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Evaluation of Some Biochemical Variables among Smoker Athletes in Kirkuk City

Essa Omran Jassim[1*], Sayran Sattar Saleh[2]

[1]Master Student, College of Science, University of Kirkuk, Iraq.*
[2]Professor, College of Science, University of Kirkuk, Kirkuk, Iraq.

Email: [2]sayran.2017@uokirkuk.edu.iq
Corresponding Email: [1]essaomran62@gmail.com*

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Abstract: The study aimed to evaluate the levels of some biochemical variables such as vitamins C, E, and D, as well as albumin and uric acid levels, in addition to levels of fatty proteins in the blood serum of a group of smoking athletes and compare the results with the levels of these biochemical variables in non-smoking athletes (as a control group). The study sample included 100 individuals divided into two groups: smoking athletes (60 individuals) and non-smoking athletes (40 individuals), in addition to calculating the Body Mass Index (BMI).The study results showed lower levels of vitamins C and E in smokers athletes compared to non-smokers athletes, while vitamin D levels were low in both smokers and non-smokers athletes. The results also showed a decrease in albumin and uric acid levels in smokers' athletes. Additionally, levels of fatty proteins (T.G, Cholesterol, HDL, LDL, VLDL) were measured, and the levels were within the normal range for both smokers and non-smokers athletes.

Keywords: Smoking, Athletes, Vitamins, Antioxidants.

1. INTRODUCTION

Physical exercise and activities play a vital role in the body's functional capacities due to the physiological changes they induce. These activities and exercises help prevent muscle weakness, walking impairments and balance disorders [1]. Regular physical activity and moderate exercise enhance physical strength, motor abilities, and increase bone mineral levels [2]. Physical activity significantly contributes to the prevention of a wide range of chronic diseases and premature deaths. There is a correlation between certain medical conditions such as cardiovascular diseases and the performance of exercises and physical activities. Engaging in these practices reduces the occurrence of heart diseases, vascular disorders, colon issues, and osteoporosis [3].Regular physical exercise and sports activities

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can help alleviate the pain associated with certain diseases. Physical exercise also aids in overcoming various substance-related disorders, such as reducing or quitting smoking [4]. Antioxidants in the body act as a defense system against oxidative stress caused by free radicals or reactive oxygen species [5]. Antioxidants exist in the body in the form of enzymes, elements, enzyme cofactors, or sulfur-containing compounds like glutathione [6] [7]. Natural antioxidants are found in vegetables, fruits, and some medicinal plants, playing important roles in protecting the body against germs and safeguarding DNA molecules from damage induced by free radicals [6].Fatty proteins are compounds that are insoluble in water but soluble in organic solvents. They are of great importance to living organisms as they are involved in the structure of cell membranes, serve as essential sources of energy and storage, and also play a role in the synthesis of steroid hormones [8].Cigarette smoke (CS) contains over 4000 compounds, including at least 200 toxicant, 80 known or suspected carcinogens. Moreover, cigarette smoking generates many toxic and carcinogenic compounds harmful to the health, such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide and free radicals [9]. Nicotine is commonly consumed via smoking cigarettes, cigars or pipes [10]. Carbon monoxide in tobacco smoker exerts a negative effect on the heart by reducing the blood's ability to carry oxygen .Although cigarette smoking is a strong risk factor for cardiovascular disease, its relationship with hypertension remains unclear [11].

2. RELATED WORKS

[12] Reported that current smokers had over a seven-fold odds ratio of deficiency compared with non-smoker, [13] showed two- to three-fold more deficiency in smokers respectively. Smokers tended to have dietary intakes that were lower in vitamin C, e.g., lower fruit and vegetable intake and higher fat intake. [14] Revealed a decrease in the level of uric acid in the blood serum of smoking athletes compared to non-smoking athletes. This decrease is attributed to the reduced internal production due to the generation of free radicals caused by smoking in the body, leading to oxidative stress that lowers the uric acid levels in the blood. The results of the two studies above were consistent with the findings of our current study.

3. METHODOLOGY

Study Design, Subjects, and Sample Collection

The study was conducted from 1st September 2023 to 10th December 2023, involving 100 male athletes aged between 18 and 30 years, collected from various sports clubs in Kirkuk city. The study samples were divided into two groups: the first group included 60 smoking athletes, and the second group included 40 non-smoking athletes as a control group.Blood samples were collected for current study by pulling (5 cc) of the vein blood with a one-time syringe, after which blood was placed in the gel tube tubes with a tight hood, then the tubes were placed in the centrifuge for 5 minutes at 3,000 cycles/minutes, then the serum was pulled by a micropipette microscopic absorber, after which the serum was divided into several divisions and distributed in an Eppendorf tube at 1.5 ml, and those samples were Preserved at -2 °C until measuring biochemical variables.

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Biochemical Assays Vitamin D Concentration
Vitamin D concentration was determined using VIDAS 25 OH Vitamin D TOTAL (VITD) is an automated quantitative test for use on the instruments of the VIDAS family for the determination of 25-hydroxyvitamin D Total in human serum or plasma using the ELFA technique (Enzyme Linked Fluorescent Assay). The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).

