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Biochemistry

STUDY OF ASSOCIATION BETWEEN VITAMIN D AND ENDOTHELIAL DYSFUNCTION IN HYPERTENSIVE PATIENTS: A HOSPITAL BASED STUDY

Adil Rahman	Department of Biochemistry, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Republic of India.
Kuldeep K. Gupta*	Department of Biochemistry, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Republic of India. *Corresponding Author
Vijay K. Verma	Department of Medicine, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Republic of India.
Javed A. Siddiqui	Department of Radio diagnosis, Uttar Pradesh University of Medical Sciences, Saifai, Etawah. Republic of India.

ABSTRACT

HIGHLIGHTS

- Vitamin D deficiency can abate the endothelial function and support the pathogenesis of atherosclerosis.
- Supplementation of vitamin D in deficient subject can markedly improve the endothelial function and cardiac health.

Background: Endothelia cells produce endogenous vasodilator nitric oxide (NO) in response to local shear stress. Brachial artery flow - mediated vasodilatation (FMD), a measure of endothelium-dependent dilation. Vitamin D is known to affect function of endothelial cells through various regulatory mechanisms and its deficiency associated with decreased bioavailability of NO, leads to endothelial dysfunction associated with arterial stiffness and increase in cardiovascular disease risk.

Aims and Objectives: To study the effect of vitamin D on arterial stiffness and endothelial function, and explores its potential mechanisms for the cardio protective effect.

Material and Methods: In this study hypertensive subjects (N=57) of vitamin D deficient (< 20 ng /ml) and insufficient (20- 29 ng /ml) were supplemented to 60000 IU of vitamin D weekly for 6 weeks. Brachial artery flow mediated dilatation (FMD) was calculated before and after supplement. The sample had been processed by enzymatic immune assay method, and brachial artery FMD had been measured by ultrasound (5-7MHz linear array transducer).

Results: The obtained records of all the selected 57 patients were analyzed in pre and post supplement vitamin D. Before and after supplement the mean serum vitamin D level was 15.71±3.69 SD and 50.9±6.87SD. The mean brachial artery FMD before and after supplement with vitamin D was 6.86±1.87 SD and 14.34±3.17SD respectively with $p<0.001$, which shows a significant association between serum level of vitamin D and FMD.

Conclusion: This study support the hypothesis that serum vitamin D status is associated with vascular endothelial function among middle-aged/older adults, also demonstrate that lower level of serum vitamin D strongly associated with suppression of endothelial function.

KEYWORDS : FMD, Vitamin D, Hypertension

1. INTRODUCTION-

Endothelial cell lining of the artery is susceptible for the shear stress and it release endogenous nitric oxide (NO) in response to local shear stress [1]. NO is a potent vasodilator, which is a local effect of NO. Any cause of shear stress spontaneously induces vasodilation, which is the mechanical property of large arteries and also an important determinant of circulatory physiology of cardiovascular health [2]. Brachial artery flow - mediated vasodilatation (FMD), a measure of endothelium-dependent dilation [3], is a homeostatic response to short-term increase in local shear stress. Though FMD can be measured in different arteries, such as the radial, brachial, or femoral artery, but it is feasible in Brachial artery due to the ease of the procedure [4]. Arterial stiffness due to atherosclerosis leads to endothelial dysfunction and dwindle the elastic property of artery [5]. In atherosclerotic artery, brachial artery FMD is impaired in response to post ischemic reactive hyperemia because of endothelial dysfunction[6]. Atherosclerosis is a disease of the arterial system, generated by injury to the vasculature [7], lead to either cardiovascular or cerebral complication [8]. Hypertension is the major traditional factor for the pathogenesis of atherosclerosis [9]. In atherosclerosis distensibility of arterial wall decreases and it become stiff. A stiffer vessel has a very poor elasticity and will dilate very little in response to NO, than a more distensible and complaint vessel [10,11]. In the ageing process there is multifactorial gradual loss of the endothelial function leads to arterial stiffness such as- blood pressure, obesity, life style, smoking habit, lipid profile, diet and nutrition [12,13]. Many recent studies establish beneficial role of vitamin D in cardiovascular health besides its role in skeletal health [14]. These include vitamin D ability to affect function of endothelial cells through regulation of rennin-angiotensin-aldosterone system or smooth muscle proliferation or through pro-inflammatory state [15,16]. Vitamin D receptors (VDR) are present on many tissues such as vascular smooth muscle cells, cardiomyocytes and coronary artery [17,18,19]. Vitamin D inhibits vascular smooth muscle cell proliferation, vascular calcification, and atherogenesis via anti-inflammatory pathways [20]. Vitamin D deficiency is associated with endothelial dysfunction and plays an important role in the pathogenesis of arterial stiffness which leads to cardiovascular disease [21]. Flow mediated vasodilatation (FMD), is one of the parameter to measure arterial stiffness non invasively [22].

