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Effects of Drug Addiction on Antioxidant Vitamins and Nitric Oxide Levels

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ABSTRACT

Background: The drug addicts are at risk of high oxidant stress and reduced antioxidant vitamins including A, C and E that could be influenced by drug habit and life style factors. So the aim of the present study was to determine plasma levels of vitamin C, E, β-carotene and nitric oxide concentrations in drug dependents and at the same time, to detect the effect of multiplicity and duration of addiction on these antioxidant vitamins in such subjects.

Subjects& Methods: The study was conducted on 150 subjects, their ages ranged between 12 and 58 years. They were addicted to the consumption of certain drugs of abuse, specifically cannabis, morphine, barbiturate and benzodiazepine. In addition to, 30 age, height and socioeconomic matched healthy volunteers as a control group. Detection of substance abuse by enzyme immunoassay method and thin layer chromatography was done. Also measurement of Vitamin C, β-carotene, α- tocopherol, and plasma levels of nitric oxide for drug addicts and controls.

Results: There were significant higher levels of the plasma nitric oxide in drug dependent group than control group (59.04±18.69 versus 30.62±9.46 umol/L, P<0.001) and significant lower plasma β-carotene (43.49±23.33 versus 62.73±22.44 ug/dl), vitamin C (0.61±0.18 versus 1.09±0.14 mg/dl) and α-tocopherol (8.65±3.94 versus 13.38± 4.03 mg/l, p<0.001) mean values in the drug dependent group than control group "P<0.001" Multiplicity of abusing substances by the same person was common and gave marked affection in the plasma nitric oxide and antioxidant vitamins levels. The plasma nitric oxide level is directly related while β- carotene, vitamin C and α- tocopherol are inversely related with duration of substances abuses.

Conclusion: Drug addicts had high nitric oxide and low antioxidant vitamins levels. The most important factors affecting an oxidant "nitric oxide", antioxidants "β-carotene, vitamin C and α-tocopherol" levels inside the body is the duration and number of substances abuse. By increasing the number and period of dependency the victims may be more exposed to free radical which consumed the antioxidants in their body.

KEY WORDS: Addiction- Nitric Oxide- Antioxidants- Duration of Addiction- Number of Abused Substances.

INTRODUCTION

It is widely accepted that drug addiction is a social worldwide health problem (1). It affects every sphere of human life (2). young adults' lives are damaged (3); teenagers experiment with drugs and use of illicit drugs has soared with the 'baby-boom' generation (4). Although research on drug abuse is too much in the Western world, there are few reports regarding drug abuse effects on antioxidant status. In spite of its fatal consequences worldwide, research on illicit drug use has received relative little attention in Egypt.

Drug addiction impairs nutritional status and immunity (5). It is well documented that abuse of heroin, cocaine, morphine, marijuana etc., induces immunonutritional deficiencies, which leads to infection and malnutrition. In addition to affecting food habits and under-mining appetite, the use of illicit drugs leads to malnutrition, which is a composite syndrome of multiple nutrient deficiencies. Cigarette smoking among addicts may also be responsible for nutritional deficiencies (3).

In general, the consumption of fruit and vegetables in drug addicts is lower than general population and they are more inclined to consume food that has low vitamin content (6,7). Therefore, drug addicts have micronutrient deficiencies, including antioxidant vitamins (8). Vitamin E, C and A, in addition to their antioxidant functions, they play an important role in immunity (9). Deficiencies of these vitamins may contribute to the development of immunodeficiency in drug addicts.