Vitamin C Concentration
Vitamin C concentration was determined using the sandwich method, an ELISA technique. This involves detecting antigens in blood serum using antibodies specific to the PEPD protein. Antibodies labeled with HRP are added to the antigen, creating a sandwich-like structure. The enzyme's base material (TMB) is added, forming a blue-colored solution that turns yellow after adding a stop solution. The absorbance of the solution is measured at a wavelength of 450nm.

Vitamin E Concentration
Vitamin E concentration was determined using the sandwich method, an ELISA technique. This involves detecting antigens in blood serum using antibodies specific to the PEPD protein. Antibodies labeled with HRP are added to the antigen, creating a sandwich-like structure. The enzyme's base material (TMB) is added, forming a blue-colored solution that turns yellow after adding a stop solution. The absorbance of the solution is measured at a wavelength of 450nm.

Albumin Concentration
The estimation of albumin concentration in serum relies on the quantity of albumin, which interacts with the reagent 3,3,5,5 – Tetra Bromocresol Green (BCG). Albumin exhibits a high affinity for binding with these dyes, forming a chromogenic complex known as Albumin-BCG with a green color, and its absorbance is measured at a wavelength of 630 nm[15].

Uric Acid
Uric acid is the final product of purine metabolism, where the enzyme uricase oxidizes uric acid to allantoin and hydrogen peroxide. In the presence of the enzyme peroxidase (POD), hydrogen peroxide is further oxidized with the compounds 4-AminoAntipyrine (4-AA) and 3, 5-Dichloro-2Hydroxybenzene Sulphonate to form the complex Quinonimine, representing the concentration of uric acid in the serum.

Triglycerides
The level of serum triglycerides is estimated using the Fossati and Princip method associated with the Trinder reaction.

Cholesterol
The level of cholesterol in the blood serum was estimated using the enzymatic method, by converting cholesterol into a dye (Quinone imine) in the presence of three enzymes: cholesterol esterase, cholesterol oxidase, and peroxidase enzyme.

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High-Density Lissspoprotein Cholestero

The level of high-density lipoprotein cholesterol in the blood serum was estimated using the enzymatic method, where chylomicrons and lipoproteins precipitate for both VLDL-ch and LDL-ch by adding phosphotungstic acid and magnesium ions, leaving HDL-ch in the blood serum after ultracentrifugation.

LDL Serum

LDL serum was determined by applying Friedewald formula [16]: LDL (mg/dL) = Total cholesterol – HDL cholesterol – TG/5. VLDL-C level was determined by derivation from the following formula: VLDL-C cholesterol (mg/dL) = Triglycerides/5.

Body Mass Index (BMI) Measurement

In order to calculate BMI, it is necessary to measure current weight without bulky clothing, using the same technique for all participants, with kilograms for weight and meters for height. The BMI was calculated by using the formula weight/height2 (kg/m2).

Table 1 Values of Body Mass Index (BMI) for the Studied Groups

Groups	**Smoker athletes**		**Non-smoker athletes**	
	No.	%	No.	%
(18-24.9)kg/m^2	49	81.6	28	70%
(25-30) kg/m^2	11	18.4	12	30%
Total	60	100%	40	100%

Table 2 illustrates the mean and standard deviation of the body mass index for the studied groups

Groups	**Mean±SD**		**p-value**
	Smoker athletes n= 60	Non-smoker athletes n=40	
(18-24)kg/m^2	22.26±1.941	21.92±1.601	0.4371
(25-30) kg/m^2	27.15 ±1.771	27.40 ±1.336	0.4074

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism v8.0 (Graph Pad Software, San

Diego, CA, USA), and mean standard deviation (SD) was used to express the results. The comparison of mean±SD was performed using the T test, and statistical significance was defined as $P \leq 0.05$.

4. RESULTS AND DISCUSSIONS

Table 3 represents the levels of Vitamins (D, C and E), albumin, uric acid, and fatty proteins (T.G, Cholesterol, HDL, LDL, VLDL) in smoker athletes, compared with results with nonsmoker athletes.

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Table 3 shows the Levels of some biochemical parameters as mean±SD for studied groups

Parameters	Smoker Athletes (n=60) mean±SD	Non -Smoker Athletes (n=40) mean±SD	P-value
Vit. D (ng/mL)	18.75 ±5.776	19.25 ±6.446	P≤0.74
Vit .C (ng/mL)	1.848 ±0.8840	3.470 ±1.266	P≤0.02
Vit .E (Pg/mL)	3.392±1.068	4.641±2.182	P≤ 0.01
Uric acid (mg/dL	5.457± 1.014	5.988± 1.053	P≤ 0.01
Albumin (mg/dL)	4.578±0.3915	4.796±0.4594	P≤ 0.01
T.G (mg/dL)	149.2±77.21	155.9±66.41	0.65 P≤
HDL (mg/dL)	37.43±6.471	38.18±7.639	0.60 P≤
Cholesterol(mg/dL)	162.3±56.39	178.9±60.94	0.16 P≤
LDL (mg/dL)	96.46±56.26	110.8±60.73	0.22 P≤
VLDL (mg/dL)	29.94±15.55	31.18±13.28	P≤ 0.68

