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EMERGING ROLE OF VITAMIN D AND REACTIVE OXYGEN SPECIES AS A NON-CONVENTIONAL RISK FACTORS IN ONSET OF MYOCARDIAL INFARCTION

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ABSTRACT

BACKGROUND: Recent advancement in the field of medicine, scientists are able to understand the role of vitamin D and oxidative stress in the development of myocardial infarction. The generation of free radicals has harmful effects on cardiovascular system such as myocardial infarction (MI). The aim of the current study is to evaluate the relationship of vitamin D and oxidative stress in the onset of myocardial infarction. **METHODOLOGY:** In this case and control study fifty diagnosed cases of MI with the help of ST-changes on ECG and Troponin I levels (Group B) and fifty age and sex matched healthy participants (Group A), as a controls, were selected. Sampling was done from different cardiac institutes of Lahore in the duration of six months from February, 2020 to July, 2020. An informed consent was taken from all participants before sampling. Five milliliters of blood were drawn from cubital vein; serum was stored at -70^{o}C after centrifugation for further analysis. All of them were screened for the oxidative stress markers, anti-oxidants and vitamin D with the help of their respective methods. Independent t- test was used to check the significance of different variable with the help of software SPSS (version 21). *P*-valve less than 0.05 remained the significant. **RESULTS:** Current study shows that levels of oxidative stress markers such as Isoprostanes (IsoP-F2α), Malondialdehyde (MDA), 4-hydroxynonenal (4-HNE)

and 8-hydroxydeoxyguanosine (8-OHdG) were significantly elevated in Group B as compared to the Group A. Statistically significant low levels of natural anti-oxidants of human body, Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT), were found. Vitamin D levels of participants also measured and when compared with control group we found sever vitamin D deficiency in Group B. **CONCLUSION:** From the results of the current study, it can be concluded that increased oxidative stress species (HNE, MDA, IsoP, 8OHG) and deficient anti-oxidants (GSH, CAT, SOD) or vitamin D may contribute as a risk factor in the of myocardial infarction.

Keywords: Myocardial Infarction, Oxidative Stress Markers, Risk Factors, Reactive Oxygen Species, Vitamin D

INTRODUCTION

Myocardial infarction (MI) is the leading cause of death worldwide despite significant progress in primary prevention, intervention and treatment strategies. Vitamin D is mainly produced endogenously and almost 80 to 100% of its nutritional requirements are derived after the exposure to U.V radiations of medium wavelength, 290 to 315 nm. Chemically it is a hormone that is fat solvable and formed in the deep skin layer by non-enzymatic process. A small amount can be obtained from food that naturally contain vitamin D (mushrooms, yeast, and fish) or vitamin D-fortified milk, bread and orange juices **[1]**. In the human body it is formed by the conversion of 7-dehyrdocholesterol into cholecalciferol after ultraviolet radiation acquaintance. This process is also termed as photochemical synthesis. Successive hydroxylation is required for the activation of vitamin D**.** In liver, the hydroxylase enzymes metabolizes vitamin D, through a substrate-dependent route which transpires mainly hepatocytes and then renal 1α-hydroxylase converts vitamin D into its active form under the influence of parathyroid hormone **[2].** Finally, both the 1,25-(OH)2D and 25-OHD are deactivated by 24- hydroxylase **[3**]. Calcitriol, the active type of vitamin D, binds to vitamin D binding protein (DBP) and then acts functionally on vitamin D receptor (VDR) and subsequently heterodimerizes **[4]**. Normal levels of Vitamin D are considered to be >30ng/ml (>75nmol/L) compared to inadequate levels measured as 21 to 29ng/ml **[5]**. Levels less than 20 (50nmol/L) are categorized as deficient **[6]**. Deficiency of 25-hydroxy vitamin D can be treated and is linked to many abnormalities including muscle weakness, bone pain, osteoporosis, osteopenia, bone fracture, and other non-skeleton diseases such as cancer, auto-immune, infectious diseases,

neuropsychological **[7]** and cardiovascular diseases (CVD) **[8]**. It has been reported that CVD is linked with vitamin D deficiency by various mechanism other than having a direct impact on cardiomyocytes. Vitamin D deficiency affects myocardial infarction risk factors indirectly like obesity, diabetes mellitus, high blood pressure and cholesterol levels **[9]**. Biological mechanisms that support leading role of vitamin D in MI are blood vessels calcification, apoptosis, angiogenesis, vascular smooth muscle cell proliferation, cardiac hypertrophy, inflammation, disturbed regulation of blood pressure through the renin-angiotensin system (RAS) **[10]**. Vitamin D triggers some second messengers including mitogen-activated protein kinase (MAP) and cyclic AMP that in turn affect calcium channels. Hence the increased calcium storage as a modulator of vascular wall has emerged as a strong predictor of cardiovascular risk **[11]**. Insufficiency of vitamin D also modulates tissue distribution of V1 and V3 myosin chains by supporting V1 isotopes. This modulation in myosin isotopes alters myocyte contractility **[12]**. Furthermore, hypovitaminosis D upsurge the danger of body fluid and arteries wall calcification and thus may block coronary arteries which results in hypoxia to cardiac muscles **[13]**.