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Vitamin C, α-tocopherol (vitamin E) and β-carotene (vitamin A precursor) are important antioxidants in human body, playing an important role in catching and eliminating excess free radicals such as O.-2, hydroxyl free radical (OH.), hydrogen peroxide free radical (HO.2), singlet oxygen (1O2) or hydrogen peroxide (H_2O_2) (10). Antioxidants prevent the physiological or pathological aggravation of free radical chain reactions. These protecting biological membranes of cells against injuries by oxidation, peroxidation and lipoperoxidation (11). Moreover, vitamin C, α-tocopherol and β-carotene can promote synthesis and stabilization of immunoglobulin in the human body and obstruct formation of carcinogens such as nitrosamine and others (12). Marked decrease of antioxidant levels and antioxidases activities can cause pathological metabolic disorders and aggravation of a series of free radical chain reactions. Thus seriously damaging DNA, proteins, enzymes, biological membranes and immunologic functions (10,13).
Nitric oxide (NO) is a common gaseous free radical, derived by the endothelium reduces the tone of vascular smooth muscle, and plays an important role in the physiological equilibrium in human (14). Therefore, the metabolic and functional states of NO are closely related to human health (13). On the other hand, as NO is a very active free radical, an excess NO in the human body can induce physiological or pathological aggravation of oxidative stress, destroying the dynamic balance between oxidation and antioxidation (15, 16).
So the aim of the present study was to determine plasma levels of vitamin C, E and β-carotene concentrations and at the same time, nitrite/nitrate 'the stable end products of nitric oxide metabolism' was also estimated in drug dependents. Also to detect the effect of multiplicity and duration of addiction on these antioxidant vitamins in such subjects.

MATERIALS AND METHODS

I- Subjects:

The present study realized in a group of 150 subjects of them 148 males and two females. Their ages ranged between 12 and 58 years with a mean of 24.19 ± 7.84 years. They were addicted to the consumption of certain drugs of abuse, specifically cannabis, morphine, barbiturate and benzodiazepine, which were transported or visited the Toxicology Unit of Emergency Hospital, Mansoura University. Thirty of them were arrived to the hospital in a comatose condition. They were of different levels of education and social states. All of them were cigarette smoking.
The diagnosis of psychoactive drugs and substances was based on the history taken and positive clinical signs and symptoms. Moreover, all were subjected to urine analysis for psychoactive drugs and substances. Exclusion criteria included medical disorders including a current acute or chronic illness, impaired hepatic or renal functions, cardiac disorders, diabetes, tuberculosis, cancer, severe asthma and using prescribed medicines including antioxidants. The studied cases were divided into 3 groups according to the number of the abused substances detected by thin layer chromatography (TLC): group (a) one abused substance, group (b) two abused substances, group (c) three or more abused substances. Also cases were divided into 3 groups according to the duration of the abused substances: subgroup (a) less than three years, subgroup (b) from three to five years and subgroup (c) more than five years.
At the same time 30 age, height and socioeconomic matched healthy volunteers who never used these drugs even therapeutically for at least one month and not taking any raw tobacco or cigarette were selected as a control group. Their ages ranged between 15 and 53 years with a mean of 24.8 ± 4.64 years.
Written consent from all subjects (patients and controls) was obtained. The study protocol was approved by local ethics of the hospital.

II- Methods:

All subjects included in this study were subjected to the followings: history taking, physical examination, laboratory investigations (substance abuse detection, routine laboratory tests, and the objected oxidants and antioxidants measurements).

A- Methods for detection of abused substances:
1- Sampling:
From each patient with positive history of substance abuse 100 ml urine were obtained “at the time of arrival and again before receiving any treatment”. Catheter was used if the patient was comatose or unable to void urine. Each sample was collected in a clean, dry, labeled container without preservation.
2- Preliminary tests:
One ml of different urine samples were analyzed for the presence of opiates, barbiturates, benzodiazepines, cannabis, alcohol, tricyclic antidepressants and amphetamnines by Syva, Solaris SLN 1067 Version 3.00 L. using Emit® d.a.u. Assay on the same day.
3- Chromatographic confirmation test:
Thin layer chromatography (T.L.C) was used to confirm positive results obtained by Emit system and detect drugs that could not be detected by Emit. Confirmation of the presence of morphine and opiates (tramadol) were detected using the method of Meadway *et al*., 1998(17). While, Delta 9 THC-11-oic acid (the metabolite of Cannabis), benzodiazepine and barbiturates, were detected by using the method of George and Braithwaite, 1995 (18).

