INFLUENCE OF VITAMIN D ON ARTERIAL STIFFNESS IN HYPERTENSIVE WITH SPECIAL REFERENCE TO OXYGEN SENSING PROTEIN EXPRESSION.

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ABBREVATION

AS	**Arterial Stiffness**
AHA	**American Heart Association**
ASA	**American Stroke Association**
ASI	**Arterial Stiffness Index**
AIx	**Augmentation Index**
ANOVA	**Analysis of Variance**
BP	**Blood Pressure**
BMI	**Body Mass Index**
bpm	**beats per minute**
CVD	**Cardiovascular Diseases**
CCB	**Calcium Channel Blocker**
cms	**centimeter**
CHOD-PAP	**Cholesterol oxidase-peroxidase**
Chol.	**Cholesterol**
CKD	**Chronic Kidney Disease**
DBP	**Diastolic Blood Pressure**
DAM	**Diacetyl monoxime**
dl	**Deci liter**

EPO	**Erythropoietin**
ELISA	**Enzyme Linked Immunosorbent Assay**
GPO-PAP	**Glycerol phosphate-oxidase**
GOD-POD	**Glucose oxidase-peroxidase**
HTN	**Hypertension**
HDL	**High Density Lipoprotein**
HC	**Hip Circumference**
IHCI	**Indian Hypertension Control Initiative**
IU	**International Unit**
IEC	**Institutional Ethical Clearance**
kg	**kilogram**
LVH	**Left Ventricular Hypertrophy**
L Bra ASI	**Left Brachial ASI**
L Ank ASI	**Left Ankle ASI**
L	**Liter**
MAP	**Mean Arterial Pressure**
MDA	**Malondialdehyde**
MI	**Myocardial Infarction**
m^2	**meter square**

mg	**milligram**
mmHg	**millimeter of mercury**
NO	**Nitric Oxide**
ng	**nanogram**
nm	**nanometer**
PP	**Pulse Pressure**
PWV	**Pulse Wave Velocity**
PWV_{b-a} Right	**Right Brachial-Ankle PWV**
PWV_{b-a} Left	**Left Brachial-Ankle PWV**
PWV_{c-f}	**Carotid-Femoral PWV**
PTH	**Parathyroid Hormone**
PR	**Pulse Rate**
PTA	**Phosphotungstic acid**
pg	**pictogram**
RAAS	**Renin-Angiotensin-Aldosterone System**
RCT	**Randomized Controlled Trial**
R Bra ASI	**Right Brachial ASI**
R Ank ASI	**Right Ankle ASI**
RR	**Respiratory Rate**

ROS	**Reactive oxygen species**
SBP	**Systolic Blood Pressure**
SD	**Standard Deviation**
SPSS	**Statistical Package for Social Sciences**
Temp.	**Temperature**
TGL	**Triglyceride**
UV-A	**Ultraviolet-A**
UV-B	**Ultraviolet-B**
VEGF	**Vascular Endothelial Growth Factor**
VEGFR	**VEGF receptor**
VDR	**Vitamin D Receptor**
VSP	**VEGF protein synthesis**
WHO	**World Health Organization**
WHR	**Waist Hip Ratio**
WC	**Waist Circumference**
µmol	**micromol**

ABSTRACT

INTRODUCTION: The influences of vitamin D on arterial stiffness in hypertensive patients are still debatable. The role of oxygen sensing proteins in regulation of blood pressure is yet to be explored. In this prospective case control study we aimed to find out the correlation of vitamin D in arterial stiffness, cardiovascular pathophysiologies in hypertensive individuals.

METHODS: 108 age matched participants were taken and divided into three groups according to their hypertension status. Participants having normal blood pressure were considered as control group; group 1 and Stage I hypertensive participants were taken as group 2 and stage II hypertension participants were considered for group 3. All the participants were assessed for their anthropometric, physiological, electrophysiological, biochemical, and molecular parameters according to the study design. Comparison between stage I and stage II hypertension with control group were assessed for all the parameters. A correlation between vitamin D and all the anthropometric, physiological (ASI, PWV), biochemical (MDA, NO) and molecular (EPO, VEGF) parameters were done. The data was analyzed using Microsoft Excel Sheet and SPSS software (version 20).