2. Materials and Methods-

2.1 *Study protocol*

The study protocol has been approved by the institutional ethical committee of U.P. University of Medical Sciences, Saifai, Etawah. The study population n=57 of hypertensive patients of age group 40-70 years were recruited from the outpatients department of medicine, U.P. University of Medical Sciences, Saifai Etawah, during 2016-2019.

2.2 *Study participants*

The study population n=57 (18 female 31.5% and 39 male 68.5%) of hypertensive subjects of age group 40-70 years with history of antihypertensive drug use, their systolic BP was ≥ 140 mmHg and diastolic BP was ≥90 mmHg were enroll for this study.

2.3 *Vitamin D*

Serum 25(OH)D level were measured by commercially available assay (EDI™ Total 25-OH Vitamin D EIA Kit). The analytical sensitivity of this assay for 25(OH)D is 0.9557 ng/ml with detection limit 150 ng/mL. The inter-assay precision reported for concentration 21.4 ng/mL, 37.0 ng/mL and 40.0 ng/mL with CV% of 3.1%, 4.3% and 2.5% respectively. In order to analyze the dose- response relationship between vitamin D and its effect on FMD. Serum level of vitamin D is categorized into three group – deficient (< 20 ng /ml), insufficient (20-29 ng /ml) and sufficient (> 29 ng /ml) [23]. Value of vitamin D measured in ng/mL, in SI unit it is nmol/L with conversion factor 2.496 (1ng/mL = 2.496 nmol/L).

2.4 *FMD (Flow Mediated Dilatation)*

It is percentage increase in dilatation of artery due to flow of blood

inside the artery after a short-term increase in local shear stress. FMD is used to measure endothelial dysfunction and to evaluate the risk of atherosclerosis or other cardiovascular diseases [24] Local shear stress for the evaluation of FMD is given in the form of pressure about 50 mmHg above the baseline systolic blood pressure to the brachial artery by sphygmomanometer[25,26,27]. Endothelium-dependent brachial artery FMD was measured by using an Ultrasonography and duplex scan machine (SAMSUNG SONOACE R7) equipped with the high frequency transducer of frequency 5-12 MHz frequency is used, having high resolution capacity. Initially on B-Mode, location and morphology of the Brachial Artery was assessed, Which was confirmed by colour Doppler in both transfers and in longitudinal section. The diameter of the pulsating artery was taken both pre and post stress in right upper arm at same position. Brachial artery diameter scan was taken proximal to the bifurcation of radial and ulnar artery. Diameter measurement was taken from one media- adventitia interface to other at baseline and after ischemia.

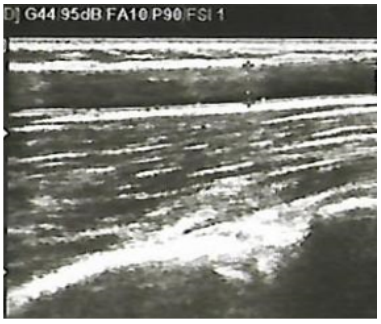

Figure : Original image showing the ulrasonographic picture of brachial artery.