Augmented invention of reactive oxygen species (ROS) and oxidative stress (OS) which is potent pro-inflammatory mediator are the important causes for the MI. Oxidative stress plays an essential character in the production or occlusion of vessels being an important character in atherogenesis. As the production of ROS increases and the bioavailability of nitric oxide (NO) decreases in the vessels partition, it leads to the disruption of endothelial wall in atherogenesis **[14]**. ROS disrupts normal endothelium and then oxidizes phospholipids which results in the generation of low density lipoprotein (LDL). Oxidized LDL is recognized as a high risk for myocardial infarction. Lipid peroxidation yields F2-isoprostanes (IsoP) which indicates oxidative damage in many diseases. Its specific structural features and mechanism of formation make it the most consistent index of lipid peroxidation compared to other free radicals. Moreover, this distinction offers a noninvasive method of dose selection for checking the response of antioxidant treatment. Lipid peroxidation-derived reactive aldehyde is known as Malondialdehyde which is one of the most consistent, accurate and commonly used markers of oxidative stress for the evaluation of oxidative damage in ischemic heart

disease. The most specific aldehydic lipid peroxidation product is 4-hydroxynonenal, an extremely cytotoxic and highly reactive α, -aldehyde. Depending upon the generation of ROS it is produced during multiple physiological and pathophysiological settings including cardiovascular diseases.

ROS also damage DNA which results in the formation of 8-hydroxydeoxyguanosine, which is also a marker of oxidative stress damage. The harmful effects of highly reactive free radical and their pathophysiological consequence is prohibited by cellular antioxidants. An antioxidant is a substance that inhibits oxidation of oxidizable matter, even though the less amount of antioxidant is present, it prevents the oxidation significantly. In other words the intracellular redox state is balanced by antioxidants such as α-tocopherol, Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), peroxiredoxin and ascorbic acid **[15]**. GSH acts most imperatively against HNE **[16]** by reducing organic lipid hydro-peroxide and H_2O_2 whereas SOD defends against oxidative stress by accelerating the dismutation of superoxide anion to H_2O_2. Since H_2O_2 is produced by SOD, it acts synergistically with H_2O_2-removing enzyme catalase which is exclusively an enzyme of peroxisome. Catalase in turn defends against oxidative stress by metabolizing H_2O to water and oxygen. Anti-oxidants scavenge free radicles and hence reduces a chance to develop myocardial infarction due to oxidative stress. This research was aimed to find a correlation among the factors for myocardial infarction such as levels of Vitamin D, oxidative stress markers and anti-oxidants molecules.

MATERIALS AND METHODS

In the present case and control study total of one hundred (n=100) participants were added. Out of one hundred, fifty subjects having ST- segment elevation myocardial infarction (STEMI) on ECG or showed elevated cardiac enzymes due to Non-ST-elevation myocardial infarction (NSTEMI) belonging to both genders (male and female) between the age of 30 to 60 years were included in case group labelled as group B. Equal number of age, sex matched healthy individuals were selected as a control and labelled as group A. Subjects with confounding factors for MI such as pregnancy, chronic kidney diseases, thyroid disease, chronic liver disease, valvular heart disease, cardiogenic shock, cardiomyopathy and any type of malignancy were excluded from this study. All work was carried out according to the Ethical review committee of Institute of Molecular Biology and

Biotechnology (IMBB), The University of Lahore. An informed consent was taken from the participants before including them in the study. Five milliliters of blood were drawn from cubital vein and serum was stored at -70^{o}C after centrifugation for further analysis. Collected samples were processed and analyzed to estimate the levels of vitamin D, 4 hydroxynonenol, 8-OHdG, and Isoprostanes by commercially available ELISA kits. Concentrations of SOD, catalase, glutathione and MDA were determined by kakkar, Aebi, Moron and Spectrophotometric methods respectively. Statistical analysis were performed by using SPSS (version 21) and, *p* value less than 0.05 was considered statistically significant.

