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The Impacts of Vitamin C on Iron Levels, Total Iron Binding Capacity, and Malondialdehyde Concentration Among Smokers and Non-smokers in Kurdistan Region of Iraq: A Comparative Study

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Abstract

The purpose of this study is to estimate the iron concentration and total iron-binding capacity in the serum of smokers and non-smokers as well as to evaluate the lipid peroxidation state by measuring malondialdehyde concentration in healthy people, as well as influence of consumption constant dose of vitamin C on malondialdehyde concentration. This study consists of two parts, the first part of our study is dedicated to the evaluation of iron concentration in smokers and non-smokers. Iron is an essential mineral that the body is using to function in the proper way, ascorbic acid has a significant enhancing effect on iron absorption when assessed by giving smokers vitamin C supplementation. Our goal is to examine the effect of vitamin C on iron levels in the body and lipid peroxidation. Iron, total iron binding capacity concentration, and transferrin saturation tests are carried out at the chemistry lab at Erbil Polytechnic University's (EPU) Medical Laboratory Department. The second part of our research concerns the concentration of malondialdehyde, lipid peroxidation is an important process in which oxidants or free radicals focus on fatty acids containing a carbon-carbon double bond (PUFA). This process produces various compounds, one of which is malondialdehyde. These compounds can be estimated as a marker of lipid peroxidation by using Thiobarbituric Acid Assay (TBA) tests, according to Aust and Buege method for MDA using a spectrophotometer at 535 nm. A total of 120 individuals were involved in the determination of the iron level and total iron binding capacity, 60 blood samples of smokers were collected from individuals of different ages, and 60 samples of control which we refer to as non-smokers, the tests measured first for the smokers, then after oral intake of vitamin c supplementation, the tests are repeated and compared to that before taking the oral supplement. And for the determination of malondialdehyde concentration, 60 healthy male volunteers from the Kurdistan region were recruited for this study, who aged between 20 and 50 years old. The participants were required to have no chronic illnesses and not be smokers. Over the course of 40 days, they were asked to take a daily dose of 180mg of vitamin C as a supplement. Under doctor permission to prevent at least minimum damage for volunteers. As a result of the first part, there was a significant difference in mean iron concentration and total-iron binding capacity with P values of (0.007, 0.0017), and a non-significant difference in transferrin saturation with a P value of 0.6011. The iron and total iron binding capacity saturations were significantly affected by the facilitating effect of vitamin C on iron absorption from vitamin C oral supplements. As a result of the second part of this study, participants out of 60 individuals, 34 (57.78%) were classified as having a normal weight, 17 (28.89%) were overweight, 6 (8.89%) were classified as obesity class I, and 3 (4.44%) were underweight. In the results indicate that the use of vitamin C supplements decrease serum level of malondialdehyde significant level at ($p < 0.001$). The results were obtained my comparing the level of malondialdehyde before taking vitamin C supplementation (7.500) and after taking vitamin C supplementation (4.688). The results show that antioxidants like vitamin C supplementation reduce free radicals. The correlation between MDA levels and weight before taking Vitamin C is very weak and not statistically significant (p=0.634). Similarly, the correlation between MDA levels and weight after taking Vitamin C is weak and not statistically significant (p=0.152). Therefore, there appeared to be no significant relationship between MDA levels and weight in both cases.

Keywords: Vitamin C, ascorbic acid, lipid peroxidation, total iron binding capacity, malondialdehyde, free radical

Introduction

Vitamin C is also called Ascorbic Acid (AA) which is a water-soluble vitamin that is present naturally in some food such as (oranges, lemon, kiwi, cabbage, broccoli, pepper, etc.), and also as a supplement available. Ascorbic acid also has antioxidant activity that protects cells from damage caused by free radicals (Devaki and Raveendran, 2017), additionally, ascorbic acid is an essential nutrient involved in the formation of certain proteins and enzymatic production of certain neurotransmitters (Devaki and Raveendran, 2017) and involves in the absorption of micronutrients especially enhancing the absorption of iron (Li et al., 2020) Ascorbic acid is important for collagen synthesis, and also participate in protein metabolism, this vitamin is vital in wound healing and immune function also it is an important antioxidant, approximately 70%-90% of AA is absorbed in a moderate rate of 30-180 mg/day, non-metabolized has been excreted in urine. However, smoker people need 35mg of AA more than non-smoker people who need 90 mg/day. Vitamin C can protect lipids, carbohydrates, and protein, even in small amounts.

