Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

'THE CORRELATION BETWEEN SERUM VITAMIN C LEVELS AND CORONARY ARTERY DISEASE'

SHILPA BHARDWAJ[1], NITIN TYAGI[2]*, CHARANJEET KAUR[3]

***Corresponding author: NITIN TYAGI**

1- Associate Professor, Department of Biochemistry, ESIC PGIMSR Basaidarapur, New Delhi, India. Orcid ID: http://orcid.org/0009-0002-6373-1794

2- Assistant Professor, Department of Biochemistry, Swaminarayan Institute of Medical Sciences and Research, Kalol, Dist. Gandhinagar, Gujarat, India. Orcid ID: http://orcid.org/0000-0001-9465-9743

3- Director- Professor, Department of Biochemistry, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. Orcid ID: http://orcid.org/0000-0002-5225-6898

Address for correspondence**: Dr.Nitin Tyagi, Department of Biochemistry, Swaminarayan Institute of Medical Sciences and Research, Kalol, Dist. Gandhinagar, Gujarat-382721, India.**

E-mail**: drnitintyagi1903@gmail.com**

Phone: **+91-9871050655**

ABSTRACT

Background: Coronary artery disease (CAD) is one of the leading causes of mortality among adults. The increase in oxidative stress and defects in antioxidant defence plays a major role in endothelium performance and are an effective factor in the progress of atherosclerosis.

Aim and Objectives: The aim of our study is to observe the correlation between serum vitamin C and coronary artery disease.

Materials and methods: In this case-control study, a total of 100 patients were included out of which 50 patients with angiographically proven CAD as the case group and 50 apparently healthy age and sex-matched adults as the control group. Serum vitamin C levels were measured in both groups using enzyme-linked immunosorbent assay (ELISA) based kits.

Results: There was a significant negative correlation between serum vitamin C levels and the CAD. On comparison, it shows that there is a significant decrease in serum vitamin C levels

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

in the case group (23.27 ± 5.74 µg/ml) than in control group (228.36 ± 38.99 µg/ml) and their difference was statistically significant ($p < 0.0001$).

Conclusion: Serum vitamin C levels decreased in patients with CAD (case group) compared with the normal coronary arteries group (control group). This decrease is more prominent with increasing levels of CAD severity, which may be helpful in risk prediction and stratification.

KEYWORDS: anti-inflammatory, anti-oxidant, serum vitamin C levels, coronary artery disease

INTRODUCTION

Coronary artery disease (CAD) refers to a reversible supply and demand mismatch related to ischemia, a history of myocardial infarction (MI), or the presence of plaque documented by catheterization or computed tomography angiography[1]. The primary pathophysiology for CAD is atherosclerosis characterized by increased oxidative stress and thereby, antioxidant defence impairment plays a major role in endothelial dysfunction[2]. CAD occurs when changes in the wall of arteries lead to the accumulation of atherosclerotic plaques that ultimately results in narrowing the vessel lumen and restricting the flow of blood. The complete blockage of blood flow due to the frequent occurrence of a thrombotic event induces myocardial infarction that makes the cardiac muscle devoid of oxygen[3]. There are multiple factors involved in the development of CAD that can be divided into modifiable and non-modifiable factors. The non-modifiable factors encompass old age and a family history of CAD whereas, the modifiable factor involves a high rate of blood pressure, diabetes, and smoking[4]. However, the clinical phenotype of cardiovascular disease can be determined by the interaction of genetic factors and environmental factors with one another[5]. The CAD is linked to the occurrence of diseases associated with civilization and an unhealthy lifestyle[6].

Through numerous metabolic and biosynthetic routes for energy status, both hydro-soluble and lipo-soluble vitamins are accounted for in the regulation of sustaining cardiovascular function[7]. During the onset of several forms of cardiovascular disease such hypertension, atherosclerosis, diabetes, ischemic heart disease, heart failure, and stroke, decreased levels of certain vitamins have been found [4]. The deficiency of vitamins such as vitamin A, B6, B9, C, D and vitamin E are in association with cardiovascular dysfunctionality. However, presence of these vitamins lower the amount of cardiovascular risk[7]. Antioxidants