RESULTS: Arterial stiffness was found to be increased in age matched both the hypertensive groups as compared to the control group. Vitamin D level was also

found to be lower in both the hypertensive group. In stage I and stage II hypertension, EPO was found to be higher whereas VEGF was found to be lower as compared to control group.

CONCLUSION: We conclude by finding that vitamin D influences arterial stiffness, vascular pathophysiology including cardiovascular diseases like hypertension.

KEYWORDS: Arterial Stiffness, Hypertension, Vitamin D, Oxygen sensing proteins, Cardiovascular Diseases.

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INTRODUCTION

Hypertension (HTN), an established major independent risk factor for cardiovascular diseases (CVD), is one of the major rising causes of secondary illness of modern society which can lead to morbidity and mortality in our country. As per World Health Organization (WHO) recent report, the deaths due to non-communicable diseases are nearly 63% in our country and around 27% of all non-communicable deaths are attributed to CVD out of which nearly half of the numbers are coming from the middle aged group. [1] The present scenario is showing that most of the current population of hypertensive patients may remain undiagnosed and don't seek medical attention. [2]

Deficient vitamin D level is a recognized worldwide concern now as it is having role in controlling the risk of different CVD including HTN and when nearly 1 billion people round the globe is either having a insufficient or deficient level of vitamin D, it create a more worse scenario as far as overall world health is concerned. If we compare the scenario of India then the picture is even worse where nearly 40% of the young adults belong to a deficient vitamin D level. [3]

The debate regarding role of vitamin D in control of blood pressure (BP) is still ongoing in spite of a number of studies suggesting vitamin D deficiency to be considered as a new risk factor for HTN. Many studies have also shown that vitamin D levels modulate the BP indirectly and there are studies which showed

the increase prevalence of HTN during winter and in the areas where the exposure to the sunlight is reduced like in the zones which are far away from the equator. These studies have also shown that there is a rise of 2.5 millimeters of mercury (mmHg) of blood pressure for every 10 degrees of equator deviation. [4]

Studies has shown that hypertensive patients are having lower levels of serum nitric oxide (NO) and higher oxidative stress and increased free radical production which alter the vascular architecture. [5]

Serum Erythropoietin (EPO) level is correlated with a rise in blood pressure [6] and Vascular Endothelial Growth Factor (VEGF) targeted therapies cause hypertension in 30-80% of patients. [7] Although research data from current studies show that EPO have significant role in the physiological maintenance of cardiovascular system but the relationship between serum EPO levels and arterial stiffness (AS) is still yet to be studied. [8]

In this prospective case control study, we aimed to assess the role of serum vitamin D on cardiovascular pathophysiology in the perspective of oxygen sensing protein (EPO, VEGF) expression in stage 1 and stage 2 hypertensive patients. The Indian Hypertension Control Initiative (IHCI) 2020 is aimed towards reduction of 25% of prevalence of hypertensive patients in our country by 2025. [1] Our study will act as a bridge between the gaps known in the field of HTN and oxygen sensing molecules (EPO, VEGF) from this part of the country.

OBJECTIVES OF THE STUDY

Primary Objective:

1. To find out the relationship between serum vitamin D and cardiovascular pathophysiology in hypertensive patients.

Secondary Objectives:

2. To find out serum vitamin D in relation with arterial stiffness in hypertensive patients (stage 1 and stage 2).

3. The influences of vitamin D level and oxygen sensing protein expression (EPO, VEGF) in hypertensive patients.

4. To find out the Co-relation between oxygen sensing proteins and arterial stiffness in hypertensive patients.

REVIEW OF LITERATURE

BLOOD PRESSURE (BP):

BP is the lateral pressure exerted by the flowing blood against any unit area of the vessel wall which is measured in mmHg. Unless specified otherwise, BP usually refers to pulsatile systemic arterial pressure. The highest pressure is achieved during systole and the lowest pressure is achieved during diastole of the cardiac cycle. Hence these are referred as Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) respectively. Having different determinants, the SBP and DBP alter differentially. The difference between SBP and DBP is considered as Pulse Pressure (PP) and the average pressure exerted during a cardiac cycle is considered as Mean Arterial Pressure (MAP). [9]