2.5 *Statistical analysis*

All the parameter (FMD, vitamin D and blood pressure) measurement were taken two times first, before supplement of vitamin D then after 6 week, when patients were supplemented with 60000 IU of vitamin D weekly for 6 week. Continuous variables have been expressed as mean (standard deviation) and the difference in distribution of continuous variable were tested using paired student t test.

p-value <0.05 were considered statistically significant. All statistical data were analyzed by using SPSS software Version 23.

All the routine test as hemogram, lipid profile, liver function test, kidney function test, serum calcium with general and systemic examination were performed in all patients to exclude any other chronic illness, other than hypertension.

1. Results:

The obtained records of all the selected 57 subjects were analyzed in pre and post supplement vitamin D. Table -1shows distribution of hypertensive patients based on their vitamin D status which observed, 45 cases (78.94%) with deficient, 9 cases (15.78%) with insufficient and 3 cases (5.26%) with sufficient vitamin D. Table-2 depict the comparison between mean values of two variable i,e. vitamin D and FMD. Before supplement the mean serum vitamin D level was 15.71±3.69 SD and after supplement with vitamin D of 60000 IU weekly for six week it was found to be 50.9±6.87SD. The mean brachial artery FMD before and after supplement with vitamin D was 6.86±1.87 SD and 14.34±3.17SD respectively. On applying paired student t test the p <.001, which shows a significant positive association between serum level of vitamin D and FMD.

Table 1: Distribution of vitamin D in study population

Total patients enrolled for study (n=57)	Vitamin D deficient (<20 ng/ml)	Vitamin D insufficient (20-29 ng/ml)	Vitamin D sufficient (>29 ng/ml)
	n=45 (78.94%)	n=9 (15.78%)	n=3 (5.26%)

Table 2: Statistical analysis of the patients between pre and post vitamin D supplement.

Parameters	Before Vitamin D supplement (Mean±SD)	After Vitamin D supplement (Mean±SD)	P value (t test)
S. Vit. D ng/dl	15.71±3.69	50.9±6.87	<0.001
FMD (%)	6.86±1.87%	14.34±3.17	<0.001

1. DISCUSSION:

This study demonstrates that the impaired brachial artery FMD indicates local endothelial dysfunction and we suspect that the brachial artery would be susceptible to atherosclerosis. In the atherosclerotic artery there is decreased bioavailability of NO so there is decreased vasodilation and FMD. This study has been conducted in a tertiary care hospital in 57 subjects of age group40-70 years. Before supplement the mean vitamin D level was 15.71±3.69SD and mean FMD was 6.86±1.87SD, in the same subjects after supplements with vitamin D of 60000 IU weekly for six week at a mean value of serum vitamin D 50.9±6.87SD, FMD was found to be 14.34±3.17SD. This study shows that with increase in serum vitamin D (34.42±9.72) there is also increase in FMD (7.31±2.55). **Syal S K *et al* (2012)** found a graded relationship between 25(OH)D levels and FMD, and observed 13.3%, 38.6%, and 62.2% had impaired FMD in patients with 25(OH)D levels >20 ng/mL, 10-20 ng/mL, and <10 ng/mL, respectively[28]. **Harris RA *et al* (2011)** in a randomized control trial Following 16 weeks of 60,000 IU of oral vitamin D monthly supplementation there is significant improvements in FMD were observed[29].

In this study we also found that in 27% subjects which are 60 year or above, with increase in vitamin D from deficient to sufficient level, there is less increase in FMD (5.27±1.23) as compared to younger age group where increase in FMD (7.96±1.58) is higher, which means where atherosclerosis already developed and artery become stiff, effect of vitamin D to improve endothelial function is less. The meaning of this finding is that there is positive association between plasma vitamin D concentration and endothelial function, suggests the greater association between low level of vitamin D with pathogenesis of artery atherosclerosis leads to cardiovascular compromise. Though in many studies vitamin D deficiency have been shown to be associated with established cardiovascular disease risk factors, higher cardiovascular death and overall mortality, but recent meta-analysis have been conflicting, with both positive and neutral associations reported [30,31]. Effect of vitamin D on endothelial function is postulated to be either direct via modulation of calcium influx or by indirect mechanisms, including protection against oxidative stress and lipid peroxidation [32,33]. It has also been shown that vitamin D supplementation in deficient individuals is associated with improvements in parameters of vascular function, thus further strengthening its association with impaired FMD [34,35]. Given the postulated role of vitamin D in pathogenesis of cardiovascular disease, it is important to study its effect on endothelial function in larger patient numbers, since this would provide a mechanistic insight into how vitamin D deficiency could lead to higher risk of development of cardiovascular disease.