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

containing diets and vitamin C have been shown in epidemiologic studies to reduce inflammation, which lowers the chance of developing CAD[8]. The vitamin C is a water soluble molecule that can be administer through the oral or intravenous route[9]. Pharmacological dosage of vitamin C boost the endothelium's production of nitric oxide (NO), that widens the coronary arteries and reduces the risk of CAD[4]. The intravenous infusion of vitamin C before percutaneous coronary intervention (PCI) could be an useful method for cardiac protection towards reperfusion injury[10]. Vitamin C is a well-known antioxidant that functions as a scavenger of reactive oxygen species (ROS)[11]. ROS scavenge nitric oxide and produce a reactive peroxynitrite intermediate that promotes lipid oxidation and atherogenesis[12]. Vitamin C acts as a cofactor of the monooxygenase and dioxygenase enzymes and thus, plays a significant role for the synthesis of several crucial biomolecules. The enzymatic reactions depended on vitamin C are involved in the biosynthesis of collagen and cellular procollagen secretion, norepinephrine, L-carnitine, epinephrine, and for the regulation of the biosynthesis of other molecules[13]. The intake of fruits rich in vitamin C and green leafy vegetables shows a protective effect by preventing the oxidation of lipoproteins with lower density and prevents atherosclerosis, which significantly lowers the risk of CAD[14]. Vitamin C lowers the level of reactive oxygen produced at the time of phagocytosis. The requirement of dietary vitamin C can be regulated by estimating the amount required to increase its concentration in neutrophils. Administration of vitamin C prevents the cardiac injury marked from the elevation of troponin and creatinine kinase (CK-MB), along with increase in the storage of antioxidant, and reduced inflammatory markers[10]. The elevated troponin and CK-MB are associated with myocardial damage. However, the troponin elevation is associated with reduced left ventricular ejection and poor prognosis in patients with coronary heart failure[15]. The lower levels of have been associated with a number of conditions, including high blood pressure (BP), endothelial dysfunction, heart disease, atherosclerosis, and stroke[13]. The aim of our study is to observe the correlation between the serum vitamin C and coronary artery disease.

METHODOLOGY

Study design:

The present study is a case control study where the study population included 50 patients with angiographically proven CAD as case group and 50 apparently healthy age and sex matched adults as control group. The study was conducted at department of biochemistry &

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

department of cardiothoracic & vascular surgery, VMMC & Safdarjung hospital, New Delhi. Prior to the conduction of study, clearance from ethical committee of Vardhman Mahavir medical college and Safdarjung hospital was obtained.

Study Patients and Selection Criteria:

The qualified individuals entered the study after being informed of the study goals; a written informed consent was signed by every patient or their legally authorized representative prior to participation in the study. The simple sampling method was employed and the patients with stenosis > 60% in one or more than vessels confirmed by a cardiologist based on coronary artery angiography were part of the case group. However, the control group constituted individuals with no coronary artery stenosis and was matched with the case group in terms of age, sex, body mass index (BMI), geographical region, and physical activity. The obese patients with BMI > 30 kg/m^2 and waist-hip ratio ≥ 0.90cm in men and ≥ 0.85 cm in women were not the part of study. We excluded the patients with history of intake of drugs like peroxisome proliferator-activated receptors (PPAR) agonists such as thiazolidinediones, acetylcholinesterase (ACE) inhibitors, angiotensin receptor type 1(AT1R) blockers, valproate anticonvulsant and glucocorticoids like dexamethasone. We also excluded the patients with diabetes mellitus, hypertension, end stage renal disease, liver disease and chronical inflammatory disease. The entire case history of the patients was recorded and the clinical examination was performed throughout the conduct of the study.

Experimental Conditions and Reagents:

The experiments were carried out at an ambient temperature in the centrally air-conditioned laboratory. As per the requirement of the study, the glasswares and plasticware sterilized under autoclave were used. The molecular biology graded chemical were used for the gel electrophoresis and to prepare the buffer solutions

Vitamin C determination:

The vitamin C level was evaluated by collecting the fasting blood samples of both case and control groups from 7:30 am to 9:00 am. Participants were asked to refrain from eating, drinking, chewing gum, brushing teeth, as well from using any mouthwashes, in the last 12 h before the sampling. The blood samples were transferred to ethylenediaminetetraacetic acid tubes. The serum was prepared by centrifuging the samples for 10 minutes at 3000 g. Serum samples were stabilized immediately using metaphosphoric acid in order to avoid oxidization

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

of vitamin C. Serum and saliva samples were stored at −20◦C until analysis. The obtained serum was then transferred to the capped microtube and the vitamin C level was estimated[4,16].