HYPERTENSION (HTN):

HTN is an epidemic effecting more than one billion people in the world and is the commonest risk factor for death with stroke and CVD. It is one of the major rising causes of secondary illness of modern society which can lead to morbidity and mortality in our country. In the guidelines issued by the American Heart Association (AHA) and American Stroke Association (ASA) in the year 2017, they claimed the normal BP to be defined as "SBP of <120 mmHg and DBP of <80 mmHg where SBP of 120-129 mmHg and DBP of <80 mmHg" [2] is to be taken as

elevated BP. According to these guidelines the "SBP of 130-139 or DBP of 80-89 is to be considered as HTN stage 1 whereas stage 2 HTN took the criteria of SBP ≥140 mmHg or DBP ≥90 mmHg". [2] If the SBP is >180 mmHg and/or DBP is >120 mmHg at any point of time, the person will be in hypertensive crisis. [2] The different categories of BP according to AHA and ASA are shown in the Figure 1.

Blood Pressure Categories

American Heart Association | American Stroke Association

BLOOD PRESSURE CATEGORY	SYSTOLIC mm Hg (upper number)		DIASTOLIC mm Hg (lower number)
NORMAL	LESS THAN 120	and	LESS THAN 80
ELEVATED	120 – 129	and	LESS THAN 80
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 1	130 – 139	or	80 – 89
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 2	140 OR HIGHER	or	90 OR HIGHER
HYPERTENSIVE CRISIS (consult your doctor immediately)	HIGHER THAN 180	and/or	HIGHER THAN 120

Figure 1: Classification of different stages of HTN as per AHA and ASA. [12]

ARTERIAL STIFFNESS (AS):

AS is a state where arteries become stiff, due to thickening of the wall of arteries which leads to loss of elasticity and it might reflect in the form of compliance and expansibility reduction. Lifestyle modification like daily routine exercise, improvement in the dietary habits can reduce an acute increase in AS. Chronic increase might result into damage of the principal organs like brain, heart

and kidney [10]. Heart is affected by AS due to its effect of afterload increase, which becomes a long-standing stress, over a period of time, affecting their ventricular performance which results into the cardiac output inadequacy that might be insufficient in situations where demand is high.

AS make the redistribution of blood in body in such a way that it results into kidney hypoperfusion which might lead to Renin-Angiotensin-Aldosterone System (RAAS) activation, which leads to a permanent state of elevated BP in the body. It should be corrected soon enough, or else it might also lead to kidney hypoperfusion without any rescuing compensatory mechanism.

The parameters used to directly reflect AS, non-invasively are the Arterial Stiffness Index (ASI) and Pulse Wave Velocity (PWV). The causes of AS are summarized in the figure 2.

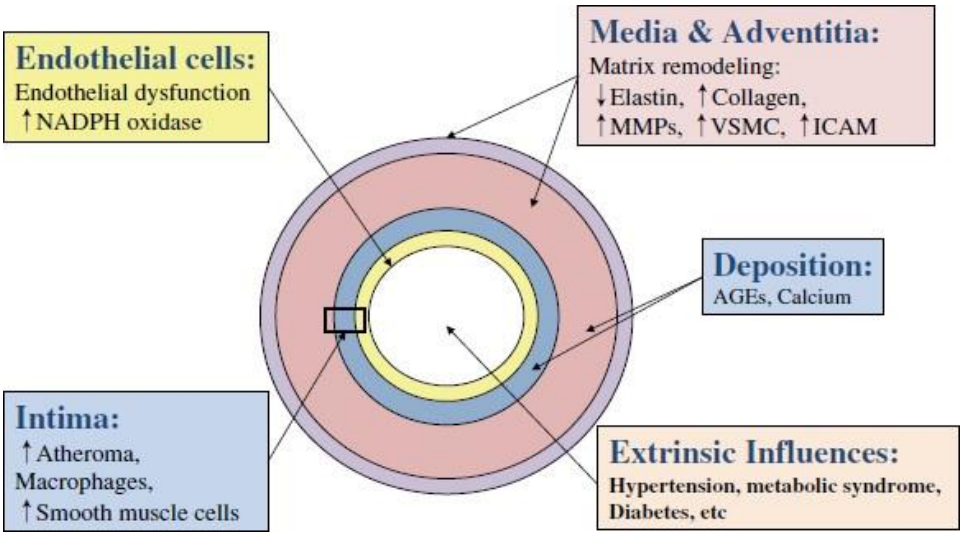


Figure 2: Causes of arterial stiffness (Reference: Lee HY & Oh BH., 2010)