2. CONCLUSION:

FMD, a measure of endothelial function has been recognized as a marker of cardiovascular disease and associated with long-term prognosis in the general population. This prospective experimental study support the hypothesis that serum vitamin D status is associated with vascular endothelial function among middle-aged/older adults. Our findings also demonstrate that lower level of serum vitamin D strongly associated with suppression of endothelial function. In the subjects of deficient and insufficient serum vitamin D, supplemented with vitamin D at sufficient level markedly improve endothelial function and a significant increase in FMD suggest protective role of vitamin D in cardiovascular disease.

Conflicts of interest: The author declared that there are no conflict of interest regarding this manuscript.

REFERENCES

1. Vanhoutte PM, Zhao Y, Xu A, Leung SW. Thirty Years of Saying NO: Sources, Fate, Actions, and Misfortunes of the Endothelium-Derived Vasodilator Mediator. Circ Res. 2016 Jul 8;119(2):375-96.
2. Meredith IT, Currie KE, Anderson TJ et al. Post ischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide. Am J Physiol. 1996; 270: H1435-H1440.
3. Tremblay JC, Pyke KE. Flow-mediated dilation stimulated by sustained increases in shear stress: a useful tool for assessing endothelial function in humans?
4. Kirthi Bellamkonda, Matthew Williams, Ashok Handa et al. Flow Mediated Dilatation as a Biomarker in Vascular Surgery Research. J Atheroscler Thromb. 2017 Aug 1; 24(8): 779–787.
5. Jessica E. Wagenseil, Robert P. Mecham. Elastin in large artery stiffness and hypertension. J Cardiovasc Transl Res. 2012 Jun; 5(3): 264–273.
6. Gary F. Mitchell, Helen Parise, Joseph A et al. Local shear stress and brachial artery flow mediated dilatation. Hypertension. 2004; 44:134-139.
7. Rodriguez-Flores M, Rodriguez-Saladana J, Cantu- Brito C et al. Prevalence and severity of atherosclerosis in different arterial territories and its relation with obesity. Cardiovascular Pathology. 2013; 22(5):332-338.