Tobacco smoke contains many toxic, carcinogenic, and mutagenic chemicals, as well as stable and unstable free radicals with the potential for biological oxidative damage. Cigarette smokes contain about 4,720 mutagenic and toxic substances (a toxic blend of poisons and cancer-causing materials) such as aromatic hydrocarbon, carbon monoxide, and nicotine, nicotine is one of the majors that cause harmful body effects. Smoking has been one of the main oxidative stress causes, various free radicals are formed by smoking and cause vital macromolecular damage, for instance, proteins, carbohydrates, and lipids. Increasing free radical production or reducing the antioxidant protective system will increase free radical activity. Cigarette smoke is one of the free radical producers, nicotine induces oxidative stress and it has been reported malondialdehyde serum concentration is higher in smoker people compared with non-smokers. Free radicals are normal byproducts of human metabolism as oxygen is utilized, FRs are atoms or groups of atoms that have at least one unpaired electron and are constantly on the hunt for additional electrons, and are highly reactive with other chemicals in the body.

Iron is a mineral essential for growth and development, and has the capacity to donate and accept electrons easily, this capability makes it physiologically essential as a useful component of cytochromes and oxygen-binding molecules. Iron mainly exists in complex forms bound to proteins, such as heme compounds, heme enzymes, or non-heme compounds (Transferrin, Ferritin) (Abbaspour et al., 2014). However, iron is also biochemically dangerous it can damage tissue by catalyzing the conversion of hydrogen peroxide to free radical ions that attack cellular membranes, proteins, and DNA. Ascorbic acid facilitates iron absorption by forming a chelate with the ferric iron at an acid pH that remains soluble in the alkaline pH of the duodenum. Ascorbic acid regulates cellular iron uptake, ferritin expression, and cell iron efflux (Lane and Richardson, 2014), and also plays an important role in the defense system. Therefore, it may be possible to relieve the smoke-induced damage by increasing the protective defense system.

Malondialdehyde (MDA) is an organic compound with a $CH_2(CHO)_2$ chemical formula, it is one of the products produced by lipid peroxidation of polyunsaturated fatty acid. MDA has a low molecular weight the most mutagenic product of lipid Peroxidation, and has been the most popular indicator for cell and tissue oxidative damage. Using Antioxidants is suitable and effective to scavenge free radicals and inactivate peroxides and could delay and prevent oxidation (Mansouri et al., 2018)

Many human diseases are associated with increased oxidative stress resulting from altered free radical production or from altered antioxidant content or reactive oxygen species (ROS) activity. Free radicals are a persistent presence within the human body, with their harmful effects largely dependent on the level at which they are present. The existence of these radicals can be amplified through either internal factor such as electron phosphate chain and normal metabolism, or external factors including smoking, pollution, irritation, and excessive sun exposure. The process of lipid peroxidation occurs in the human body when free radicals target lipids that possess a carbon–carbon double bond, particularly polyunsaturated fatty acids (PUFAs) (Ayala et al., 2014). Lipid peroxidation, a chain of oxidative degradation reactions of lipids, can be categorized into three distinct stages - initiation, propagation, and termination. In each of these stages, free radicals extract electrons from lipids in cell membranes, which can lead to cellular damage (Saieva et al., 2016). The chemical products of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs). One of the consequences of uncontrolled oxidative stress (imbalance between prooxidants and antioxidants levels) in cells, tissues, and organ injuries caused by oxidative damage, it has been recognized that high levels of free radicals can cause direct lipid damage products of lipid peroxidation are hydroperoxides (LOOH) among aldehyde products which formed as secondary lipid peroxidation product, malondialdehyde (MDA) hexanal, propanal, and 4-hydroxenonenal (4-HNE).