RESULTS

Total of one hundred participants were recruited for the analysis and equally divided in two groups (group A, B). Selection of variable of age was limited between 30 to 60 years and stratified into three subgroups. Total of 66% were the males and 44% females. Those who had ST-elevation MI constituted 33% of group B and participants had non ST elevated MI contributed 17% of group B. **(Table 1)**. Results depicted the significantly elevated levels of reactive oxygen species were observed as compared to low levels of vitamin D and anti-oxidants in group B.

Reactive Oxygen Species

Oxidative stress was measured by measuring the levels of lipid peroxidation and DNA damage. A statistically significant rise in MDA, 8-OHdG, IsoP and 4-HNE (4.56±1.09 nmol/mL *p*=0.022, 2.18±0.015 µmol/L *p*=0.025, 73.29±12.58 pg/ml *p*=0.014, 23.25±5.66 µmol/L *p*=0.03 respectively) were seen as compared to control group (0.932±0.056 nmol/mL, 0.281±0.004 µmol/L, 1.59±3.85 pg/mL, l 9.25±2.05 µmol/L respectively) **(Graph-1)**.

Anti- Oxidants

Comparison and analysis of antioxidant showed significantly low levels of SOD, CAT and GSH (*p*=0.031, *p*=0.014 and *p*=0.025 respectively) **(Graph-2)**.

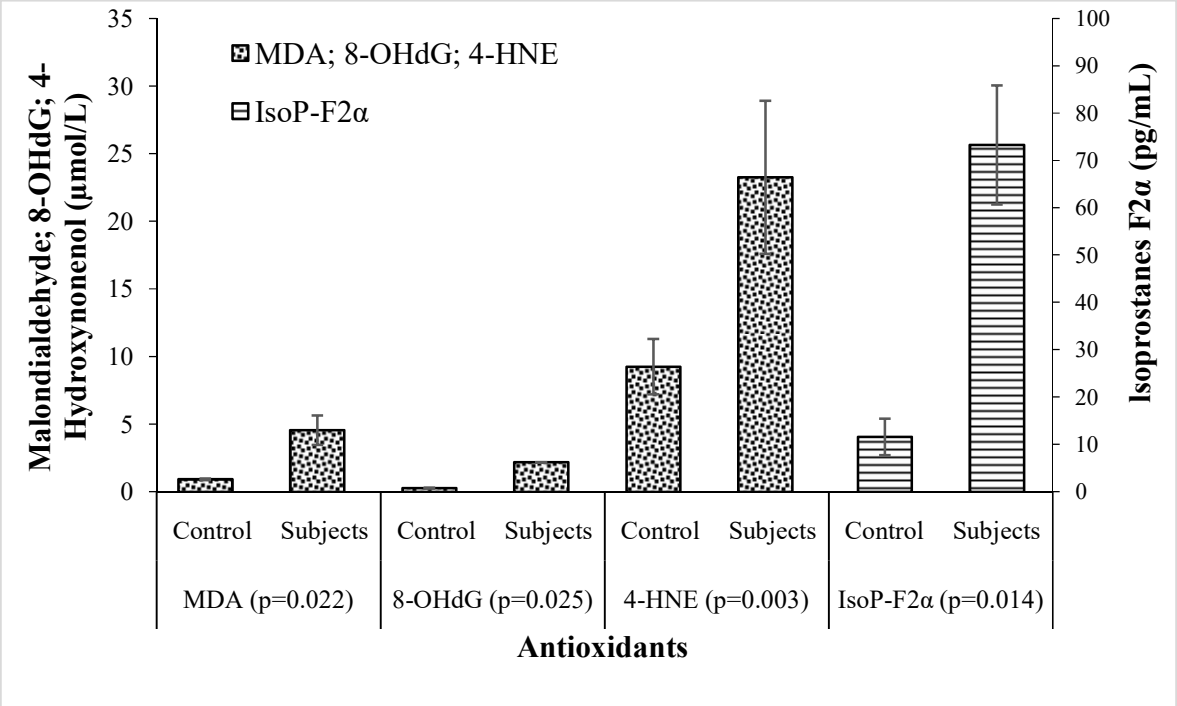
Vitamin D Levels

The vitamin D levels were found to have an inverse correlation with myocardial infarction. As compared to control group, MI patients had decreased levels of Vitamin-D (13.29±1.28 vs. 33.26±5.26 ng/ml), which was statistically significant (p=0.025) **(Graph-3)**.

