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The intricate relationship of vitamin-D with Arterial Stiffness: A case control study on middle-aged Indian population with an insight from the mechanistic pathways providing special reference to endothelial dysfunction and oxygen sensing proteins.

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ABSTRACT

Introduction: Arterial stiffness (AS) is recognized as a crucial predictor of cardiovascular events, particularly in middle-aged individuals. Meanwhile, the role of vitamin D in cardiovascular health has garnered significant attention. Emerging evidence suggests a potential link between the level of serum total vitamin D status and AS, although the underlying mechanisms remain incompletely understood. Our case control study aims to elucidate the intricate relationship of serum total vitamin D with AS and explore the potential mechanistic pathways in the middle-aged population.

Materials and Methods: 108 age-matched participants of both genders were divided into three groups according to their hypertension (HTN) status following the latest American Heart Association (AHA) guidelines. Individuals having normal blood pressure (BP) were considered participants of Group 1 (control group) whereas the stage-I hypertensive individuals were considered participants of Group 2 and stage-II hypertensive participants were considered participants of Group 3. All the participants were assessed for their level of serum total vitamin D, arterial stiffness index (ASI) and pulse wave velocity (PWV). The data was entered into a Microsoft Excel Sheet and analyzed using SPSS software (version 25).

Results: ASIs of different limbs and PWVs were found to be increased in age-matched both the hypertensive groups participants as compared to the normotensive (control) group. Serum total vitamin D level was also found to be lower in both the hypertensive groups when compared with the normotensive (control) group. We found a negative correlation between parameters of the arterial stiffness and level of serum total vitamin D. We also found a negative correlation between the components of BP and serum total vitamin D.

Conclusion: We conclude our study by finding a beneficial role of vitamin D for reducing AS which in turn influences to decrease the BP and results in lower events of cardiovascular morbidities.

Keywords: *Arterial Stiffness Index (ASI), Pulse Wave Velocity (PWV), Hypertension (HTN), Blood Pressure (BP), Vitamin D*

1. INTRODUCTION

Arterial stiffness (AS), characterized by decreased elasticity and compliance of the arterial wall, is a hallmark of vascular ageing, and a strong predictor of cardiovascular events, such as hypertension (HTN), stroke, and coronary artery disease (CAD). This is an independent responsible factor for morbidities and mortalities anyone can suffer globally due to Non-

communicable diseases (NCDs) [1]. Arterial stiffness index (ASI) of different limbs and different pulse wave velocities (PWVs), commonly used to assess AS, can predict ongoing vascular damage and alteration in vascular architecture [2]. Elevated AS is associated with HTN, atherosclerosis, and other cardiovascular diseases, highlighting the clinical importance of identifying factors contributing to its development and progression [3]. Vitamin D, primarily known for its role in calcium homeostasis and bone health, has garnered increasing attention for its potential effects on cardiovascular health [4]. Vitamin D receptors (VDRs) are widely expressed in vascular endothelial cells, smooth muscle cells, and immune cells, suggesting a direct role for vitamin D in vascular function and inflammation [5]. Several studies have reported an association between vitamin D deficiency and an increased risk of cardiovascular events, including HTN, myocardial infarction (MI), and stroke [6, 7]. However, the underlying mechanisms linking vitamin D and cardiovascular health, particularly AS, remain poorly understood. Our case control study aims to elucidate the intricate relationship of serum level of total vitamin D with AS and explore the potential mechanistic pathways in the middle-aged Indian population underlying this relationship with a special reference to endothelial function and oxygen sensing proteins. Thus, we also aim to provide insights into the role of serum level of total vitamin D in AS and its implications for cardiovascular health.

2. MATERIALS AND METHODS

Type of study: Case Control study.

Sample size calculation: With Anticipated correlation coefficient between Vitamin D and PWV - 0.555 at 95% confidence level and 90 power in the study [8], the sample size worked out is 36 per group. Total sample size= 36+36+36=108

Formula used: $N=\left[\left(\frac{Z_\alpha+Z_\beta}{C}\right)\right]^2+3$

$C=0.5*\ln\left[\frac{1+r}{1-r}\right]= 0.2758$

The standard normal deviate for $\alpha = Z_\alpha = 1.960$

The standard normal deviate for $\beta = Z_\beta = 1.649$

Study design: Our study included 108 participants of both genders (54 males and 54 females) divided into group 1 (control, normotensive individuals, n = 36), group 2 (stage-I hypertensive individuals, n = 36) and group 3 (stage-II hypertensives, n = 36) from the age range of 35 to 50 years. Each study group consisted of age-matched 18 male and 18 female participants. Chronic smokers, alcoholics, diabetics, patients with any chronic diseases or malignancies, and patients taking antihypertensive treatments were excluded. We also excluded the female participants with pregnancy and 1 year post childbirth. We excluded all the participants taking regular medications which can alter the vascular architecture.

Assessed parameters: The following parameters were assessed for all the individuals accepting to be participants of the study after following the proper study design:

I. Anthropometric parameters [9]:

- Height: Measured using a device (BIOCON^{TM}) mounted on the wall and was expressed in centimeters (*cms*).
- Weight: Measured using a manually calibrated digital weighing machine and was expressed in Kilograms (*Kg*).
- Body Mass Index (BMI): Calculated manually from weight in Kilograms (*Kg*) divided by height in meters square (m^2) and was expressed as Kg/m^2.
- Waist Circumference (WC): Measured by following the World Health Organization (WHO) guidelines and was expressed in cms.
- Hip Circumference (HC): Measured by following the World Health Organization (WHO) guidelines and was expressed in cms.
- Waist Hip Ratio (WHR): Calculated manually from weight circumference (cms) divided by hip circumference (cms) and the value was recorded.