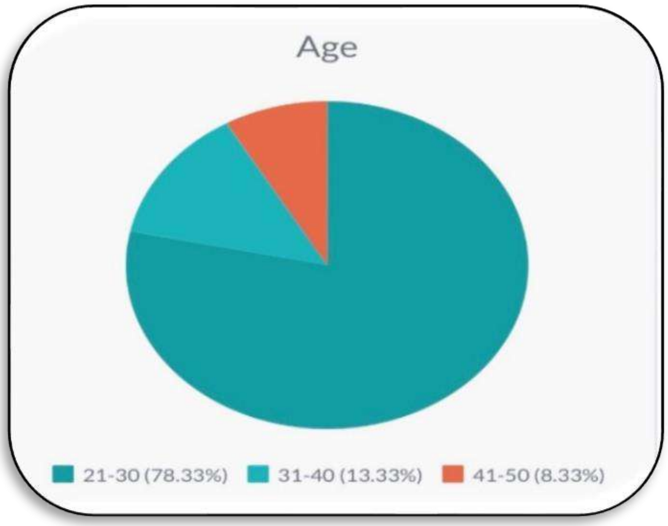
Aim of the study

1. The present study aims to evaluate lipid peroxidation by measuring the concentration of malondialdehyde in both smoker and non-smoker populations.
2. The objective of this study is to determine the efficacy of vitamin C supplementation on malondialdehyde levels in both smokers and non-smokers.
3. This study seeks to determine the effectiveness of vitamin C supplementation on iron concentration and total iron-binding capacity in both smoker and non-smoker populations.

Samples and Methods

In this research conducted in Iraqi Kurdistan - Erbil, we focused on both smokers and non-smokers without chronic diseases as our volunteers. With a doctor's approval, the participants were given a daily vitamin C supplement of 180mg for a period of 40 days, ensuring their well-being throughout the study. To measure the impact, we assessed iron concentration, total iron binding capacity, and malondialdehyde were estimated both before and after the supplementation, aiming to understand the potential effects on these parameters in the context of vitamin C intake.

At first, 120 people's histories are collected (60 smokers and 60 non-smokers) and were asked questions about (age, sex, height, weight, chronic disease, and also about blood samples), the age of the volunteers were between (20-50), and information was gathered, and then (60 smokers) were advised to take vitamin C.



A blood sample is obtained by taking venous blood (4 mL) from volunteers, placing the blood in a gel tube, allowing it to clot, and then separating the serum by centrifugation at 3000 rpm for 10 minutes. Stored and frozen until estimation.

The body mass index (BMI) of male volunteers was calculated using the standard formula of dividing their weight in kilograms by the square of their height in meters (kg/m2). The following parameters were used to determine the BMI classification:

Underweight $\leq$ 18.5, Normal 18.5-24.9, Overweight 25-29.9, Obesity $\geq$ 30.

In this study, the iron concentration and total iron binding capacity in all serum samples were determined using Biolabo kits.

Malondialdehyde (MDA) concentration estimation, the concentration of MDA in the serum was then determined using the Thiobarbituric acid (TBA) assay method developed by Buego and Aust in 1978, using a spectrophotometer (Buege and Aust, 1978). In smokers and non-smokers before and after taking vitamin C.

This method quantifies lipid peroxides by measuring the aldehyde breakdown products of lipid peroxidation. The basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of Thiobarbituric acid to form a red MDA-TBA complex which can be measured at 535 nm.

In this study, the vitamin C supplementation was used in the form of effervescent tablets, with a dosage of 180mg per daily portion. The nutrient reference values for the daily intake (adult) for vitamins and minerals were 225%, according to the Regulation of the European Union (EU).

To analyze the difference in MDA levels, iron concentration and total iron binding capacity before and after vitamin C supplementation, the data was analyzed using Graph pad prism 8.4.2 software one way- nova statistics were used; it is important to test for the statistical significance of the difference in original data.

Result and Discussion

Mean of Iron concentration in smokers and non-smokers groups before and after taking Vitamin C

There was a significant difference (P= <0.0001) between the iron concentration of smoker groups (389.8 mg/dL) and control groups (119.6 mg/dL) before taking vitamiv C. Also, there was highly significant increase in iron concentration after taking vitamin C for smoker.