The results of current study showed that there was a significant difference (*P*≥0.74) in vitamin D levels, a significant decrease (*P*≤0.02) in vitamin C , and significant decrease (*P*≥0.01) in vitamin E there was significant decrease (*P*≥0.01) in albumin levels, and a significant decrease (*P*≤0.01) in uric acid levels, there was a non-significant difference in fatty proteins levels(T.G (P≤0.65)/ Cholesterol (P≤0.60)/ HDL(P≤0.16)/ LDL(P≤0.22)/ VLDL(P≤0.68)) in smoker athletes, compared with results with non-smoker athletes . The current study revealed insufficient levels of vitamin D in samples of both smoking and non-smoking athletes, as vitamin D levels are considered inadequate when ranging between 20-30 nag/ml [17].The decrease in vitamin D levels in both smoking and non-smoking athletes is attributed to limited exposure to sunlight and certain diseases such as heart and vascular diseases, kidney diseases, diabetes, and others. Vitamin D levels vary according to gender, age, and region. Previous studies have indicated a 54% deficiency rate of vitamin D in the Arab region and Asia [18] , which is higher than other regions due to a diet lacking in foods rich in vitamin D such as fish, along with a lifestyle that lacks proper and consistent sun exposure to obtain vitamin D [19]. The current study also showed a decrease in vitamin C levels among smoking athletes compared to the control group. These findings are consistent with several previous studies, including a study conducted by[12] which indicated that smoking athletes are more than seven times more likely to have a vitamin deficiency compared to non-smokers[20]. This is due to the oxidative substances in cigarette smoke leading to oxidative stress in the body [21], as well as smokers tending to consume lower amounts of vitamin C [12]. In addition to the impact of smoking on vitamin C deficiency, there are other factors and causes contributing to low levels of this vitamin, such as a diet lacking in vitamin-rich foods, environmental factors, environmental pollution, body mass index, and genetic variables affecting vitamin C metabolism. Vitamin levels can also decrease during illness [12].Our current study results have shown lower levels of vitamin E in smokers compared to non-smokers among athletes. These findings align with Menotti and

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others, who demonstrated in their study that vitamin E levels are negatively affected and decreased due to cigarette smoking [22]. This is attributed to the accumulation of excess free radicals and other reactive molecules generated by cigarette smoke. These free radicals and reactive molecules reduce the levels of vitamin E while it acts as an antioxidant by neutralizing the activity of free radicals, depleting its concentrations and consequently lowering its levels in the body [22] .The current study results align with previous findings by [14] indicating a decrease in uric acid levels among smoking athletes compared to non-smoking athletes. This decrease in uric acid levels is attributed to the reduced internal production due to smoking-induced free radical production in the body, leading to oxidative stress that lowers the acid levels in the blood [14]. These findings are consistent with a study by [23] which demonstrated lower uric acid levels in smokers compared to the control group due to chronic exposure to cigarette smoke, a source of oxidative stress, and reduced intake of dietary antioxidants [23].The current study results align with findings showing a decrease in uric acid levels in smoking athletes compared to non-smoking athletes, as concluded by [14]. The reduction in uric acid levels is attributed to decreased internal production due to smoking-induced free radical production in the body, leading to oxidative stress that diminishes blood acid levels. The study also revealed lower albumin levels in the serum of smoking athletes compared to non-smokers. Cigarette smoke chemicals directly and indirectly affect serum protein, resulting in alterations in albumin binding properties. Furthermore, albumin possesses antioxidant properties, and high levels of free radicals lead to increased protein degradation activity [23][24].Despite the close relationship between smoking and body fat levels by reducing HDL-C levels through altering important fat-carrying enzymes [25], thereby reducing the activity of the lecithin cholesterol enzyme and altering cholesterol ester transfer protein and hepatic lipase activity in the blood [26]. Fatty protein levels did not show significant differences in increase or decrease among smoking athletes compared to non-smoking athletes because the physical exercises and activities done by athletes are associated with an increase in plasma [27].concentrations of beneficial cholesterol and antioxidant enzymes. On the other hand, physical exercises contribute to accelerating metabolic processes in athletes' bodies, resulting in burning larger amounts of fat and reducing its levels in the body. The results of the current study align with [27], indicating that physical exercises performed by athletes significantly contribute to reducing body fat levels and mitigating the risks of heart and vascular diseases and other medical conditions[27] .

5. CONCLUSIONS

The current study findings indicate that vitamin D levels are decreased in both smoking and non-smoking athletes, with smoking not having an impact on vitamin D levels as they are closely related to sun exposure, the primary source of this vitamin, as well as dietary patterns and other factors. Additionally, the study shows a decrease in vitamin C and E levels, as well as a decrease in uric acid and albumin levels in smoking athletes compared to non-smoking athletes, showing that smoking does have an effect on these biochemical variables in the body. The results of this study demonstrated that exercise has a significant relationship with lipid profiles and body composition.

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