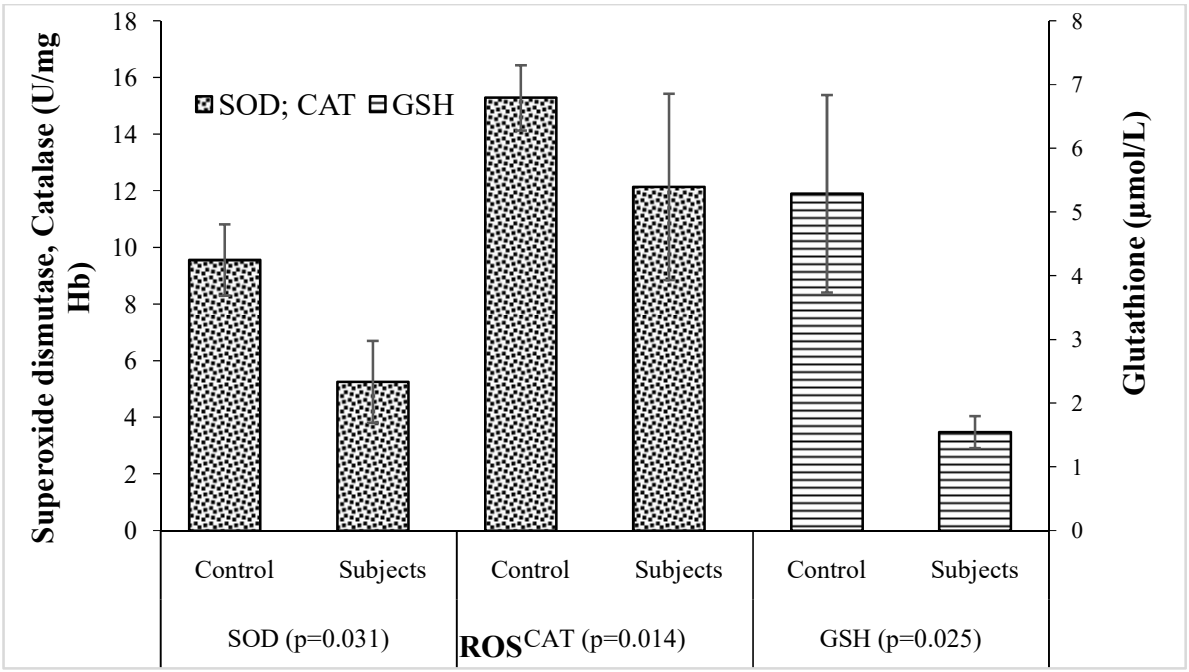
Table 1: Frequencies and percentages of all variables

Individual factors	Category	Frequency (%)		
		[1]Group A n=50	[2]Group B n=50	Total N=100
Disease Status	Healthy	50 (100%)	0 (0%)	50 (50%)
	Myocardial Infarction	0 (0%)	50 (100%)	50 (50%)
*Age	30-40 years	14 (28%)	15 (30%)	29 (29%)
	41-50 years	14 (28%)	13 (26%)	27 (27%)
	51- 60 Years	22 (44%)	22 (44%)	44 (44%)
Gender	Male	32 (64%)	34 (68%)	66 (66%)
	Female	18 (36%)	16 (32%)	34 (34%)
[a]STEMI	Yes	0 (0%)	33 (66%)	33 (33%)
	No	50 (100%)	0 (0%)	50 (50%)
[b]NSTEMI	Yes	0 (0%)	17 (34%)	17 (17%)
	No	50 (100%)	0 (0%)	50 (50%)