STATISTICAL ANALYSIS:

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Quantitative variables were compared using Mann-Whitney Test between the two groups. Qualitative variables were correlated using Fisher exact test. Odds ratio with 95% CI was calculated. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

Table 1 lists the sex distribution of case group and control group. As shown in Figure 1, the case group included 86 % males and 14 % females as compared to control group with 74 % males and 26 % females. The difference of age between the two groups (cases and controls) was not statistically significant.

Table 1: Sex distribution of the study groups

Gender	CAD		Total	p value
	Case	Control		
Male	43 (86.00%)	37 (74.00%)	80 (80%)	0.134
Female	7 (14.00%)	13 (26%)	20 (20%)	

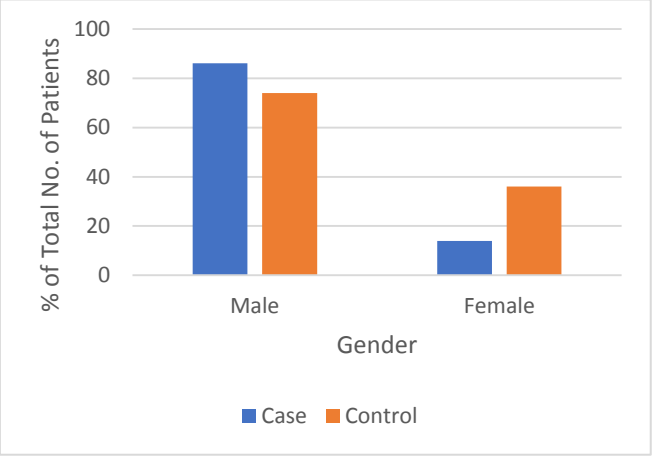

Figure 1: Bar chart showing sex distribution of the study groups

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

Table 2 summarizes the baseline parameters of cases and controls. The mean age (p = 0.0002), height (p = 0.637), weight (p = 0.534) and BMI (p = 0.552) were not significantly different between the two groups. It was observed that serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea and creatinine were significantly higher in cases as compared to controls. On the other hand, levels of low-density lipoproteins (LDL), total cholesterol and triglycerides were lesser in the cases as compared to controls.

Table 2: Baseline parameters of the study groups

	Case Group	Control Group	P value
Age (years)	52.74 ± 12.42	42.06 ± 13.14	0.0002
Height (cm)	162.66 ± 6.09	162.12 ± 5.61	0.637
Weight (kg)	60.92 ± 7.07	59.94 ± 6.09	0.534
BMI (kg/m^2)	23.01 ± 2.35	22.77 ± 1.64	0.552
Triglycerides (mg/dL)	130.26 ± 44.38	133.3 ± 97.11	0.081
Cholesterol (mg/dL)	127.94 ± 26.58	154.48 ± 39.11	0.0001
LDL (mg/dL)	84.94 ± 27.29	104.94 ± 36.8	0.008
Bilirubin (mg/dL)	0.72 ± 0.42	0.57 ± 0.31	0.065
AST (U/L)	56.66 ± 45.16	27.54 ± 15.12	<0.0001
ALT (U/L)	42.94 ± 32.5	24.68 ± 22.53	0.0001
ALP (U/L)	87.3 ± 30.28	90.64 ± 28.9	0.364
Blood Urea (mg/dL)	29.3 ± 12.48	24.96 ± 9.25	0.036
Creatinine (mg/dL)	0.75 ± 0.16	0.72 ± 0.21	0.283

The results are presented as the mean ± standard deviation. Independent t-test was performed among the groups, $p<0.05$ was considered to be statistically significant.

ALT-alanine transaminase, ALP-alkaline phosphatase, AST-aspartate transaminase, BMI-body mass index, LDL-low density lipoproteins

Results of the comparison in the level of vitamin C in case group and control group are shown in Table 3. There was a significant negative correlation between serum vitamin C levels and severity of CAD ($p < 0.0001$). The level of vitamin C in both the groups was

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

determined using Fisher exact test whereas the mean ± standard deviation (SD), median and range was determined using Mann Whitney test. On comparing the outcomes of both the groups, it shows that there is significant decrease in serum vitamin C levels in case group (23.27 ± 5.74 µg/ml) then in control group (228.36 ± 38.99 µg/ml) and their difference was statistically significant ($p < 0.0001$). The median of case group was found to be 24.15 with interquartile range (IQR) of 18.325 to 27.575 and 228.1 for the control group with IQR between 193.075 to 268.625. Whereas in control group, the median was 193.075 to 268.625 and range was between 10.1 to 288.6.