Table 1: Mean of Iron concentration in smokers and non-smokers groups

Groups	Sample size	Mean (mg/dL)	Standard deviation	P value
Smoker Before vit C	60	389.8	114.4	<0.0001
Control (Non-smokers) Before vit C	60	119.6	77.26	
Smoker after vit C	60	543.9	162.9	
Control (Non-smokers) after vit C	60	233.5	79.8	

Mean of TIBC concentration in smokers and non-smokers

There was no significant difference (P= 0.0439) between the mean of TIBC of smoker groups (379.0 mg/dL) and control groups (301.8 mg/dL). However, there was highly significant increase in TIBC after taking vitamin C for smoker (P=0.0017).

Table 2: Mean of TIBC concentration in smokers and non-smokers before and after taking Vitamin C

Groups	Sample size	Mean (mg/dL)	Standard deviation	P value
Smoker Before vit C (group A)	60	379.0	125.0	0.0439*
Control (Non-smokers) Before vit C (group B)	60	301.8	102.7	0.0041**
Smoker after vit C (group C)	60	632.7	180.6	0.0017***
Control (Non-smokers) after vit C (group D)	60	399.6	113.2	0.0019****

*P value between A and B
**P value between B and D
***P value between A and C
****P value between C and D

Mean percentage of transferrin saturation in smokers and non-smokers before and after taking Vitamin C

There was a significant difference (P= 0.0009) between the mean concentration of smoker groups (97.60 %) and control groups (45.80 %).

Table 3: Mean percentage of transferrin saturation in smokers and non-smokers

Groups	Sample size	Mean (mg/dL)	Standard deviation	P value
Smoker Before vit C (group A)	60	97.60	77.26	0.0009*
Control (Non-smokers) Before vit C (group B)	60	45.80	24.18	0.5833**
Smoker after vit C (group C)	60	109.8	162.9	0.6011***
Control (Non-smokers) after vit C (group D)	60	53.7	26.3	0.0008****

*P value between A and B
**P value between B and D
***P value between A and C
****P value between C and D

Mean of MDA concentration in Smokers before taking Vitamin C, after taking Vitamin C, and Control groups

There was no significant difference (P= 0.0439) between the mean concentration of MDA of smoker groups (4.694 nmol/ml) and control groups (4.580 nmol/ml) before taking vitamin C. However, there was highly significant decrease in MDA concentration (2.688 nmol/ml) after taking vitamin C for non-smoker group comparing with before taking vitamin C (4.580 nmol/ml) (P<0.001). In contrast there was highly significant increase (P<0.001) in MDA concentration in smoker group after taking vitamin C (7.264 nmol/ml) comparing with before taking the vitamin (4.580 nmol/ml).

Table 4: Concentration of MDA in control and smokers before and after taking Vitamin C

Groups	**Sample size**	**Mean nmol/ml**	**Standard deviation**	**P value**
Smoker Before vit C (group A)	60	4.694	5.506	0.1144*
Control (Non-smokers) Before vit C (group B)	60	4.580	24.18	P<0.001**
Smoker after vit C (group C)	60	7.264	7.279	P<0.001***
Control (Non-smokers) after vit C (group D)	60	2.688	1.247	P<0.001****

*P value between A and B
**P value between B and D
***P value between A and C
****P value between C and D

These results suggest that Vitamin C supplementation may be have an effective strategy for reducing oxidative damage and improving the overall health of individuals at risk of chronic diseases related to oxidative stress for non-smoker individuals, while there is opposite effect for smokers.

Table 5: The relation between the concentration of MDA and age in smoker groups

	18-30 years old		**31-50 years old**	
	Before vitamin C	**After vitamin C**	**Before vitamin C**	**After vitamin C**
Mean	4.981	6.350	3.988	9.513
Standard deviation	6.035	5.473	4.040	10.45
P value = 0.1182				

Table 6 A: Descriptive statistics for the individuals in the study, including their ages, height, weight, and body mass index (BMI)

Variables	**Mean**	**Standard Deviation**	**Variance**	**Minimum**	**Maximum**	**Range**
Age (Years)	23.24	6.25	39.01	19.00	52.00	33.00
Height (cm)	174.13	6.10	37.21	165.00	188.00	23.00
Weight (kg)	74.53	12.99	168.71	50.00	115.00	65.00
BMI	24.51	3.52	12.41	17.30	34.30	17.00

The participants in this study had a mean age of 23 years, with a minimum age of 19 years and a maximum age of 52 years. The average height was 174.13 cm, ranging from 165 cm to 188 cm, while the average weight was 74.53 kg, ranging from 50 kg to 115 kg. The mean BMI was 24.51, ranging from 17.30 to 34.30. These findings indicate that the study included a diverse group of individuals with a wide range of ages, heights, weights, and BMIs.