II. Physiological parameters [9]:

- Pulse rate (PR): Determined manually and was expressed in beats/minute.
- Respiratory rate (RR): Examined manually and was expressed in breaths/minute.
- Temperature (Temp): Examined by using a digital thermometer and was expressed in ºF.
- Systolic Blood Pressure (SBP): Recorded by using mercury sphygmomanometer and was expressed in mmHg.
- Diastolic Blood Pressure (DBP): Recorded by using mercury sphygmomanometer and was expressed in mmHg.
- Pulse Pressure (PP): Calculated manually by deducting the value of DBP (mmHg) from SBP (mmHg) and was

expressed in mmHg.

- Mean Arterial Pressure (MAP): Calculated manually by adding the value of DBP (mmHg) with the one-third value of PP (mmHg) and expressed in mmHg.

III. Electrophysiological parameters [9]:

- Arterial Stiffness Index (ASI) of all four limbs:
- Right Brachial Arterial Stiffness Index (R Bra ASI, mmHg)
- Left Brachial Arterial Stiffness Index (L Bra ASI, mmHg)
- Right Ankle Arterial Stiffness Index (R Ank ASI, mmHg)
- Left Ankle Arterial Stiffness Index (L Ank ASI, mmHg)
- Pulse Wave Velocities (PWVs):
- Right Brachial-Ankle Pulse Wave Velocity (PWV_{b-a} Right, cm/sec)
- Left Brachial-Ankle Pulse Wave Velocity (PWV_{b-a} Left, cm/sec)
- Carotid-Femoral Pulse Wave Velocity (PWV_{c-f}, cm/sec)

IV. Biochemical parameters [4, 10]:

- Serum total vitamin D level (Vit D, ng/ml) was estimated by Enzyme-linked immunosorbent assay (ELISA) method.
- Serum Creatinine level (Creat, mg/dl) was estimated by using Jaff's Method.
- Serum Urea level (Urea, mg/dl) was estimated by Diacetyl Monoxime (DAM) method.
- Serum Fasting blood glucose level (FBS, mg/dl) was measured by Glucose oxidase-peroxidase (GOD-POD) method.
- Serum Malondialdehyde level (MDA, µmol/L) was measured by using UV Spectrophotometer at 535 nm.
- Serum Nitric Oxide (NO, µmol/L) was measured by using UV spectrophotometer at 535 nm.
- Serum Triglyceride (TGL, mg/dl) was estimated by glycerol phosphatase-oxidase (GPO-PAP) method.
- Serum total Cholesterol (Choles, mg/dl) was estimated by using cholesterol oxidase-peroxidase (CHOD-PAP) enzymatic method.
- Serum High Density Lipoprotein (HDL, mg/dl) was estimated by using phosphotungstic acid (PTA) method.

V. Molecular parameters [10]:

- Serum Erythropoietin (EPO, pg/ml) was estimated by Enzyme-linked immunosorbent assay (ELISA) method for its quantitative measurement.
- Serum Vascular Endothelial Growth Factor (VEGF, pg/ml) was estimated by Enzyme-linked immunosorbent assay (ELISA) method for its quantitative measurement.

Study setting: The present study is conducted in the Shri B M Patil Medical College Hospital and Research Centre, BLDE (Deemed to be University) of Vijayapura district of Karnataka, India. The study was conducted after obtaining the ethical clearance (IEC/No-09/2021 Dated 22/01/2021) from the Institutional Ethical Committee (IEC) of BLDE (Deemed to be University) following which voluntary written informed consent was obtained from all the study participants.

Method of selection of study participants and collection of data: Participants were selected from the outpatient department clinic of the Department of Medicine, Shri B M Patil Medical College Hospital and Research Centre after obtaining their consent provided that they fit into the proposed study design. The selected participants were called the very next working day to the Centre for Yoga and Exercise, Department of Physiology of Shri B M Patil Medical College Hospital and Research Centre between 7 AM to 8 AM for assessment of their anthropometric parameters, physiological parameters and electrophysiological parameters in the supine posture after giving them rest for 10 minutes at room temperature. Then all the study participants were taken to the Laboratory of Vascular Physiology and Medicine, Department of Physiology of Shri B M Patil Medical College Hospital and Research Centre between 8 AM to 9 AM for collection of the overnight fasting blood samples in plain vials for assessment of the biochemical parameters. After the collection of the blood samples, all the participants were directed to the lounge area near the research laboratory of the Department of Biochemistry where they were served breakfast. All the participants who were diagnosed with hypertension were accompanied to the outpatient department clinic of the Department of Medicine of Shri B M Patil Medical College Hospital and Research Centre after breakfast between 10 AM to 11 AM where they were managed for their hypertension.

Assessment of anthropometric parameters: The anthropometric parameters were assessed by following the WHO protocol for measurement of the anthropometric parameters by using a measuring tape, and a manually calibrated digital weighing machine.

Assessment of physiological parameters: The physiological parameters were assessed by using a stethoscope, a manually

calibrated mercury sphygmomanometer, and a digital thermometer.

Assessment of electrophysiological parameters: The electrophysiological parameters were measured by using a Periscope (Genesis Medical Systems, India) which is a non-invasive automatic device, work on oscillometric method.

Study Period: 1st July 2021 to 30th June 2022, Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka

Period of Data Analysis: 1st December 2022 to 31st December 2022, Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka.