Table 3: Comparison of vitamin C (ng/mL) between cases and controls

Vitamin C (ng/mL)	Case (n=50)	Control (n=50)	Total	P value
Deficient	50 (100%)	0 (0%)	50 (50%)	<0.0001
Nondeficient	0 (0%)	50 (100%)	50 (50%)	
Mean ± SD	23.27 ± 5.74	228.36± 38.99	125.81 ± 106.73	<0.0001
Median (IQR)	24.15 (18.325-27.575)	228.1 (193.075-268.625)	101.1 (24.425-226.35)	
Range	10.1-33.1	169.1-288.6	10.1-288.6	

IQR-interquartile range, SD-standard deviation

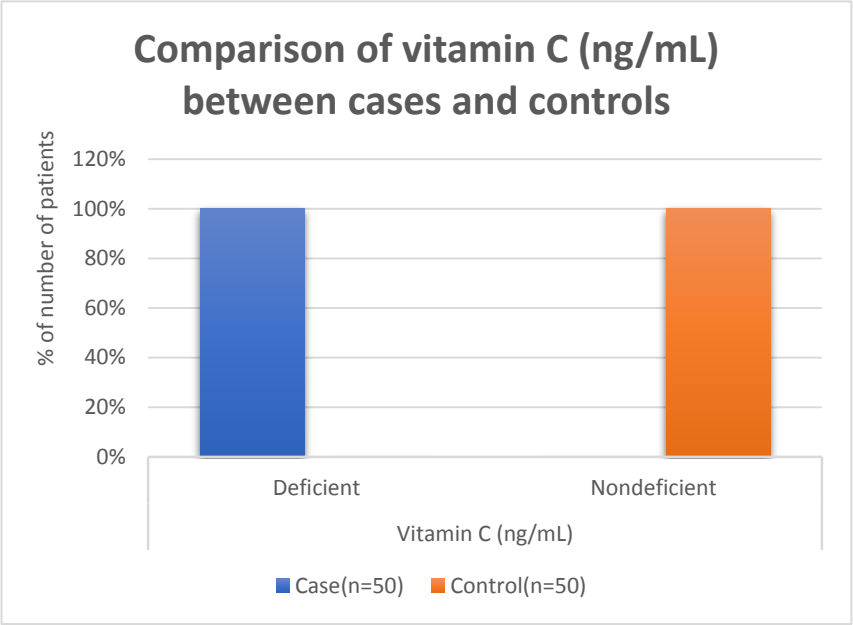

Figure 2: Comparison of vitamin C (ng/mL) between cases and controls.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

DISCUSSION

The increase of oxidative stress and defects in the antioxidant defence system contributes in endothelium function disorders and have been considered as major factors in atherosclerosis progress (8).The vitamin C has been investigated to assess its effect on preventing the development and progression of atherosclerosis through its antioxidant properties[17]. It is known to be involved in the synthesis of collagen, the maintenance of healthy gums, and the absorption of iron. Vitamin C can neutralize reactive oxygen species (ROS) which deplete tetrahydrobiopterin, a cofactor required for endothelial cell nitric oxide synthase (eNOS). The findings revealed that serum levels of vitamin C were significantly correlated with CAD related risk. In the present study, we subjected 50 patients with CAD in case group to compare with 50 healthy adults as control group. The assessment of serum vitamin C level in both the groups was performed. It was observed that there is significant decrease in serum vitamin C levels in case group (23.27 ± 5.74 µg/ml) then in control group (228.36 ± 38.99 µg/ml) and their difference was statistically significant ($p < 0.0001$) The epidemiological studies revealed that the balanced level of antioxidant vitamin C in the body is correlated with the decrease of inflammation markers along with the lowered risk of cardiovascular disease[18]. The increase of oxidative stress and defects in the antioxidant defence system plays a significant role in endothelium function disorders and have been considered as the contributing factors in atherosclerosis progress[19].

Torkzaban A et al. study indicated that the mean serum level of vitamin C was significantly higher in the healthy subjects in comparison with that in the patients[4]. Prevalence studies have demonstrated a correlation between the consumption of foods high in antioxidants, such as vitamin C, and the treatment of inflammation markers and the risk of coronary heart events[20]. Similarly, Shi R et al. observed that 48.8% of patients received vitamin C supplementation and 51.2% of control developed post atrial fibrillation (POAF) after angiography. The pooled relative risk (RR) using a random-effects model showed that vitamin C reduced the incidence of POAF (RR 0.68, 95% CI 0.54 to 0.87, $P = 0.002$)[21]. Baker WL et al. reported ascorbic acid use was associated with a reduction in ICU length of stay (LOS) after post angiography, although appreciable statistical heterogeneity was seen[22]. Khan SA et al. perform randomised control trial and reported that among the eight included trials, six trials reported positive results of vitamin C measure by various measurements to report the outcomes of myocardial injury, cardiac contractility, antioxidant