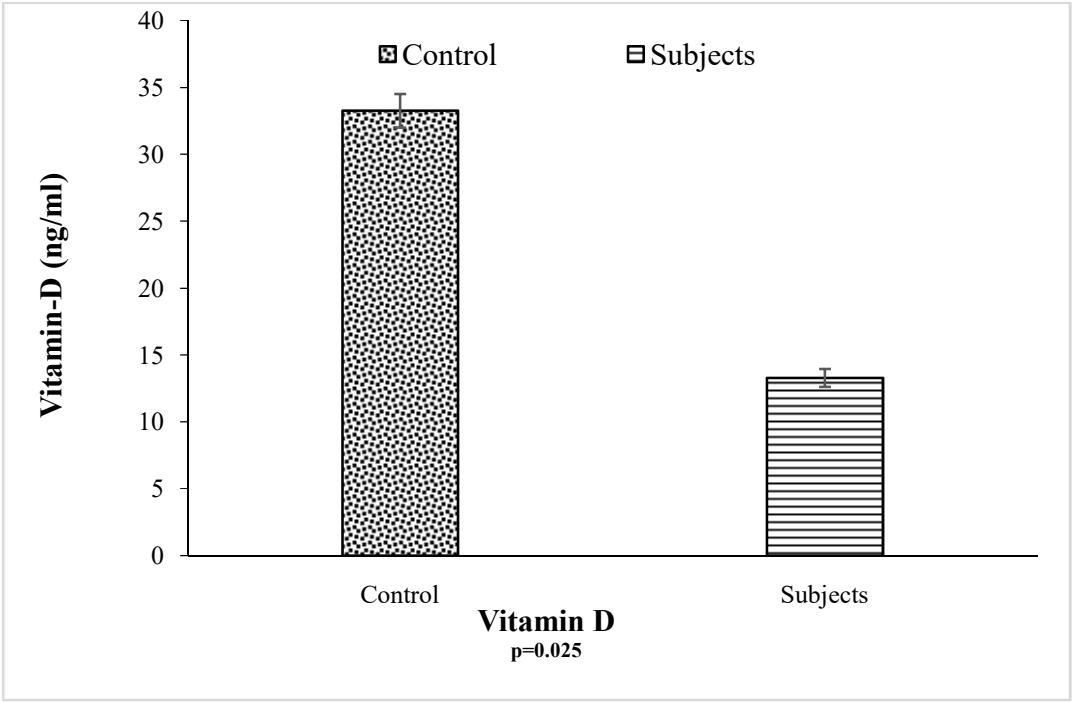
*Age is stratified into three groups; a. ST elevation myocardial infarction; 1. Healthy population; b. Non ST elevation myocardial infarction; 2. Confirmed cases of Myocardial infarction



Graph 1: Compression of Reactive Oxygen Species Between the Groups



Graph 2: Compression of Anti-Oxidant Between the Groups



Graph 3: Compression of Vitamin D Levels Between the Groups

DISCUSSION

Epidemic of myocardial infarction dates back to 1920. In 1918, Mellanby was the first to prove that cod liver oil would prevent rickets **[17]** and Hess in 1917 initially described that rickets can be cured by sunlight exposure **[18, 19**]. Vitamin D insufficiency is linked with ischemic heart disease and it can be treated. The gold standard is to analyze 25 (OH)D levels when AMI has occurred and start prompt treatment subsequently **[20]**. In the current study low levels of vitamin D were found to be allied with MI. This is in agreement with numerous studies which have identified that vitamin D deficiency is a risk factor for MI in every season **[21]**. Another strong evidence comes from the studies in which it was found that 25 (OH) D reduces MI. However, another study conducted on 2686 individuals allocated to receive 830 IU of 25OHD daily or placebo for 5 years, where vitamin D was administrated as 100000 IU of oral cholecalciferol every 4 months. Levels of vitamin D in placebo group were 21.4 ng/mL and in vitamin D group were 29.7 ng/mL. A large study conducted on 6436 MI patients, followed up for 8 years showed an inverse association of vitamin D3 with cardiovascular disease events. Reduced concentrations of 25(OH)D were related with greater incident of cardiovascular events in white race individuals **[22]**. Our results are consistent with Shanker and his colleagues who documented that the vitamin D hormone levels were considerably decreased in 287 MI patients as compared to healthy normal controls as a result cardiovascular disease risk was 254 times greater in patients falling in first vitamin D quartile than fourth quartile. Severe hypovitaminosis D has been proved to be a strong linkage with severe cardiovascular disease outcome, showing that patients with levels < 10 ng/mL are at a greater cardiovascular risk **[23]**. Contrary to our findings Veloudi and his colleagues who could not find, the association of prevalence of MI with vitamin D, neither retrospectively nor prospectively. During retrospective study history was obtained from patients and during prospective study patients were looked for any cardiovascular event. No association was found during either study **[24]**.

Subjects with ischemic heart disease has over production of ROS, which is known to quench NO **[25]**. The oxidative byproducts determine oxidative stress status. Earlier researches validated the association of ischemic heart disease with oxidative products of proteins and lipids i.e. Malonaldehyde **[26]**. Our results are similar