Period of Original Draft Preparation: 1st September 2023 to 1st December 2023, Teerthanker Mahaveer Medical College & Research Centre, Moradabad, India.

Period of Draft Review: 31st December 2023 to 21st January 2024, Teerthanker Mahaveer Medical College & Research Centre, Moradabad, India.

Period of Final Manuscript: 21st January 2024 to 31st January 2024, Teerthanker Mahaveer Medical College & Research Centre, Moradabad, India.

Statistical Analysis: The data was entered into a Microsoft Excel Sheet and analyzed using SPSS software (version 25). We compared our parameters between the study groups by using the Kruskal Wallis test and Post hoc test. The comparison between the genders were done using Mann-Whitney U test. The comparative results between the study groups were expressed as mean ± SD and $p \leq 0.05$ was considered statistically significant. The correlations between the variables were performed using Pearson's correlation for the entire study population and the values of the correlation coefficient (r-value) were expressed. All statistical tests are performed two tailed.

3. RESULTS

All the participants (n = 108) examined were age matched.

Comparative assessment of the anthropometric parameters: Comparison between anthropometric parameters of three groups is shown in Table 1. It is clearly visible that mean values of all the anthropometric parameters (BMI, WC, HC, WHR) of stage I HTN group are significantly greater than the control group (P<0.001). All these anthropometric parameters of stage II HTN group participants were also found to be significantly higher than stage I HTN group participants (P<0.001). When compared between the genders of the anthropometric parameters of all the groups, there were no significant changes observed (Table 2). The detailed Post hoc analysis report is depicted in Table 3.

Comparative assessment of the physiological parameters: While analyzing the physiological parameters between three groups by Kruskal-Wallis test, it showed significant higher values of PR (P<0.001) in the stage II HTN group participants as compared to the control group participants while other physiological parameters like RR, Temp. did not showed any significant difference between all the three groups which is depicted in Table 4. We did not find any significant difference of any of the physiological parameters between the genders by using Mann Whitney U test (Table 5). The detailed analysis report of the Post hoc test of the Physiological parameters is shown in Table 6.

Comparative assessment of the electrophysiological parameters: After using Kruskal-Wallis test, the report of comparison of all the Electrophysiological parameters between all the three groups are shown in Table 7 where it is clearly visible that all the arterial stiffness parameters like PWV_{b-a} Right, PWV_{b-a} Left, PWV_{c-f}, R Bra ASI, L Bra ASI, R Ank ASI, L Ank ASI were found to be higher in stage II hypertension group as compared to stage I hypertension group (P<0.001). Both the hypertensive groups were also found to be significantly greater than control group (P<0.001). We did not find any significant difference of any of the electrophysiological parameters between the genders by using Mann Whitney U test (Table 8). The detailed Post hoc analysis report of the electrophysiological parameters is depicted in Table 9.

Comparative assessment of the biochemical parameters: After using Kruskal-Wallis test, the report of comparison of all the biochemical parameters between the three groups is shown in Table 10.

Serum total Vitamin D concentration: It is clearly visible that the concentration of serum total vitamin D level found to be lower in stage II HTN group participants as compared to stage I HTN group participants (P<0.001). The serum total vitamin D concentration in both the HTN groups were also found to be significantly lesser than the control group (P<0.001).

Concentrations of renal profile (Blood Urea and Serum Creatinine) and FBS: We also found a significant (P<0.05) higher levels of Blood Urea and Serum Creatinine, FBS concentration in stage II hypertensive participants as compared to stage I hypertensive participants. Both the HTN group participants were found to be having significantly greater (P<0.05) FBS, Blood Urea and Serum Creatinine level concentration in comparison to their respective control group.

Concentration of parameters of lipid profile (Serum Total Cholesterol, Serum Triglyceride, HDL Cholesterol): Among the parameters of lipid profiles, we found that concentration of serum total cholesterol and serum TGL are significantly higher (P<0.05) in stage II HTN group participants as compared to stage I HTN group participants and both the HTN group

participants were having a significant higher (P<0.05) values of serum cholesterol and serum TGL level concentration as compared to control group participants. We also found that the concentration of serum HDL cholesterol is significantly lower (P<0.001) in stage II hypertensive participants as compared to stage I hypertensive participants and both the HTN group participants are having significantly lower (P<0.001) serum HDL cholesterol level concentration as compared to control group participants.

Parameters of oxidative stress (Serum MDA) and endothelial function (Serum Nitric Oxide): We also found that the oxidative stress parameter like concentration of serum MDA is significantly higher (P<0.001) in stage II HTN group participants as compared to stage I HTN group participants and both the HTN group participants were having significantly higher (P<0.001) serum MDA concentration as compared to control group participants. The concentration of serum NO level, which is a marker for endothelial dysfunction, were found to be significantly lower (P<0.001) in stage II HTN group participants as compared to stage I HTN group participants and both HTN group participants were having significantly lower (P<0.001) levels of serum NO concentration as compared to control group participants.

There were no significant differences of all these biochemical parameters between the Male and the Females of each group (Table 11). The detailed Post hoc analysis report of the biochemical parameters is depicted in Table 12.

Comparative assessment of the molecular parameters: After using Kruskal-Wallis test, the report of comparison of all the Molecular parameters between all the three groups is shown in Table 13.

Concentration of serum EPO: It is clearly visible that the concentration of Serum EPO is found to be higher in stage II HTN group participants as compared to stage I HTN group participants (P<0.001). Both the HTN group participants were also found to be having significantly greater (P<0.001) level of serum EPO concentration than the control group participants.