B- Methods for detection of oxidants and antioxidants plasma levels:

Measurements of plasma levels of vitamin C, α- tocopherol, β-carotenoids and nitric oxide metabolites were done.

1- Sampling:

15 ml blood samples were collected from every subject "patients and controls". Of them three ml blood samples were collected into plain tube and non haemolysed sera were separated by centrifugation and used for assay of liver and kidney functions and lipid profile. Five ml blood samples were separated immediately after extraction, serum was treated with 5% trichloroacetic acid and then centrifuged at 3000 rpm for 10 min, the clear supernatant thus obtained and stored at -20°C until analysis of vitamin C.

The remaining blood were collected into tube containing EDTA "1mg/ml of blood" and aprotinin "500 KIU/ml of blood" and mixed gently. The non haemolysed plasma was separated by centrifugation. These samples were preserved in aliquots at -20°C till being used for determination of α-tocopherol, β-carotene and nitric oxide metabolites.

2- Determination of vitamin C "ascorbic acid" (19):

The concentration of ascorbic acid in the serum was determined by spectrophotometry using phenyl hydrazine indicators. Ascorbic acid was oxidized by Cu^{+2} to form dehydroascorbic acid, which reacted with acidic 2, 4 dinitrophenyl-hydrazine to form a red bis-hydrazone, which measured at 520 nm. The chemicals used were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Co, USA.

3- Determination of β- carotenoids and α-tocopherol (20):

β-carotene and α-tocopherol plasma levels were assayed by HPLC, kit supplied by Immundiagnostik Bensheim, Germany. The chromatographic system is a reverse phase (C18) using system gold, Model 126 pump and 166 variable wave length Uv/vis detectors, Beckman, USA. Separation was performed using 5 um ultrashere ODS column (150 x 4.6 mm). Flow rate 1.2 ml/min. β- carotenoids was detected at 325 nm, and α-tocopherol detected at 300 nm. Wavelength switched after 7 min. Results were calculated according to the following equation:

$$\text{Concentration of sample: } \frac{\text{Peak height of sample X conc. of calibrator}}{\text{Peak height of internal standard in the sample}} \text{ X F}$$

F: is the peak height of internal standard in the calibrator/peak height of calibrator

4- Determination of nitric oxide metabolites (21):

This assay determines total nitric oxide based on the enzymatic conversion of nitrate to nitrite. The reaction was followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction. The Griess Reaction is based on the two-step diazotization reaction in which acidified NO2 produces a nitrosating agent, which reacts with sulfanic acid to produce the diazonium ion. This ion is then coupled to N- (1-naphthyl) ethylenediamine to form chromophoric azo-derivative, which absorbs light at 540 nm.

Statistical analysis:

Statistical Package Social Science (SPSS), version 11 was used. Normality of data was detected by Kolmogorov Samirnov test. Data was expressed as mean ± SD, being parametric. A probability value (P) less than 0.05 considered to be significant. Analysis of one-way variance (ANOVA) was used to find the effect of drug multiplicity and duration etc, on the serum vitamin levels.

RESULTS

Table (1): Showed the demographic characters of the studied drug dependents and controls. Most of drug dependants (78.9 %) lived at urban areas and 70.7% were of low social state. Also most of them were of a low educational level (68.7%), 28% were in a moderate educational level and 3.3% were at a high education level.

All patients gave positive history (cannabis 76%, benzodiazepine 65.3%, barbiturates 25.3% and opiates 22.3%) and results by TLC (cannabis 76.6%, opiates72.6%, benzodiazepine 52.6% and barbiturate 4%) of drug abuse "150 patient'' **(Figure 1).**

There were significant higher level of the plasma nitric oxide metabolites "NO" in drug dependent group than control group "P <0.001" and significant lower plasma β-carotene, vitamin C and α-tocopherol mean values in the drug dependant group than control group "P<0.001" **(Table 2).**