with the previous study in which patients of MI were confirmed on the basis of angiography showed increased concentrations of MDA than control group of healthy individuals **[27]**. Contrarily, Bridges and Stanger reported no significant association between MDA and angiographic findings that specify severity of MI **[28] [29].** Another significant marker of DNA damage is 8-OHdG, which is also produced via burst of oxygen radicals **[30].** Our results are in accordance with a study which showed that pro-oxidant biomarkers were higher in MI patient **[31].** Our results are also in agreement with the report by Suzuki *et al.,* (2011) in which 8-OHdG levels were higher in cardiac disease patients. Yet these higher levels of oxidative stress can harm mitochondria themselves and a supplementary oxygen radical production thus a vicious cycle goes on. Group with no cardiac event showed lesser levels of 8-OHdG than cardiac event group, where higher 8-OHdG concentrations reflect more severe damage to heart. Here the presence of higher levels of 8-OHdG represent cellular damage which is exaggerated by ROS. Anti-oxidant drug treatment can be effective in such cases **[32]**. Myocardial infarction was correlated with reduced concentrations of antioxidants such as CAT, GSH, and SOD. Whenever cells are exposed to average ROS burst, they initiate a survival and cytoprotective response through antioxidants which in turn enhance scavenging the ROS **[33]**. Similar to our results, Kumar and Das (1993) also found that levels of SOD were significantly decreased in patients group ($p<0.05$) and indicated that greater production of free radical with concomitant decline in NO and antioxidants i.e., CAT, SOD and alpha tocopherol follow essential hypertension which is a traditional risk factor for MI **[34**]. It has been already established in other studies that concentrations of HNE are raised in atherosclerotic lesions of humans plus animal models of ischemic heart which can result in MI **[35]**. In coronary arteries atherosclerotic lesions block blood supply to cardiac tissues which result in MI. The demise of cardiac myocytes and the degree of damage is positively associated with HNE levels **[36]**. Some studies failed to approve a contributory correlation between HNE levels and factors leading to myocardial infarction. This can be attributed to varying study designs, selection of different study population and mismatched demographics **[37]**. Similar to our findings, previous studies also shed light on the role of Isoprostanes in cardiovascular pathology. Platelets from acute myocardial infarction and angina

pectoris patients showed an enhanced expression of Isoprostanes receptors that was associated with chest pain time interval **[38]**.

Indeed, Isoprostanes production is high in cardiovascular disease or any other disease that is related with oxidative stress. Hence, Isoprostanes levels predict atherosclerotic burden so it directly adds to oxidant stress through activation of TxA2 prostanoid receptor (TP) that could influence vascular tone, endothelial regeneration, homeostasis, cellular function and reperfusion injury as well **[39-52]**. It is proposed that vascular tone accounts for its role in the development of hypertension and ischemia **[39]**.

CONCLUSION

This study concluded that elevated levels of reactive oxygen species or deficient levels of Vitamin D and naturally produced anti-oxidant in human body could be considered as the risk factors for the onset of myocardial infarction because decrease in antioxidant causes imbalance between pro-oxidants and anti-oxidants. Vitamins D also acts as anti-oxidants which strongly protect against myocardial infarction. Hence therapies including anti-oxidants and vitamin supplementation can play an important role in the treatment and prevention of myocardial infarction.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

REFERENCES

[1] Black, L.J., *et al*., *An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification.* The Journal of nutrition, 2012. **142**(6): p. 1102-1108.

[2] Deleskog, A., *et al*., *Serum 25-hydroxyvitamin D concentration, established and emerging cardiovascular risk factors and risk of myocardial infarction before the age of 60 years.* Atherosclerosis, 2012. **223**(1): p. 223-229.

[3] Marcos Pereira-Santos, M., *et al*., *Vitamin D deficiency and associated factors among pregnant women of a sunny city in Northeast of Brazil.* 2018.

[4] Weishaar, R.E. and R.U. Simpson, *Vitamin D3 and cardiovascular function in rats.* The Journal of clinical investigation, 1987. **79**(6): p. 1706-1712.

[5] Holick, M.F. and T.C. Chen, *Vitamin D deficiency: a worldwide problem with health consequences.* The American journal of clinical nutrition, 2008. **87**(4): p. 1080S-1086S.

[6] Bischoff-Ferrari, H.A., *et al*., *Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes.* The American journal of clinical nutrition, 2006. **84**(1): p. 18-28.

[7] Muscogiuri, G., *et al*., *Can vitamin D deficiency cause diabetes and cardiovascular diseases? Present evidence and future perspectives.* Nutrition, Metabolism and Cardiovascular Diseases, 2012. **22**(2): p. 81-87.

[8] Baz-Hecht, M. and A.B. Goldfine, *The impact of vitamin D deficiency on diabetes and cardiovascular risk.* Current Opinion in Endocrinology, Diabetes and Obesity, 2010. **17**(2): p. 113-119.