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

level, ROS, inflammation, reperfusion efficiency and endothelial dysfunction[10]. In the present study, it was reported that there was a significant negative correlation between serum vitamin C levels and severity of CAD ($p < 0.0001$). A study by Moser MA et al. found a modest inverse association between vitamin C intake and CAD risk[14]. The studies along with the present investigation suggested that vitamin C supplements may have a beneficial effect on risk factors for CAD, such as high blood pressure and inflammation. Vitamin C may help to lower blood pressure and improve the function of the endothelium, the lining of the blood vessels, which can help to prevent the progression of CAD.

The following study is associated with several limitations. The small sample size is the first amongst them. Second, despite the preponderance of confounding factors were managed appropriately, it was not feasible to entirely control the potential effect of other underlying conditions and the medications used to treat them on the results since some study subjects may have had multiple chronic diseases. To support these results, additional research with larger sample numbers and additional markers of oxidative stress is required.

CONCLUSION

Overall, the present study showed a significant relationship between serum vitamin C levels and CAD risk. According to the outcomes, CAD patients possessed lower levels of vitamin C in comparison with healthy subjects. Increased oxidative stress is one of the potential common aetiologies in various cardiovascular diseases. These diseases are very complex in their pathogenesis and no single mechanism explains their physiopathology. The results showed a significant negative correlation between serum vitamin C levels and severity of CAD. The intake of vitamin C in the patients was significantly higher than the healthy subjects. There was significant decrease in serum vitamin C levels in case group compared with control group and their difference was statistically significant.

ACKNOWLEDGMENT

The authors would like to extend their gratitude to Mrs.Mamta ,Mrs.Sugandha and Mr. Dhananjay, who helped us in the sampling and examination process in this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of this study.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

AUTHORS CONTRIBUTION

The authors agree on this final form of the manuscript, and attested that all authors contributed equally in preparing and revising the final draft of the manuscript.

FINANCIAL SUPPORT

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

REFERENCES

1. Cassar A, Holmes DR Jr, Rihal CS, Gersh BJ. Chronic coronary artery disease: diagnosis and management. Mayo Clin Proc. 2009 Dec;84(12):1130-46. doi: 10.4065/mcp.2009.0391. PMID: 19955250; PMCID: PMC2787400.
2. Matsuzawa Y, Lerman A. Endothelial dysfunction and coronary artery disease: assessment, prognosis, and treatment. Coron Artery Dis. 2014 Dec;25(8):713-24. doi: 10.1097/MCA.0000000000000178. PMID: 25365643; PMCID: PMC4220301.
3. Sharma B, Chang A, Red-Horse K. Coronary Artery Development: Progenitor Cells and Differentiation Pathways. Annu Rev Physiol. 2017 Feb 10;79:1-19. doi: 10.1146/annurev-physiol-022516-033953. Epub 2016 Dec 9. PMID: 27959616; PMCID: PMC5513160.
4. Hajar R. Risk Factors for Coronary Artery Disease: Historical Perspectives. Heart Views. 2017 Jul-Sep;18(3):109-114. doi: 10.4103/HEARTVIEWS.HEARTVIEWS_106_17. PMID: 29184622; PMCID: PMC5686931.
5. Kathiresan S, Srivastava D. Genetics of human cardiovascular disease. Cell. 2012 Mar 16;148(6):1242-57. doi: 10.1016/j.cell.2012.03.001. PMID: 22424232; PMCID: PMC3319439.
6. Maroszyńska-Dmoch EM, Wożakowska-Kapłon B. Clinical and angiographic characteristics of coronary artery disease in young adults: a single centre study. Kardiol Pol. 2016;74(4):314-21. doi: 10.5603/KP.a2015.0178. Epub 2015 Sep 14. PMID: 26365941.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

7. Shah AK, Dhalla NS. Effectiveness of Some Vitamins in the Prevention of Cardiovascular Disease: A Narrative Review. Front Physiol. 2021 Oct 8;12:729255. doi: 10.3389/fphys.2021.729255. PMID: 34690803; PMCID: PMC8531219.