24.7% of cases were positive to one abused substance, 45.3% cases were positive to two abused substances. 29.3% cases were positive to three abused substances and one (0.7%) case gave positivity to four substances abused. There was a significant increase of the mean plasma NO level with increasing the number of the abused substances "P=0.005". However no significance difference was found between plasma nitric oxide metabolites level in group "b" when compared with group "c" "P=0.995". Overall, there was no significant difference in between the mean plasma β-carotene levels, vitamin C, α-tocopherol in the three studied groups having different number of abused substances "P=0.382, 0.587, 0.820 respective1y **(Table 3).**

As regard the duration of the abused substances, 51.2% had a duration of the abuse substance <3 years, 19.4% had a duration of substance abuse 3-5 years and 29.4% of the studied cases gave a history of more than 5 years of the substance abuse. However, there was, a non significant increase in the mean plasma nitric oxide metabolites level with prolongation of duration of the substance abuse "P=0.546". Also there was non significant decrease in the mean plasma β-carotene levels with prolongation of the duration of the substance abuse "P=0.301". On the other hand there was a significant difference of the plasma vitamin C level of the three studied subgroups with different duration of drug abuse "P=0.02". However, there was no significant

difference in between the mean plasma vitamin C level in the subgroup "b" when compared with subgroup "c" "P=0.946". Drug dependents with longer duration of substances abuse " b & c" subgroup had a significant lower plasma vitamin C level compared to drug dependants with shorter duration of substance abuse subgroup "a" "P=0.050 & P=0.050" **(Table 4).**

There was a significant decrease in the mean plasma α-tocopherol level with increase of the duration of drug abuse in the drug dependants "P=0.03". Drug dependents with longer duration of substance abuse subgroup " b" had a significant lower value when compared with those of shorter duration of the substance abuse subgroup "a" "P=0.034". However there was a non significant lower value when compared "c" subgroup with "a & b" subgroups "P=0.198 & P=0.634" respectively **(Table 4).**

Table (1): The demographic characters of the studied drug dependant and control groups.

Studied groups	***Drug dependant group***	***Control Group***
Parameter	N = 150	N = 30
Body mass index (kg/m²): Means ± SD	22.49 ± 3.34	28.85 ± 4.64
Sex: Male Female	148 2	30 ---
Social State: Low Moderate High	106 39 5	19 7 4
Educational Level: Low Moderate High	103 42 5	12 10 8

Low educational level: Not end the primary stage.
Moderate educational level: End the preparatory or secondary stages.
High educational level: End the university study.
Low social level: social score "0-7".
Moderate social level: score: "8-13".
High social level: social score: "above 13".

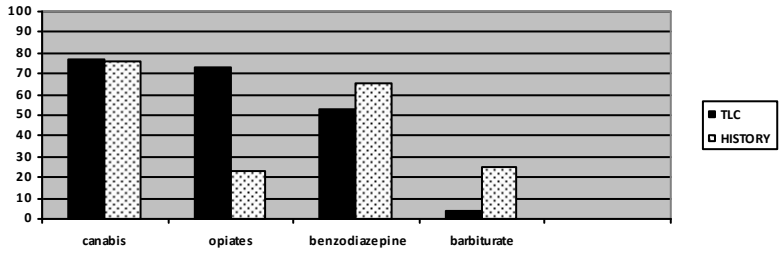

Figure (1): Percentage of drug abuse detection and identification by history recording and TLC in the studied drug dependent group.

Table (2): Plasma nitric oxide, β-carotene, vitamin C and α-tocopherol levels in both drug dependent and control groups.

Studied group **Parameters**	**Drug dependant group** **n="150"**	**Control group** **n="30"**	**Significance**
Nitric oxide (µmol/L) Range Mean ± SD	24.6 - 99.0 59.04 ± 18.69	10.3 - 46.5 30.62 ± 9.46	P= 0.001*
β-carotene (µg/dl) Range Mean ± SD	11.0 - 91.0 43.49 ± 23.33	19.0 - 91.0 62.73 ± 22.44	P= 0.001*
Vitamin C (mg/dl) Range Mean ± SD	0.22 - 0.99 0.61 ± 0.18	0.83 - 1.84 1.09 ± 0.14	P= 0.001*
α-tocopherol (mg/L) Range Mean ± SD	0.29 - 17.68 8.65 ± 3.94	1.05 - 18.63 13.38 ± 4.03	P= 0.001*

* Significant at P ≤ 0.05.