Concentration of serum VEGF: It is clearly visible that the concentration of Serum VEGF is found to be lower in stage II HTN group participants as compared to stage I HTN group participants (P<0.001). Both the HTN group participants were also found to be having significantly lesser (P<0.001) level of serum VEGF concentration than their respective control groups.

There were no significant differences of all these molecular parameters between the genders of all the 3 groups by using Mann-Whitney U test (Table 14). The detailed Post hoc analysis report of the molecular parameters is depicted in Table 15.

Correlation between parameters: Correlation between serum total vitamin D concentration and blood pressure is depicted in Table 16 and Table 17 depicts the correlation between serum total vitamin D concentration, arterial Stiffness, and blood pressure. Table 18 shows the correlation between serum total vitamin D concentration and oxygen sensing proteins and Table 19 depicts the correlation between parameters of oxidative stress (MDA), endothelial function (NO), and oxygen sensing proteins (EPO, VEGF) with BP (SBP, DBP, MAP). It is self-explanatory from these tables that level of serum total vitamin D is negatively correlated with arterial stiffness and blood pressure while blood pressure and arterial stiffness is positively correlated. When we compared the level of serum total vitamin D with the oxygen sensing proteins, we found a negative correlation with EPO and positive correlation with VEGF. It is also visible from these tables that the components of BP are negatively correlated with serum NO and VEGF while we also found a positive correlation of components of BP with serum MDA and EPO.

Table 1: Comparison of Anthropometric Parameters between three groups

Parameters	Control Group (n=36) Mean ± SD	Stage I HTN (n=36) Mean ± SD	Stage II HTN (n=36) Mean ± SD	Kruskal-Wallis test	P Value
BMI (Kg/m^2)	23.06 ± 1.31	25.81 ± 3.87	27.9 ± 3.21	45.531	<0.001
WC (cm)	84.17 ± 1.59	92.14 ± 9.08	97.5 ± 5.68	52.517	<0.001
HC (cm)	85.97 ± 2.91	94.08 ± 9.65	97.8 ± 6.59	44.440	<0.001
WHR	0.97 ± 0.03	0.98 ± 0.05	0.99 ± 0.04	29.881	<0.001
Statistically significant					

Table 2: Comparison of Anthropometric Parameters between Genders

Parameters	Male (n=54) Mean ± SD	Female (n=54) Mean ± SD	Mann-Whitney U test	P Value
BMI (Kg/m^2)	25.65 ± 4.071	25.52 ± 3.039	U=1361.500	0.550
WC (cm)	91.74 ± 9.341	90.80 ± 7.133	U=1457.500	0.998
HC (cm)	92.13 ± 9.051	93.15 ± 7.968	U=1329.000	0.427
WHR	0.9936 ± 0.021	0.992 ± 0.019	U=1448.000	0.940

Statistically insignificant

Table 3: Post hoc test of Anthropometric Parameters between three groups

Parameters	Control Group (n=36)		Stage I HTN (n=36)		Stage II HTN (n=36)	
	Stage I	Stage II	Control	Stage II	Control	Stage I
BMI (Kg/m^2)	0.001	0.001	0.001	0.012	0.001	0.012
WC (cm)	0.012	0.001	0.001	0.001	0.001	0.001
HC (cm)	0.001	0.001	0.001	0.069	0.001	0.069
WHR (WC:HC)	0.001	0.001	0.001	1.000	0.001	1.000

Table 4: Comparison of Physiological Parameters between three groups

N=108	Control Group (n=36) Mean ± SD	Stage I HTN (n=36) Mean ± SD	Stage II HTN (n=36) Mean ± SD	Kruskal-Wallis test	P Value
Pulse (bpm)	75.08 ± 7.17	73.47 ± 6.566	81.89 ± 7.797	21.550	<0.001*
RR (cpm)	13.25 ± 1.05	13.33 ± 1.146	13.25 ± 1.052	0.135	0.935
Temp. (F)	97.44 ± 0.51	97.56 ± 0.504	97.44 ± 0.504	1.176	0.555
SBP (mmHg)	115.1 ± 3.39	134.2 ± 2.762	148.1 ± 8.342	90.197	<0.001*
DBP (mmHg)	73.28 ± 4.76	83.17 ± 3.621	90.33 ± 6.076	79.440	<0.001*
PP (mmHg)	41.83 ± 5.05	51.00 ± 4.623	57.72 ± 10.33	51.850	<0.001*
MAP (mmHg)	87.22 ± 3.65	100.2 ± 2.684	109.7 ± 4.911	92.191	<0.001*
*Statistically significant					

Table 5: Comparison of Physiological Parameters between Genders

Parameters	Male (n=54) Mean ± SD	Female (n=54) Mean ± SD	Mann-Whitney U test	P Value
PR (bpm)	75.81 ± 8.143	77.81 ± 7.833	U=1210.500	0.127
RR (cpm)	13.44 ± 1.058	13.11 ± 1.076	U=1206.000	0.106
Temp. (F)	97.46 ± 0.503	97.50 ± 0.505	U=1404.000	0.701
SBP (mmHg)	133.3 ± 15.68	131.6 ± 13.53	U=1394.500	0.696
DBP (mmHg)	82.59 ± 9.410	81.93 ± 7.672	U=1382.000	0.639
PP (mmHg)	50.67 ± 9.292	49.70 ± 10.05	U=1344.000	0.482
MAP (mmHg)	99.54 ± 11.09	98.54 ± 8.855	U=1384.500	0.651
Statistically insignificant				