[9] Lauckner, J.E., B. Hille, and K. Mackie, *The cannabinoid agonist WIN55, 212-2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins.* Proceedings of the National Academy of Sciences, 2005. **102**(52): p. 19144-19149.

[10] Levin, A. and Y.C. Li, *Vitamin D and its analogues: do they protect against cardiovascular disease in patients with kidney disease?* Kidney international, 2005. **68**(5): p. 1973-1981.

[11] Vitamin 2nd, D., *edition; Feldman, D.; Pike, JW; Glorieux, FH, Eds*. 2005, Academic Press: San Diego.

[12] O'Connell, T.D., R.E. Weishaar, and R.U. Simpson, *Regulation of myosin isozyme expression by vitamin D3 deficiency and 1, 25-dihydroxyvitamin D3 in the rat heart.* Endocrinology, 1994. **134**(2): p. 899-905.

[13] Razzaque, M.S., *The dualistic role of vitamin D in vascular calcifications.* Kidney international, 2011. **79**(7): p. 708-714.

[14] Levine, G.N., *et al*., *Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease.* Circulation, 1996. **93**(6): p. 1107-1113.

[15] Leopold, J.A. and J. Loscalzo, *Oxidative risk for atherothrombotic cardiovascular disease.* Free Radical Biology and Medicine, 2009. **47**(12): p. 1673-1706.

[16] Bahorun, T., *et al*., *Free radicals and antioxidants in cardiovascular health and disease.* Internet Journal of

Medical Update, 2006. **1**(2): p. 25-41.

[17] Mellanby, S.E., *The part played by an accessory factor in the production of experimental rickets.* 1918.

[18] Lindén, V., *Vitamin D and myocardial infarction.* Br Med J, 1974. **3**(5932): p. 647-650.

[19] Hess, A.F. and L.J. Unger, *Prophylactic therapy for rickets in a Negro community.* Journal of the American Medical Association, 1917. **69**(19): p. 1583-1586.

[20] Lee, J.H., *et al*., *Prevalence of vitamin D deficiency in patients with acute myocardial infarction.* The American journal of cardiology, 2011. **107**(11): p. 1636-1638.

[21] Giovannucci, E., *et al*., *25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study.* Archives of internal medicine, 2008. **168**(11): p. 1174-1180.

[22] Scialla, J.J., *et al*., *Race, mineral homeostasis and mortality in patients with end-stage renal disease on dialysis.* American journal of nephrology, 2015. **42**(1): p. 25-34.

[23] Shanker, J., *et al*., *Role of vitamin D levels and vitamin D receptor polymorphisms in relation to coronary artery disease: the Indian atherosclerosis research study.* Coronary artery disease, 2011. **22**(5): p. 324-332.

[24] Veloudi, P., G. Jones, and J.E. Sharman, *Effectiveness of vitamin D supplementation for cardiovascular health outcomes.* Pulse, 2016. **4**(4): p. 193-207.

[25] Perła-Kaján, J. and H. Jakubowski, *Paraoxonase 1 and homocysteine metabolism.* Amino acids, 2012. **43**(4): p. 1405-1417.

[26] Mutlu-Türkoðlu, Ü., *et al*., *Increased plasma malondialdehyde and protein carbonyl levels and lymphocyte DNA damage in patients with angiographically defined coronary artery disease.* Clinical biochemistry, 2005. **38**(12): p. 1059-1065.

[27] Jung, H.H., D.H. Choi, and S.H. Lee, *Serum malondialdehyde and coronary artery disease in hemodialysis patients.* American journal of nephrology, 2004. **24**(5): p. 537-542.

[28] Bridges, A., *et al*., *Relationship between the extent of coronary artery disease and indicators of free radical activity.* Clinical cardiology, 1992. **15**(3): p. 169-174.

[29] Stanger, O., *et al*., *NADH/NADPH oxidase p22 phox C242T polymorphism*

and lipid peroxidation in coronary artery disease. Clinical Physiology, 2001. **21**(6): p. 718-722.

[30] Steinberg, D. and J.L. Witztum, *Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis?* Circulation, 2002. **105**(17): p. 2107-2111.

[31] مقـــدم, ع.پ., *et al*., *Serum MDA as a diagnostics biomarker in stable coronary heart disease.* Research Journal of Biological Sciences, 2008. **3**(2): p. 206-210.

[32] Suzuki, S., *et al*., *8-hydroxy-2'-deoxyguanosine is a prognostic mediator for cardiac event.* European journal of clinical investigation, 2011. **41**(7): p. 759-766.