Table (3): The relation between plasma total nitrites, β-carotene, vitamin C and α-tocopherol levels and number of abused substances.

Number of abused substances **Parameter**	***One abused Substance*** **Group "a" (n=37)**	***Two abused substances*** **Group "b" (n=68)**	***Three abused substances*** **Group "c" (n=45)**	***Significance test*** **"ANOVA test"**
Nitric oxide (umol/L) Range Mean ± SD	24.60 - 81.5 50.40 ± 14.51	25.00 - 99.0 61.73 ± 18.49	25.0 - 98.0 62.06 ± 20.06	P=0.005*
β-carotene (ug/dl) Range Mean ± SD	11.0 - 91.0 38.97 ± 23.04	13.0 - 91.0 45.52 ± 23.64	11.0 - 91.0 44.13 ± 23.12	P=0.382
Vitamin C (mg/dl) Range Mean ± SD	0.32 - 0.99 0.63 ± 0.19	0.22 - 0.95 0.61 ± 0.18	0.32 - 0.92 0.59 ± 0.16	P=0.587
α-tocopherol (mg/L) Range Mean ± SD	2.29 -17.60 8.99 ± 3.94	2.62 - 17.37 8.56 ± 3.99	2.81-17.68 8.48 ± 3.88	P=0.820

n = number. Significant at P ≤ 0.05.

Table (4): The relation between plasma "total nitrites, β-carotene, vitamin C and α-tocopherol levels and duration of intake of substance abuse.

Duration of abuse **Parameter**	***<3 years*** **n=77 Subgroup "a"**	***3-5 years*** **n=29 Subgroup "b"**	***> 5 years*** **n=44 Subgroup "c"**	***Significance test*** **"ANOVA test"**
Nitric oxide (umol/L) Range Mean ± SD	25.0 - 99.0 57.41 ± 18.13	24.6 - 97.5 60.41 ± 18.22	25.0 - 90.0 62.98 ± 20.09	P=0.546
β-carotene (ug/dl) Range Mean ± SD	11.0 - 91.0 45.53 ± 22.57	13.0 - 91.0 45.03 ± 24.59	11.0 - 91.0 38.91 ± 23.73	P=0.301
Vitamin C (mg/dl) Range Mean ± SD	0.32 - 0.99 0.65 ± 0.17	0.34 - 0.93 0.56 ± 0.17	0.22 - 0.92 0.57 ± 0.18	P=0.020*
α-tocopherol (mg/L) Range Mean ± SD	2.62-17.68 9.42 ± 3.89	2.93- 17.6 7.32 ± 3.60	2.29-16.5 8.16 ± 3.98	P=0.030*

Significant at P ≤ 0.05.

DISCUSSION

Drug addiction has been recognized as a social, health and also political problem and it need to be addressed urgently (3). The present study analyzed the plasma nitric oxide metabolites, vitamin C, β-carotene and α-tocopherol levels in drug dependants cases and control subjects, in an attempters to assess the influence of illicited drugs on the nitric oxide and antioxidant vitamins status, also to detect the effect of multiplicity and duration of addiction on their blood levels.

The present study realized in a group of 150 subjects, they were addicted to the consumption of certain drugs of abuse, specifically cannabis, morphine, barbiturate and benzodiazepine. The majority of them were below 30 years, which reflected that, the consumption of drugs of abuse is affecting fundamentally to the young sector. Also, most of them were of low social state (68.7%). In this respect some authors found that the users of marijuana and other drugs were mainly in the late adolescence and twenty years old (4,22), and there is marked affection of the social and educational state on addicted person(2,23,24).