Pulse Wave Velocity (PWV) [11]**:** It is the wave velocity which precedes the column of blood which is ejected out in a ventricular systole, to reach the peripheries. Therefore, when AS increases, PWV increases because the elasticity of the arterial wall decreases, this is seen in diseases like atherosclerosis, diabetes mellitus, etc.

Arterial Stiffness Index (ASI)**:** ASI is estimated by estimating the oscillometric envelopes resulted from the oscillations in the respective arteries (Naidu MUR et al., 2012).

Central Blood Pressure [11]**:** The regular way of BP measurement is with the help of sphygmomanometer which gives BP of the peripheral arteries, most often brachial artery. The measurement of central blood pressure is the unconventional way of BP measurement. The recording of peripheral BP measurement showed discrepancy with that of the aortic BP such that it is higher than the peripheral BP that is measured and relied upon usually in order to categorize the patient into hypertensive or normotensive. It is the central BP, which exerts its influence on the vital organs like heart, brain and kidney. Therefore, if this parameter is used for management of HTN, the trajectory of the disease can be altered for a better picture, in future. As increased arterial stiffness also corresponds significantly with BP level, this parameter can also be used to assess the situation of AS in human body.

VITAMIN D:

Vitamin D is a fat soluble vitamin which is having role in calcium homeostasis, development of skeletal muscles, smooth functioning of the cardiovascular health. There are two main sources of vitamin D known till now which are as follows:

1. Direct sunlight exposure through Ultraviolet-B (UV-B) rays,
2. Dietary supplements.

Previtamin D is formed from 7-dehydrocholesterol once UV-B rays of sunlight penetrates the skin which later converts to vitamin D_3. Fish oil like cod liver oil, oily fishes like salmon, egg yolks, fortified milk and yogurt are some examples of dietary sources of vitamin D. Depending on the availability of sunlight exposure, the synthesis of vitamin D from skin may vary. It also varies to the bare skin exposure to available sunlight. On the other hand, pigmentation of the skin is a limiting factor for vitamin D synthesis from the skin. Melanin, the natural sunscreen for the body, reduces the synthesis of vitamin D_3 from skin. [23]

Cutaneous vitamin D is metabolized in liver to 25-hydroxyvitamin D, metabolized in kidney to form 1,25-dihydroxyvitamin D, the active form of

vitamin D. Parathyroid Hormone (PTH), serum phosphorus and calcium closely regulate the production of vitamin D from kidney. [24]

Vitamin D Receptor (VDR) which is a steroid hormone nuclear receptor that binds to 1,25-dihydroxyvitamin D, is present in many tissues and organs such as vascular smooth muscles, endothelium, skin, heart, and many cells of immune system. [24]

There are various factors influence vitamin D nutritional status. Following are some examples: [25]

1. Racial factors: high number of populations of vitamin D deficiency in African American populations due to increase melanin secretion
2. Geographical factors: Population of Edmonton, Canada suffer from vitamin D deficiency from October to April every year because it is situated 52 degrees North to the equator.
3. Social Factors: Covering the entire body with clothing, applying sunscreen, etc may reduce cutaneous vitamin D production.

The production of vitamin D from skin, its metabolism, regulation and relationship with the RAAS is depicted in the figure 3 which clearly tells about its possibility of regulating BP.