[33] Patil, N., V. Chavan, and N. Karnik, *Antioxidant status in patients with acute myocardial infarction.* Indian Journal of Clinical Biochemistry, 2007. **22**(1): p. 45-51.

[34] Kumar, K.V. and U. Das, *Are free radicals involved in the pathobiology of human essential hypertension?* Free radical research communications, 1993. **19**(1): p. 59-66.

[35] Ylä-Herttuala, S., *et al*., *Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man.* The Journal of clinical investigation, 1989. **84**(4): p. 1086-1095.

[36] Abbate, A., *et al*., *Increased myocardial apoptosis in patients with unfavorable left ventricular remodeling and early symptomatic post-infarction heart failure.* Journal of the American College of Cardiology, 2003. **41**(5): p. 753-760.

[37] Singh, S.P., *et al*., *Fat accumulation in Caenorhabditis elegans triggered by the electrophilic lipid peroxidation product 4-hydroxynonenal (4-HNE).* Aging (Albany NY), 2009. **1**(1): p. 68.

[38] Dorn 2nd, G., *et al*., *Increased platelet thromboxane A2/prostaglandin H2 receptors in patients with acute myocardial infarction.* Circulation, 1990. **81**(1): p. 212-218.

[39] Bauer, J., *et al*., *Pathophysiology of isoprostanes in the cardiovascular system: implications of isoprostane-mediated thromboxane A 2 receptor activation.* British journal of pharmacology, 2014. **171**(13): p. 3115-3131.

[40] Hafeez M, Yasin T, Safdar U, Waquar S, Rana M, Malik A. An evidence

based assessment of most common risk factors of myocardial infraction: analysis from a local population. Biol Clin Sci Res J 2020(1):e044.

[41] Khalil R, Ali Q, Hafeez M, Malik A. Phenolic acid profiling by rp-hplc: evaluation of antibacterial and anticancer activities of *Conocarpus erectus* plant extracts. Biol Clin Sci Res J 2020(1):e010.

[42] Hameed B, Ali Q, Hafeez MM, Malik A. Antibacterial and antifungal activity of fruit, seed and root extracts of Citrullus colocynthis plant. Biol Clin Sci Res J. 2020;33.

[43] Ali J, Ali Q, Hafeez MM, Malik A. Clinical features, diagnosis and treatment of COVID-19. Biol Clin Sci Res J. 2020;2020:e032.

[44] Siddique A, Fateh A, Idrees N, Hafeez MM, Ali Q, Malik A. The epidemics of COVID-19. Biol Clin Sci Res J. 2020:e030.

[45] Rashid M, Kari M, Rashid R, Rana M, Amjad A, Hafeez M. Uterine artery doppler indices as predictive measures for the pre-eclampsia and intrauterine growth restriction. Biol Clin Sci Res J; 2020(1):e023.

[46] Omer M, Malik S, Anjum M, Riaz A, Ali R. Diagnostic accuracy of ultrasound in detecting meniscal tears taking magnetic resonance imaging as gold standard. Biol Clin Sci Res J 2020(1):e040.

[47] Ali Q, Khalil R, Nadeem M, Hafeez, MM, Malik, A. Antibacterial, antioxidant activities and association among plant growth related traits of *Lepidium draba*. Biol Clin Sci Res J. 2020:011.

[48] Khalid A, Anjum M, Daraaz U, Hussain K, Omer M. Predictive accuracy of cervical length in mid trimester on transabdominal ultrasound for cesarean section. Biol Clin Sci Res J 2020(1):e043.

[49] Chudhary H, Amin A, Malik M, Hafeez M, Rana M, Malik A. Risk assessment of non-conventional contributory factors in onset of diabetes mellitus type II. Biol Clin Sci Res J 2020(1):e036.

[50] Ali S, Gillani S, Afzal M, Parveen K. Assessment of nurses management skills for critically ill patients. Biol Clin Sci Res J 2020(1):e013.

[51] Tabassum S, Bibi T, Tariq F, Tariq S, Raza S, Hafeez M, Rana M. Unusual leukemoid reaction in a covid-19 patient: a case report. Biol Clin Sci Res J 2020(1):e034.

[52] Mady A, Ramdan O, Al Yousef R, Ishag A, Bakirova G, Kuhail A, Shahzad S, El-Etreby W, Mumtaz S, Almozainy S, Palacio K, Aldamahshi D, Alcazar A, Alodat M, Abdelrahman B, Harthy A. COVID 19 critical care training surge experience for physicians in riyadh health cluster one, Saudi Arabia. Biol Clin Sci Res J 2020(1):e041.