
16.1- 16.9	0	19.5±1.9	5.0(1)	19.0±2.4	t=0.79
17.0- 18.4	36.4(16)		40.0(8)		p=0.430
18.5- 25.0	59.1(26)		50.0(10)		
25.1- 28.0	4.5(2)		0		
Immunoglobulin (g/L)					
IgG 3.50- 4.50	0		0		t=6.44
4.51- 5.50	20.5 (9)	6.06±0.65	70.0 (14)	5.04±0.40	p=0.000
5.51- 6.50	54.5 (24)		30.0(6)		
6.51- 7.50	25.0 (11)		0		
IgA 1.50- 2.50	22.7(10)		70.0(14)		t=0.51
2.51 – 3.50	77.3(34)	2.29±0.50	30.0(6)	2.22±0.42	p=0.61
3.51- 4.50	0		0		
IgM 0.50- 1.00	22.7(10)		10.0(2)		t=3.02
1.01- 2.30	77.3(34)	1.18±0.27	90.0(18)	1.40±0.27	p=0.003
2.31- 3.00	0		0		
Immune cells					
Lymphocytes					
30- 50	6.8 (3)	68.4±13.6	35.0(7)	57.3±9.8	t=3.27
51- 80	79.5 (35)		65.0(13)		p=0.002
81- 100	13.6 (6)		0		
Monocytes					
2- 5	68.9(29)	5.2±2.3	80.0(16)	4.7±1.5	t=0.93
6- 15	31.8 (14)		20.0(4)		p=0.36
16- 25	2.3 (1)		0		
Neutrophils					
60- 80	4.5 (2)	118.5±16.2	0	132.1±11.2	t=3.35
81- 120	52.3 (23)		25.0(5)		p=0.001
121- 160	43.2 (19)		75.0(15)		
Eosinophils					
4- 10	86.4(38)	7.8±5.9	100.0(20)	6.1±2.3	t=1.23
11- 15	4.5 (2)		0		p=0.222
16- 32	9.1(4)		0		

*Significant P< 0.05

Legend Replicate analysis was carried out for every sample.

Descriptive statistics: Frequencies, descriptive, crosstabes.

Compare means: Independent-sample t- test.

DISCUSSIONS

Miconutrient malnutrition is induced by deficiencies of multiple micronutrients. Isolated micronutrient deficiency is rare with the exception of iron, vitamin A, and zinc (Chandra, 2001). Even, it is reported that function of certain micronutrients needs the presence of others (Hurrell, 2001). Therefore, in recent time multiple micronutrients intervention has become a leader to address the micronutrient deficiencies (Bachou, 2001; Hurrell, 2001).

Supplementation of immunoenhancing antioxidant vitamins to immunomicronutrients deficient drug addicts was observed to increase IgG, lymphocytes, and eosinophils. It has been documented that micronutrient deficiencies is associated with lymphoid atrophy and impaired development, proliferation and function of lymphocytes, thus suppresses synthesis of antibodies, and a slight excess of micronutrients like antioxidant vitamins, zinc improve immunity (Chandra, 1997; 2001; Blackburn, 2001; Shankar and Prasad, 1998). Therefore, supplementation of vitamins E, C and beta-carotene to the immunodeficient drug addicts may repair the lymphoid atrophy, and thus induce lymphocytosis and synthesis of IgG. Supplementation of micronutrients resulted in decrease of mean IgM level, but it was observed that before supplementation, 30% (n=13 subjects were with lower IgM which turned into 23% (n=10) after intervention indicating IgM level was improved to some deficient drug addicts. Further initially 71% (n=31) deficient subjects had mid level IgM that became 78% (n=34). This is also telling that supplementation has increased IgM level. The higher neutrophils and eosinophils in the immunodeficient drug addicts would be involved in clearing pathogens. The decrease of the leucocytes after supplementation of micronutrients suggests upregulation of immunity.

This study reveals that supplementation of immunoenhancing antioxidant vitamin E, C and β-carotene to immunodeficient subjects upregulates immunity.

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