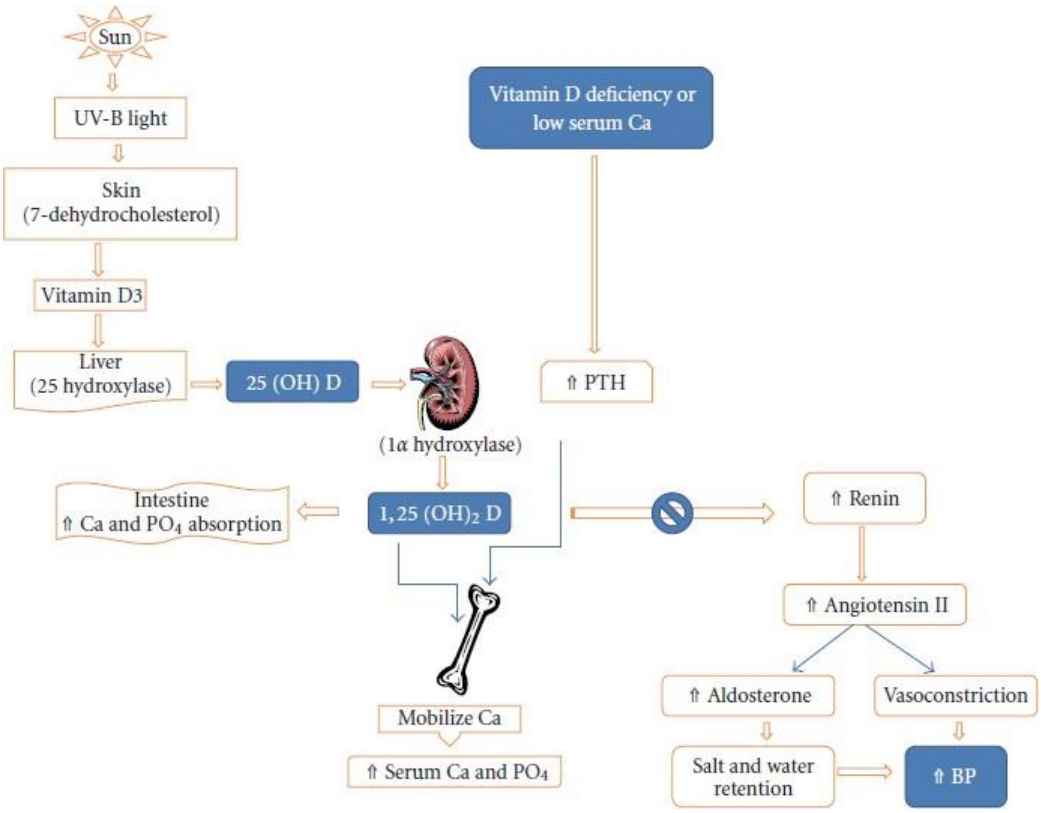


Figure 3: Vitamin D metabolism and its relationship with RAAS (Ullah MI et al. 2010) [26]

VTAMIN D AND BLOOD PRESSURE:

There was a time when many healthcare professionals used to think that the complications arising from vitamin D deficiency has been conquered once rickets were taken care of by supplementing vitamin D along with food but till now we came to know that rickets were just tip of an iceberg of deficient vitamin D concentration. Most of the experts believes that a level of <20 ng/ml is a deficient range of vitamin D whereas 20-29 ng/ml is considered as insufficient level and ≥30

ng/ml is considered as normal vitamin D level for an average adult. It is also to be mentioned that >150 ng/ml is considered as vitamin D intoxication. After we came to know that most of our body cells and tissues have a vitamin D receptor and a number of them also possess the capability of converting 25-hydroxyvitamin D, the primary circulating form to 1,25-dihydroxyvitamin D, the active form, we got a new insight regarding the function of this hormone.

Getting it more intensified 1,25-dihydroxyvitamin D also controls more than 200 genes directly or indirectly, including the genes which are responsible for angiogenesis, cellular proliferation, apoptosis, cell differentiation and so on. It also acts as an immunomodulator and several studies have also shown its importance in reducing the risk of type-1 diabetes in childrens. [3]

The relationship between level of serum vitamin D concentration and increased AS in were reported in several studies.

Following studies have been done to show the relationship between vitamin D and AS in normotensive subjects:

Iain Bressendorff, et al. in 2015 [13] did a double blinded randomized Controlled Trial (RCT) to examine the effect of cholecalciferol on AS and BP. In the study 40 healthy normotensive adults were examined for their changes in the peripheral and central BP, 24-hour ambulatory BP, PWV, Augmentation Index

(AIx) after receiving oral cholecalciferol 3000 International Unit (IU)/day for 16 weeks. Their results showed no difference in changes in BP and AS between 18 subjects of placebo arm and 22 subjects of cholecalciferol arm. They concluded that BP and arterial stiffness in healthy normotensive adults does not get affected by 16 weeks of treatment with 3000 IU/day cholecalciferol.