In the present study, the majority of cases "75.3%" gave positive results for more than one substance by thin layer chromatography. This declared the problem of substance abuse in Egypt due to multiplicity of abusing substances by the same person. In accordance with the present results are those reported by Islam *et al*, (3) in Bangladesh who found that 39.5% of drug dependants take one to two abused substances and 60.5% of drug dependants take three or more of abused substances.

On the other aspect, in developed countries the economic level is high that allows the drug dependants to buy the drug by any price. But in developing countries the economic level is low, so any available cheap psychoactive substances, producing effect or nearby it may be taken. Also, the addict may take more than one type of different drugs to produce synergism of their actions (25).

There are much free radical, such as NO, O., .OH in bango are inhaled and entered the blood when the abusers were abusing bango, or result from the metabolism of other drug as opiates, benzodiazepines or barbiturates (26). These free radicals inactivated antioxidants. Moreover, the NO may combine with O.-2 to produce the super-oxide nitroso free radicals "ONOO-"

which has strong oxidative abilities (27). The free radicals attacked DNA, proteins, enzymes, biological membranes and various biochemical components in the cells, resulting in damaging cell function (15,16). In the present study there was a significant higher concentration of nitric oxide metabo1ites in the drug dependant group than does in control group. Zhou *et al.*, (2002) (25) measured the oxidants antioxidants status in 114 heroine abusers. They found significant higher nitric oxide levels in the heroin abusers than in healthy volunteers. At the same time, the plasma levels of β- carotene, vitamin C and α-tocopherol were significantly decreased in the drug dependents (13).

Results of the present study showed significant lower concentrations of antioxidants vitamins E, C and A in the drug addicts than those in the non-addict controls. This is because of the illicit drug use and alteration of food habits. Drug abusers are prone to malnutrition for several reasons e.g. reduced intake of food calories owing to the anorexic effect of addictive stimulants; decreased income for food purchases owing to under employment or diversion of funds for drug (28). Also ignorance of or indifference to basic principles of healthful nutrition and direct adverse effects of drugs on nutrient absorption and/or metabolism considered additional causes to malnutrition (29). So it is difficult for drug dependents to acquire sufficient amount of vitamins from dietary sources (3,5,8,30). Also, a partial reduction of the vitamin levels may be due to cigarette smoking (3). In addition, the drug dependents had to use a great quantity of antioxidants vitamins in the bodies to catch and clean these excess free radicals (31).

As increase the number of abused substances, there was a highly significant increase in plasma nitric oxide levels, because more abused substance produces more free radicals that more inactivate antioxidants. In the same aspect, Zhou *et al.*, (2002) (25) found that, by increasing the amount of daily intake of heroin '0.1 gm/day, 1gm/day and 2gm /day, there was a highly significant increase in plasma nitric oxide level.

As regard the effect of duration of the abused substances, the present study showed an inverse relationship between β-carotene, vitamin C and α-tocopherol levels and the duration of substances abuse. There was a decrease in the plasma β-carotene, vitamin C and α-tocopherol levels with prolongation of the duration of abuse. However, the decrease was statistically significant only with vitamin C and α-tocopherol levels. Accordingly the abused substances dependency produces nutritional deficiency (32), and the use of multiple abused substances for long duration may result a greater deficiency of these vitamins (33). Also Islam *et al.*, (2001) (3) measured plasma β-carotene, vitamin C and α-tocopherol levels in drug dependants and found a negative correlation between the number of abused substances and duration of abuse with the levels of plasma β-carotene, vitamin C and α-tocopherol.

CONCLUSION

This study revealed that, drug addicts had high nitric oxide levels and low β-carotene, vitamin C and α-tocopherol levels. The most important factors affecting an oxidant "nitric oxide" and antioxidants "β-carotene, vitamin C and α-tocopherol" levels inside the body is the duration and number of substances abuse. By increasing the number and period of dependency the victims may be more exposed to free radical which consumed the antioxidants in their body. In view of these findings the drug dependents should acquire sufficient quantity of antioxidants such as vitamins A, C and E to abate the injuries by oxidant-antioxidant disturbance.

Conflict of interest:

There is no conflict of interest.

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