Table 6 B: Side Effects from Consuming Vitamin C

Volunteers	**Vitamin C Dose (mg/day)**	**Duration (days)**	**Side Effect**
60 Volunteers	180mg/day	40 days	No side effects reported

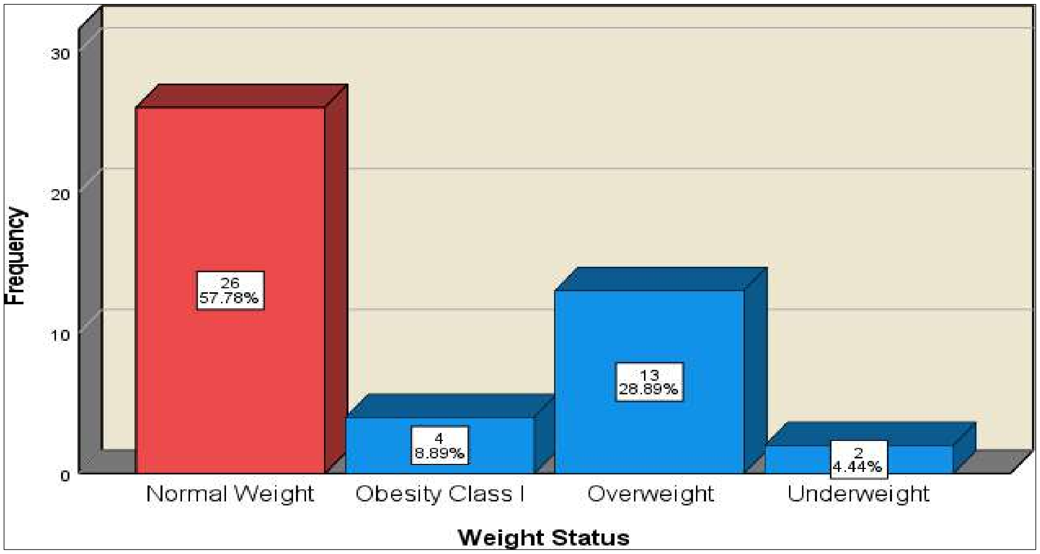


Figure 1. Weight status percentage and frequency of volunteers

Discussion

Iron plays an essential element in human tissues, and a critical role in transporting oxygen in the body as well as in cellular processes, including in this research we wanted to know the value of iron and malondialdehyde concentration in smokers and non-smokers. In this study, the result of this study was observed is a positive correlation in smokers, there was an increase in the amount of iron in the body. However, in addition to this, heavy smokers are more likely to have iron overload. The reasons for the high amount of iron in the blood are that smoking increases the amount of iron in the blood, as well as, increases the level of hemoglobin in the blood, because it causes a decrease in the proportion of oxygenated blood and an increase in the level of carbon monoxide in the blood. According to the findings of this study, there are significant differences between heavy smokers and non-smokers. To prevent iron accumulation in the body, it is necessary to avoid excessive smoking (Chełchowska et al., 2016; Pateva et al., 2015; Shibata et al., 2013; Zeng et al., 2016). This study also shows that when smokers take vitamin C supplements, the concentration of iron in their blood increases. One of vitamin C's roles is to enhance the process of iron absorption in the body via the intestine. This suggests that increased vitamin C levels may result in a rise in iron levels as well.