Table 6: Post hoc test of Physiological Parameters between three groups

Parameters	Control Group (n=36)		Stage I HTN (n=36)		Stage II HTN (n=36)	
	Stage I	Stage II	Control	Stage II	Control	Stage I
PR (bpm)	1.000	0.001	1.000	0.001	0.001	0.001
RR (cpm)	1.000	1.000	1.000	1.000	1.000	1.000
Temp. (F)	1.000	1.000	1.000	1.000	1.000	1.000
SBP (mmHg)	0.001	0.001	0.001	0.001	0.001	0.001
DBP (mmHg)	0.001	0.001	0.001	0.001	0.001	0.001
PP (mmHg)	0.001	0.001	0.001	0.001	0.001	0.001
MAP (mmHg)	0.001	0.001	0.001	0.001	0.001	0.001

Table 7: Comparison of Electrophysiological Parameters between 3 groups

Parameters	Control Group (n=36) Mean ± SD	Stage I HTN (n=36) Mean ± SD	Stage II HTN (n=36) Mean ± SD	Kruskal-Wallis test	P Value

R Bra ASI (mmHg)	25.44 ± 2.4	24.44 ± 6.1	36.50 ± 4.98	65.34	<0.001
L Bra ASI (mmHg)	26.08 ± 2.7	25.58 ± 6.2	36.17 ± 5.40	59.71	<0.001
R Ank ASI (mmHg)	31.42 ± 4.3	34.11 ± 7.9	46.03 ± 8.85	50.35	<0.001
L Ank ASI (mmHg)	33.79 ± 5.3	37.97 ± 10	47.27 ± 7.34	42.28	<0.001
PWV_{b-a} Right (cm/s)	1170.7 ± 234	1345 ± 152	1544 ± 246	36.24	<0.001
PWV_{b-a} Left (cm/s)	1136.6 ± 107	1288 ± 162	1418 ± 373	28.56	<0.001
PWV_{c-f} (cm/s)	738.73 ± 98	874.2 ± 139	989.7 ± 175	38.19	<0.001
Statistically significant					

Table 8: Comparison of Electrophysiological Parameters between Genders

Parameters	Male (n=54)	Female (n=54)	Mann-Whitney U test	P Value
	Mean ± SD	Mean ± SD		
R Bra ASI (mmHg)	29.669 ± 7.981	27.926 ± 6.372	U=1333.500	0.443
L Bra ASI (mmHg)	29.120 ± 7.867	29.431 ± 5.991	U=1349.000	0.503
R Ank ASI (mmHg)	37.70 ± 11.28	36.67 ± 7.867	U=1458.000	1.000
L Ank ASI (mmHg)	39.222 ± 9.294	40.135 ± 10.14	U=1432.000	0.873
PWV_{b-a} Right (cm/s)	1397.9 ± 242.4	1309.4 ± 275.9	U=1262.000	0.228
PWV_{b-a} Left (cm/s)	1247.3 ± 173.3	1314.9 ± 333.9	U=1279.000	0.271
PWV_{c-f} (cm/s)	868.11 ± 168.7	866.99 ± 180.5	U=1434.500	0.885
Statistically insignificant				

Table 9: Post hoc test of Electrophysiological Parameters between three groups

Parameters	Control Group (n=36)		Stage I HTN (n=36)		Stage II HTN (n=36)	
	Stage I	Stage II	Control	Stage II	Control	Stage I
R Bra ASI (mmHg)	1.000	0.001	1.000	0.001	0.001	0.001
L Bra ASI (mmHg)	1.000	0.001	1.000	0.001	0.001	0.001
R Ank ASI (mmHg)	0.365	0.001	0.365	0.001	0.001	0.001
L Ank ASI (mmHg)	0.082	0.001	0.082	0.001	0.001	0.001
PWV_{b-a} Right (cm/s)	0.002	0.001	0.002	0.001	0.001	0.001
PWV_{b-a} Left (cm/s)	0.027	0.001	0.027	0.077	0.001	0.077
PWV_{c-f} (cm/s)	0.001	0.001	0.001	0.002	0.001	0.002

Table 10: Comparison of Biochemical Parameters between three groups

Parameters	Control Group (n=36) Mean ± SD	Stage I HTN (n=36) Mean ± SD	Stage II HTN (n=36) Mean ± SD	Kruskal-Wallis test	P Value
Total Vit. D (ng/ml)	35.92 ± 4.1	25.41 ± 4.1	17.05 ± 3.51	86.58	<0.001
S. Creatinine (mg/dl)	0.714 ± 0.1	0.944 ± 0.2	1.00 ± 0.17	88.11	<0.001
Blood Urea (mg/dl)	26.58 ± 4.5	27.36 ± 5.6	29.83 ± 6.52	7.92	0.019
FBS (mg/dl)	72.50 ± 8.7	82.42 ± 10	84.17 ± 8.30	25.93	<0.001
Serum TGL (mg/dl)	118.8 ± 25	137.9 ± 56	148.5 ± 45.8	7.23	0.027
Serum Chol. (mg/dl)	169.9 ± 35	173.1 ± 42	195.4 ± 44.7	7.44	0.024
HDL (mg/dl)	55.25 ± 7.6	47.61 ± 8.2	45.94 ± 9.13	21.30	<0.001
MDA (µmol/L)	1.014 ± 0.2	1.222 ± 0.4	2.083 ± 0.55	53.09	<0.001
NO (µmol/L)	8.532 ± 1.5	5.694 ± 1.4	3.694 ± 1.16	75.08	<0.001
Statistically significant					