8. Rollo M, Tartaglion T, Pedicelli A. Atherosclerosis of carotid and intracranial arteries. Rays 2001; 26(4):247-268.
9. Li G, Hu R, Gu J, Wu HZ. Relationship between carotid artery atherosclerosis and sulfatide in hypertensive patients. Genetics and Molecular Research. 2015 May 11; 14(2):4840-6.
10. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indication. Vascular Pharmacology. 2016; 77:1-7.
11. Dickinson KM, Clifton PM, Burrell LM et al. Postprandial effects of a high salt meal on serum sodium, arterial stiffness, markers of nitric oxide production and markers of endothelial function. Atherosclerosis. 2014 Jan; 232(1):211-6.
12. Determination of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: ' establishing normal and reference values '. European Heart Journal. Volume 31, issue 19, 1 October 2010, 2338-2350.
13. Ching-Fen Wu, Pang-Yen Liu, Tsung-Jui Wu et al. Therapeutic modification of arterial stiffness: An update and comprehensive review. World Journal of Cardiology.2015 Nov 26; 7(11):742-753
14. Schnatz PF, Manson JE. Vitamin D and cardiovascular disease: an appraisal of the evidence. Clinical Chemistry. 2014 Apr; 60(4):600-9.
15. Hernandez NP, Duque GA, Hernandez MCN, et al. Vitamin D and its effects on cardiovascular disease: a comprehensive review. Korean J Internal Med.2016 Nov; 31(6): 1018-1029.
16. Nathan Carlson, Robert Mah, Maria Aburto et al. Hypovitaminosis D correction and high-sensitivity C- reactive protein levels in hypertensive adults. The Permanente Journal. 2013; 17(4): 19-21.
17. Merke J, Milde P, Lewicka S, Hugel U, Klaus G, Mangelsdorf DJ, et al. Identification and regulation of 1,25-dihydroxyvitamin D3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D3. Studies in cultured bovine aortic endothelial cells and human dermal capillaries. Journal of Clinical Investigation. 1989; 83:1903–1915.
18. Schnatz PF, Nudy M, O'Sullivan DM, Jiang X, Cline JM, Kaplan JR, et al. The quantification of vitamin D receptors in coronary arteries and their association with atherosclerosis. Maturitas. 2012; 73:143–147.
19. Schnatz PF, Nudy M, O'Sullivan DM, Jiang X, Cline JM, Kaplan JR, et al. Coronary artery vitamin D receptor expression and plasma concentrations of 25-hydroxyvitamin D: their association with atherosclerosis. Menopause 2012; 19:967-973.
20. Paloma Munoz-Aguirre, Edgar Denova-Gutierrez, Mario Flores et al. High vitamin D consumption is inversely associated with cardiovascular disease risk in an urban Mexican population. PLOS. 2016; 11(11): e0166869.
21. Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008; 117:503-511.
22. Tomiyama H, Yamashina A. Non-invasive vascular function tests: their pathophysiological background and clinical application. Circulation 210 Jan; 74(1):24-33
23. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. Hypertension.2011; 57:63-69.
24. Gary F. Mitchell, Helen Parise, Joseph A et al. Local shear stress and brachial artery flow mediated dilatation. Hypertension. 2004; 44:134-139.
25. E Bianchi, F Faita, V Gemignani et al. The assessment of Flow-Mediated Dilatation of the brachial artery. Computers in Cardiology. 2006; 33:509-512.
26. Hassan A Chami, Michelle J Keyes, Joseph A Vita et al. Brachial artery diameter, blood flow and flow mediated dilation in sleep-disordered breathing. Vascular medicine. 2009; 14: 351-360.
27. Michiel L, Bots Jan Westerink Ton J, Rabelink Eelco J et al. Assessment of flow-mediated vasodilation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. European Heart Journal 2005; 26:363-368.
28. Syal SK, Kapoor A, Bhatia E, et al. Vitamin D deficiency, coronary artery disease and endothelial dysfunction: Observations from a coronary angiographic study in Indian patients. J Invasive Cardiole. 2012;24:385-389.
29. Harris RA, Pedersen-White J, Guo DH et al. Vitamin D3 supplementation for 16 weeks improves flow-mediated in overweight African-American adults. Am J Hypertens.2011 may;24(5):557-562.
30. Elamin MB, Abu Elnour NO, Elamin KB et al. Vitamin D and Cardiovascular outcomes: a systemic review and meta-analysis. J Clin Endocrinol Metab. 2011;96(7):1931-1942
31. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med. 2007;167(16):1730-1737.
32. Wiseman H. Vitamin D is a membrane antioxidant. Ability to inhibit iron-dependent lipid peroxidation in liposomes compared to cholesterol, ergosterol and tamoxifen and relevance to anticancer action. FEBS Lett. 1993;326(1-3):285-288
33. Kuzmenko AI, Morozova RP, Nikolenko IA et al. Effects of vitamin D3 and ecdysterone on free-radical lipid peroxidation. Biochemistry (Mosc). 1997;62(6):609-612
34. Harris RA, Pedersen-White J, Guo DH, et al. Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. Am J Hypertens. 2011;24(5):557-562
35. Tarcin O, Yavuz DG, Ozben B, et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab. 2009;94(10):4023-4030