8. Leopold JA. Antioxidants and coronary artery disease: from pathophysiology to preventive therapy. Coron Artery Dis. 2015 Mar;26(2):176-83. doi: 10.1097/MCA.0000000000000187. PMID: 25369999; PMCID: PMC4315737.

9. Lykkesfeldt J, Tveden-Nyborg P. The Pharmacokinetics of Vitamin C. Nutrients. 2019 Oct 9;11(10):2412. doi: 10.3390/nu11102412. PMID: 31601028; PMCID: PMC6835439.

10. Khan SA, Bhattacharjee S, Ghani MOA, Walden R, Chen QM. Vitamin C for Cardiac Protection during Percutaneous Coronary Intervention: A Systematic Review of Randomized Controlled Trials. Nutrients. 2020 Jul 23;12(8):2199. doi: 10.3390/nu12082199. PMID: 32718091; PMCID: PMC7468730.

11. Kaźmierczak-Barańska J, Boguszewska K, Adamus-Grabicka A, Karwowski BT. Two Faces of Vitamin C-Antioxidative and Pro-Oxidative Agent. Nutrients. 2020 May 21;12(5):1501. doi: 10.3390/nu12051501. PMID: 32455696; PMCID: PMC7285147.

12. Nita M, Grzybowski A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev. 2016;2016:3164734. doi: 10.1155/2016/3164734. Epub 2016 Jan 10. PMID: 26881021; PMCID: PMC4736974.

13. Morelli MB, Gambardella J, Castellanos V, Trimarco V, Santulli G. Vitamin C and Cardiovascular Disease: An Update. Antioxidants (Basel). 2020 Dec 3;9(12):1227. doi: 10.3390/antiox9121227. PMID: 33287462; PMCID: PMC7761826.

14. Zhao CN, Meng X, Li Y, Li S, Liu Q, Tang GY, Li HB. Fruits for Prevention and Treatment of Cardiovascular Diseases. Nutrients. 2017 Jun 13;9(6):598. doi: 10.3390/nu9060598. PMID: 28608832; PMCID: PMC5490577.

15. Korff S, Katus HA, Giannitsis E. Differential diagnosis of elevated troponins. Heart. 2006 Jul;92(7):987-93. doi: 10.1136/hrt.2005.071282. PMID: 16775113; PMCID: PMC1860726.

16. Meurman JH, Murtomaa H. Effect of effervescent vitamin C preparations on bovine teeth and on some clinical and salivary parameters in man. Scand J Dent Res. 1986 Dec;94(6):491-9. doi: 10.1111/j.1600-0722.1986.tb01791.x. PMID: 3468598.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 06, 2023

17. Collins BJ, Mukherjee MS, Miller MD, Delaney CL. Effect of Dietary or Supplemental Vitamin C Intake on Vitamin C Levels in Patients with and without Cardiovascular Disease: A Systematic Review. Nutrients. 2021 Jul 8;13(7):2330. doi: 10.3390/nu13072330. PMID: 34371840; PMCID: PMC8308513.

18. Doseděl M, Jirkovský E, Macáková K, Krčmová LK, Javorská L, Pourová J, Mercolini L, Remião F, Nováková L, Mladěnka P, On Behalf Of The Oemonom. Vitamin C-Sources, Physiological Role, Kinetics, Deficiency, Use, Toxicity, and Determination. Nutrients. 2021 Feb 13;13(2):615. doi: 10.3390/nu13020615. PMID: 33668681; PMCID: PMC7918462.

19. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017;2017:8416763. doi: 10.1155/2017/8416763. Epub 2017 Jul 27. PMID: 28819546; PMCID: PMC5551541.

20. Leopold JA. Antioxidants and coronary artery disease: from pathophysiology to preventive therapy. Coron Artery Dis. 2015 Mar;26(2):176-83. doi: 10.1097/MCA.0000000000000187. PMID: 25369999; PMCID: PMC4315737.

21. Shi R, Li ZH, Chen D, Wu QC, Zhou XL, Tie HT. Sole and combined vitamin C supplementation can prevent postoperative atrial fibrillation after cardiac surgery: A systematic review and meta-analysis of randomized controlled trials. Clin Cardiol. 2018 Jun;41(6):871-878. doi: 10.1002/clc.22951. Epub 2018 May 15. PMID: 29603289; PMCID: PMC6489881.

22. Hemilä H, Chalker E. Vitamin C Can Shorten the Length of Stay in the ICU: A Meta-Analysis. Nutrients. 2019 Mar 27;11(4):708. doi: 10.3390/nu11040708. PMID: 30934660; PMCID: PMC6521194.