Cigarette smoking promotes the organic end product of lipid peroxidation MDA, and vitamin C is a powerful reducing agent that acts as an antioxidant to neutralize oxidants (Kubihal and Naik, 2019). One of vitamin C's most important functions is to increase iron absorption in the intestine. This study's findings disagree with those of (Popovic et al., 2015), who found that an adequate amount of antioxidants, such as vitamin C, reduced MDA concentrations (Popovic et al., 2015). However, (Chambial et al., 2013) research confirmed that iron overload by supplementation with vitamin C increased free radicals due to the Fenton reaction due to the enhancement of lipid peroxidation. According to (Pehlivan, 2017), Vitamin C can act as a prooxidant by reducing

hydrogen peroxide or metal ions like iron to form free radicals through the Fenton reaction (Pehlivan, 2017). In addition, research confirmed that iron increases significant mortality in patients with heart disease and cancer, and vitamin C may accelerate this mortality by increasing iron absorption.
The current study investigated the influence of vitamin C on malondialdehyde (MDA) levels in healthy individuals. The findings showed that after 40 days of taking vitamin C supplements (180mg/day), there was a significant decrease in MDA levels. In non-smokers this reduction is attributed to the antioxidant properties of vitamin C, indicating that vitamin C may have a protective effect against oxidative stress and lipid peroxidation. This result is consistent with previous research, suggesting that vitamin C supplementation can reduce MDA levels in various populations, including healthy individuals and those with chronic diseases such as diabetes and cardiovascular disease.
Furthermore, as an antioxidant, vitamin C can neutralize reactive oxygen species (ROS) and prevent lipid peroxidation. It can also regenerate other antioxidants such as vitamin E and glutathione, thereby enhancing its protective effects.
However, the findings of this study disagree with the results of Ashor et al. (2017), who reported no significant effect of vitamin C supplementation on MDA levels in healthy young men. This discrepancy may be due to differences in the dose and duration of vitamin C supplementation (Ashor et al., 2017). Ashor et al. (2017) used a higher dose of vitamin C (500mg/day) and a shorter duration of supplementation (14 days) compared to this study. It is possible that a longer duration of supplementation or a higher dose of vitamin C may have a more significant effect on MDA levels (Ashor et al., 2017).
In smokers, iron levels increase axiomatically during smoking due to the elevation of hemoglobin. Clearly, there is an observed elevation in iron levels in the bloodstream of smokers, attributed to the heightened hemoglobin, which is believed to serve as a defensive mechanism against the rise in carbon monoxide. Despite its antioxidant properties, vitamin C is paradoxically considered a prooxidant. Concurrently, vitamin C promotes iron absorption, resulting in a substantial increase in malondialdehyde (MDA) levels in smokers.

Conclusion

In the current study, serum iron concentrations were highly significant in the smoker group than in the control group. There was a significant difference in iron concentration (P= 0.0001). In addition, there was a slight significant increase in the TIBC of the smoker group compared to the control group (P= 0.0439). There were no significant differences in transferrin saturation levels in smokers before and after taking vitamin C (P= 0.6011). According to the current study, vitamin C can increase iron levels in the blood. Furthermore, in the current study, the difference in serum MDA concentration between smokers and non-smokers was not highly significant, in smoker group compared to non-smoker group non-significant difference value (P= 0.1144) was found. There was no significant increase in MDA concentration after taking vitamin C (P= 0.1182). This study proposes an increase in the smoker's lipid peroxidation state and the effectiveness of vitamin

C on these processes in smokers. In conclusion, this study provides evidence that vitamin C supplementation can decrease serum MDA levels in non-smoker individuals. This suggests that vitamin C may have a protective effect against oxidative stress and lipid peroxidation. Further research is needed to confirm these findings and to explore the potential therapeutic applications of vitamin C in the prevention and treatment of diseases associated with oxidative stress and increase MDA in smokers.

Recommendation

1. More study on iron-related proteins in smoker blood, such as ferritin, ceruloplasmin, and myoglobin, is required. Furthermore, additional studies of the lipid peroxidation state in order to determine the level of MDA in different types of smokers, such as heavy smokers, light smokers, and electronic cigarette users, may be conducted.

2. Evaluate oxidative stress to determine the effectiveness of vitamin C in smokers, as well as vitamin C levels in smokers and non-smokers before and after vitamin C administration.

3. Determine the iron concentration in different types of smokers, including heavy, light, and electronic cigarette users.

4. Limiting the amount and duration of vitamin C consumption since it may increase free radicals.

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