Table 11: Comparison of Biochemical Parameters between Genders

Parameters	Male (n=54)	Female (n=54)	Mann-Whitney U test	P Value
	Mean ± SD	Mean ± SD		
Total Vit. D (ng/ml)	26.15 ± 9.09	26.12 ± 8.31	U=1455.500	0.988
S. Creatinine (mg/dl)	0.904 ± 0.15	0.869 ± 0.23	U=1389.000	0.619

Blood Urea (mg/dl)	29.07 ± 5.58	26.78 ± 5.72	U=1140.000	0.051
FBS (mg/dl)	80.26 ± 11.1	79.13 ± 10.1	U=1366.500	0.574
Serum TGL (mg/dl)	134.6 ± 46.2	135.6 ± 45.8	U=1432.500	0.875
Serum Chol. (mg/dl)	174.6 ± 40.1	184.3 ± 43.9	U=1265.000	0.236
Serum HDL (mg/dl)	49.52 ± 9.51	49.69 ± 8.99	U=1431.500	0.871
MDA (µmol/L)	1.396 ± 0.51	1.484 ± 0.72	U=1448.500	0.952
NO (µmol/L)	5.829 ± 2.27	6.119 ± 2.55	U=1351.500	0.510
Statistically insignificant				

Table 12: Post hoc test of Biochemical Parameters between three groups

Parameters	Control Group (n=36)		Stage I HTN (n=36)		Stage II HTN (n=36)	
	Stage I	Stage II	Control	Stage II	Control	Stage I
Total Vit. D (ng/ml)	0.001	0.001	0.001	0.001	0.001	0.001
S. Creatinine (mg/dl)	0.001	0.001	0.001	0.364	0.001	0.364
Blood Urea (mg/dl)	1.000	0.048	1.000	0.195	0.048	0.195
FBS (mg/dl)	0.001	0.001	0.001	1.000	0.001	1.000
Serum TGL (mg/dl)	0.214	0.017	0.214	0.954	0.017	0.954
Serum Chol. (mg/dl)	1.000	0.028	1.000	0.068	0.028	0.068
Serum HDL (mg/dl)	0.001	0.001	0.001	1.000	0.001	1.000
MDA (µmol/L)	0.112	0.001	0.112	0.001	0.001	0.001
NO (µmol/L)	0.001	0.001	0.001	0.001	0.001	0.001

Table 13: Comparison of Molecular Parameters between three groups

Parameters	Control Group (n=36)	Stage I HTN (n=36)	Stage II HTN (n=36)	Kruskal-Wallis test	P Value
	Mean ± SD	Mean ± SD	Mean ± SD		
EPO (pg/ml)	105.6 ± 25.3	132.6 ± 18.9	151.8 ± 22.1	37.781	<0.001
VEGF (pg/ml)	410.7 ± 44.1	380.1 ± 26.5	343.3 ± 39.2	34.544	<0.001
Statistically significant					

Table 14: Comparison of Molecular Parameters between Genders

Parameters	Male (n=54)	Female (n=54)	Mann-Whitney U test	P Value
	Mean ± SD	Mean ± SD		
EPO (pg/ml)	133.39 ± 28.614	126.63 ± 29.49	U=1303.500	0.342
VEGF (pg/ml)	380.24 ± 47.977	375.83 ± 44.76	U=1408.500	0.761
Statistically insignificant				

Table 15: Post hoc test of Molecular Parameters between three groups

Parameters	Control Group (n=36)		Stage I HTN (n=36)		Stage II HTN (n=36)	
	Stage I	Stage II	Control	Stage II	Control	Stage I
EPO (pg/ml)	0.001	0.001	0.001	0.001	0.001	0.001
VEGF (pg/ml)	0.002	0.001	0.002	0.001	0.001	0.001

Table 16: Correlation between serum vitamin D concentration and Blood Pressure

N = 108 (54M, 54F)	SBP (mmHg)	DBP (mmHg)	PP (mmHg)	MAP (mmHg)
Total Vit. D (ng/ml)	-0.851	-0.793	-0.592	-0.861
Statistically significant (p <0.05)				

Table 17: Correlation of vitamin D concentration, Arterial Stiffness, and Blood Pressure

N = 108 (54M, 54F)	Total Vit. D (ng/ml)	SBP (mmHg)	DBP (mmHg)
R Bra ASI (mmHg)	-0.654	0.657	0.578
L Bra ASI (mmHg)	-0.588	0.601	0.496
R Ank ASI (mmHg)	-0.541	0.621	0.571
L Ank ASI (mmHg)	-0.513	0.606	0.486
PWV_{b-a} Right (cm/s)	-0.551	0.528	0.447
PWV_{b-a} Left (cm/s)	-0.501	0.447	0.424
PWV_{c-f} (cm/s)	-0.571	0.568	0.478
Statistically significant ($p < 0.05$)			

Table 18: Correlation between total vitamin D concentration and oxygen sensing proteins

N = 108 (54M, 54F)	Total Vit. D (ng/ml)
EPO (pg/ml)	-0.474
VEGF (pg/ml)	0.493
Statistically significant ($p < 0.05$)	

Table 19: Correlation between parameters of oxidative stress (MDA), endothelial function (NO), and oxygen sensing proteins (EPO, VEGF) with blood pressure (SBP, DBP, MAP)

N = 108 (54M, 54F)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
MDA (µmol/L)	0.601	0.565	0.623
NO (µmol/L)	-0.735	-0.711	-0.759
EPO (pg/ml)	0.512	0.457	0.511
VEGF (pg/ml)	-0.558	-0.439	-0.501
Statistically significant ($p < 0.05$)			

4. DISCUSSION

The results of anthropometric parameters in this study further showed stage I HTN and stage II HTN group participants were having higher BMI within overweight range as compared to normal subjects. Similarly, significant higher WHR in case of stage I and stage II HTN indicate abdominal obesity influences BP. From our observations on physical anthropometry in stage I and stage II HTN patients as compared to the normal controls did not show any gender biasness. Although in case of female WHR, average is showing above the normal which might be one of the reasons for inducing increase BP. Although PR in stage II HTN group shows significantly higher values than stage I HTN group and control group but it is within the normal range (60 to 100 bpm). Results also indicate stage I and stage II HTN has no influence on body temperature and RR. Further, increase MAP in stage II and stage I HTN also indicate that the sample selection was following appropriate methodology for inclusion criteria. Results also indicate that there is no gender biasness on physiological parameters among the study groups. Results of ASI of Right and Left brachial, Right and Left ankle of stage II HTN group were higher as compared to control group. Higher ASI in stage II HTN clearly indicates vascular stiffness and pathophysiological changes of vascular system. Results further indicate in case of stage I HTN, the vascular abnormalities are apparently lower but early indication of altered vascular integrity.

Melo E Silva FV et al. conducted a study [11] in 2021 to establish an association of body composition with AS concluded that there is a positive correlation between obesity and AS which may lead to cardiovascular risks such as HTN.

AS are also an indicator of early CVD and possible fatal outcome of individuals. Although ASI reflects ageing but as the subjects included in this study were age matched, hence, ageing factor may not be responsible for increase ASI in the present study. PWV_{b-a} Right, PWV_{b-a} Left, and PWV_{c-f} of stage II and stage I HTN group showed significantly higher than control group, which clearly indicate deviation of vascular integrity from normal in both the HTN group. Increase PWV also indicates stiffness of condute arteries which is a prediction of cardiovascular risk.

Kanthe PS et al. in 2015 showed in their study [12] that adiposity is directly proportional to future development of cardiovascular events such as HTN, atherosclerosis etc.

The generation of PWV occurs due to contraction of heart. Later, pulse waves travel through the vascular wall with a particular speed which is referred as PWV. If the AS increase, resulted in arterial compliances, leads to increase PWV. Loss of integrity in arterial walls, develop loss of elasticity of the arteries and make arteries become stiff. The more stiffer is the arterial wall, will lead to higher PWV. This pathophysiology changes cardiac functioning and leads to overall dearrangement of cardiovascular system. Further, these dearrangements will lead to HTN. Our results found ASI and PWV clearly indicate altered pathophysiology of cardiovascular system. Our results are also indicative that PWV is more

sensitive and early predictor of severe alteration of vascular pathophysiology as compared to ASI. The decrease elasticity of the arteries make the blood vessels more vulnerable as cardiovascular and Cerebrovascular risk factor. From our observations on electrophysiological parameters in stage I and stage II hypertensive patients as compared to the normal controls did not show any gender biasness. Results from our study show significant lower vitamin D concentration in both stage I and stage II HTN group participants. Further it is also noticed that vitamin D level in serum decreases more in stage II HTN group participants in comparison to stage I HTN group. The results indicate a clear vitamin D deficiency in stage II HTN (normal range >20 ng/ml). Although stage I HTN is having lower vitamin D in comparison to controls but it is within the normal range. Results clearly indicate a relationship between vitamin D and vascular health. Lower value of vitamin D is well linked with increased risk of HTN [13].

Hence, baseline vitamin D level may be considered as a marker for CVD including HTN. An increase relationship between vitamin D and Angiotensin II is already reported. Lower vitamin D level is correlated with higher concentration of Angiotensin II which leads to blunted renal plasma protein and over-activation of RAAS, may be one of the reason behind the link between vitamin D and HTN. As endothelial cells contain high concentration of vitamin D receptors and supplementation of vitamin D improve endothelial function and partly regulate BP, hence, lower level of vitamin D probably influences endothelial cell of vascular wall and induces HTN [14].

Further, concentration of vitamin D in blood is related to intracellular calcium homeostasis. Hence, this regulation also positively associated with BP by calcium influx to vascular smooth muscles under the influences of 1,25-dihydroxycholecalciferol. The amount of vitamin D produced by the body depending on age, sunlight exposure, color of the skin, seasons, etc. It has been found in winter, UV-B radiation is low, hence, skin produces less vitamin D. Hence, maintenance of vitamin D is very crucial for not only skeletal health but also for vascular health. The risk of myocardial infarction (MI) greatly increased when vitamin D level is found to be <15 ng/ml. Hence, clinicians must notice vitamin D level of any patient of either stage I or stage II HTN. Although Serum Creatinine and Blood Urea showed higher values in stage I and stage II HTN group participants but it is within normal range. Similarly FBS and lipid profile also found to be in normal range in stage I and stage II HTN group participants. In case of lipid profile, serum HDL was found to be lesser than normal range in both stage I and stage II HTN group participants. Hence, it may be considered as potent marker of HTN [14].

Oxidative stress parameter in case of stage I and stage II hypertensive patients were found to be remarkably altered. Increase MDA in both stage I and stage II HTN indicate altered vascular pathophysiology. Excessive MDA in stage I and stage II HTN in our study may be due to generation of more reactive oxygen species (ROS), which is a key factor of HTN pathology by modulating vasomotor system and developing vasoconstriction through Angiotensin II. Lower the NO level in stage I and stage II HTN patients indicate lesser bioavailability of NO, which is a potent vasodilator and extremely dependent on Redox signaling system. Increase level of ROS in our study probably induced vascular remodeling via oxidative damage. Hence, both MDA and NO result in our study confirm alteration of arterial smooth muscle cells and endothelial cells. The results of NO also to be considered as a degree of HTN, possibly antioxidant status might have changes simultaneously during HTN which we could not assess and we consider it is our limitation. Increase level of serum EPO in both stage I and stage II hypertensive patients indicate loss of vascular integrity due to HTN. Although different types of explanations were given for rise of BP or HTN and increased level of EPO probably due to reduced oxygen supply to the tissue in vasoconstriction induced HTN, but, role of altered angiogenesis may not be ruled out for a positive correlation between BP and serum EPO concentration, may be due to decreased angiogenesis. Report also suggested that serum EPO level has a positive correlation with vascular resistance which may also lead to HTN. A possible role of EPO induced hematocrit values and erythrocyte mass alter the integrity of vascular smooth muscles, lead to disregulation of endothelial vasodilatory factors like NO. Our results of low NO probably support this observation. It has been noticed that treating with EPO to chronic kidney disease (CKD) patients develop severe arterial HTN. The possible reason of this development may be done to EPO induced increased blood viscocity and decrease hypoxic vasodilatation. The results found serum VEGF decreases in both stage I and stage II HTN. VEGF protein synthesis depends on hypoxia signaling pathway (VSP) regulates arterial smooth muscle pathophysiology. Hence, in alteration of VEGF clearly indicate cardiovascular and cerebrovascular diseases. In our study lower level of VEGF probably influences reduced vasculogenesis and remodel vascular architecture during stage I and stage II HTN. Report also found VEGF inhibition leads to HTN as decrease VEGF also reduces NO synthesis, microvascular abnormalities and increase vascular resistance, which leads to development of HTN. Our results of lower level of NO and VEGF support these findings. Another possible reason of VEGF signaling NO synthesis is VEGF receptor (VEGFR). As in our study, we did not assay VEGFR in serum; hence, we cannot explain the role of VEGFR induced HTN. It may be considered as one of the limitation of our study. Although the exact reason behind HTN and VEGF is not clearly defined but serum VEGF need to be considered as one of the important marker for progressive HTN, especially transformation of HTN stage I and stage II.

5. CONCLUSION

Our study has been assigned to understand the role of vitamin D and its impact on stage I and stage II hypertension. The findings of the present study are suggestive that there is a beneficial role of vitamin D in maintenance of an optimal cardiovascular health by delaying the arterial stiffness and hypertension at any stage. Hence, it is suggested that each

middle aged individual should be assessed for level of vitamin D concentration if suspected for any cardiovascular risk and vitamin D supplementation may be advised to prevent or treat cardiovascular diseases such as hypertension. Our findings are also suggestive of altered anthropometric parameters as risk factors for vascular stiffening and future adverse cardiovascular diseases such as hypertension. Hence, we suggest parameters to assess arterial stiffness to be considered for screening of patients who are suspected to have future cardiovascular events. We also found that oxidative stress and endothelial function is altered in hypertensive individuals. Overall finding from our results indicate significant impact of vitamin D in relation to hypertension as blood pressure, arterial stiffness, EPO negatively correlate and VEGF positively correlate with vitamin D. So, vitamin D can be considered as one of the strongest markers in hypertension at any stage.

6. SUMMARY

The summarized form of the mechanistic pathways involved in this intricate relationship is provided in the Figure 1.

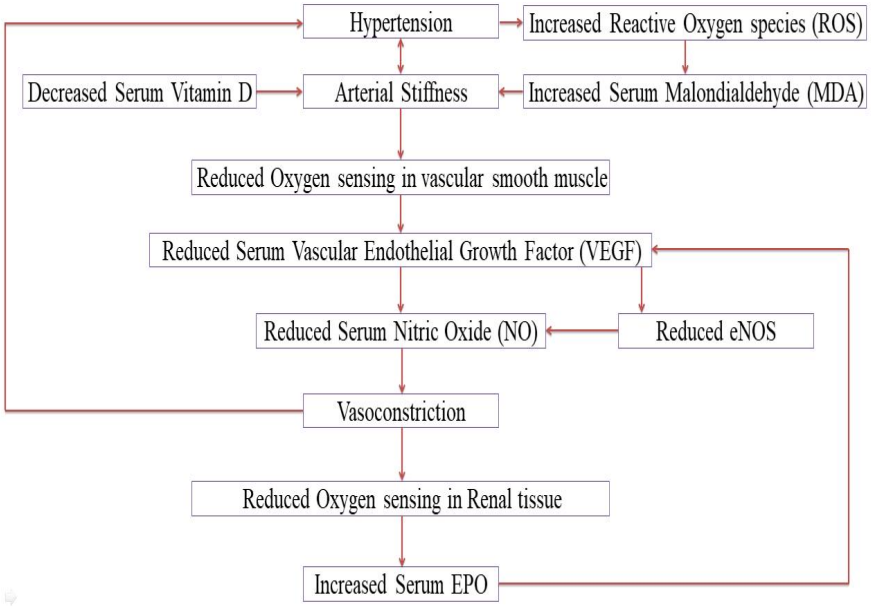

Figure 1: Summary of the intricate relationship of vitamin D and Arterial Stiffness

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