AI Mheid I et al. in 2011 [14] did a study with 554 healthy subjects aiming to elucidate the relationship between CVD and serum vitamin D and concluded that increase AS in resistance blood vessels is associated with serum vitamin D insufficiency which might indicate towards its mechanism of modulating RAAS.

In a study by Giallauria F et al. in 2012 [15] aimed to establish the independent cross-sectional relationship between AS and HTN and concluded that increase in the AS is inversely associated with serum vitamin D level in normotensive population.

Following are the epidemiological studies done to show the relationship between vitamin D and Hypertension:

McCarron DA et al. [27] in 1980 stated in their study that HTN may develop from the disorders of calcium metabolism.

Cooper R et al. [28] found a significant geographical difference of BP among the African population in 1994 suspecting a genetic predisposition of HTN in their study.

Zemel MB et al. in 1990 found in their study [29,30] that supplementation of calcium in salt sensitive black population may reduce BP but land up into left ventricular hypertrophy (LVH).

Griffith LE et al. in 1999, Allender PS et al. in 1996, Scragg R et al. in 2007 and Judd SE et al. in 2008 concluded their study with the similar finding stating towards the role of vitamin D in regulation of BP. [31-34]

Following studies done to show effect of vitamin D supplementation on BP:

Krause R et al. in their study [35] in 1998 randomly taken 18 hypertensive individuals to receive Ultraviolet-B (UV-B) or Ultraviolet-A (UV-A) light exposure 3 times weekly for 6 weeks in which they found a 162% rise in the vitamin D level among the participants who received UV-B lights along with a significant drop in the SBP and DBP by 6 mmHg. In contrast, the participants who received UV-A light did not show any change in vitamin D concentration or BP.

Another study [36] by Pfeifer M et al in 2001 done on 145 elderly women, showed significant reduction in BP up to 9.3% after 8 weeks on receiving 800 IU of vitamin D_3 and 1200 mg of calcium. They also found a reduction of BP by 4% after treatment with only 1200 mg of calcium for 8 weeks.

Following studies have shown no relationship of vitamin D with HTN:

Forman JP et al. in 2005 concluded their study [37] with no association of deficiency of vitamin D with increase risk of HTN.

A random double blinded study [38] by Margolis KL et al. done to show the effect of 1000 mg of calcium and 400IU of vitamin D_3 daily supplementation on HTN in 2008 concluded with no significant decrease in incidence of HTN after 7 years follow up.

Other studies by Orwoll ES et al. in 1990 and Scragg R et al. in 1995 concluded with similar finding showing no association of vitamin D supplementation in reduction of BP. [38-39]

Following studies have been done to show the relationship between vitamin D and Cardiovascular Pathophysiology:

Osman Kuloglu et al. in 2013 did a study [16] to know the association of level of serum vitamin D concentration with AS, LVH, and inflammation in 133 hypertensive patients in the year 2012 over a period of 6 months. They showed serum vitamin D is independently related with AS, LVH and inflammation. Vitamin D may play a significant role on pathogenesis of AS and LVH in individuals with freshly diagnosed HTN.

Ji Yeon Kang et al. did a study [17] in 2015 to find relationships of dietary and serum vitamin D with CVD and AS on 1381 subjects. They made a conclusion that concentration of Serum vitamin D level has a beneficial relationship with High Density Lipoprotein (HDL) cholesterol levels in both men and women, but the

same relationship did not founded with other cardiometabolic risk factors such as blood glucose, BP, and other parameters of lipid profiles.

Songcang Chen et al. did a study [18] on Vitamin D and Essential HTN in 2016 in which they said that if we treat vitamin D-deficient persons or normotensive persons having insufficient levels of vitamin D for a short period results in minimal effects on BP. By supplementing high doses of vitamin D daily in a cohort at the age at risk of Essential HTN, will prevent the development of HTN by eliminating deficiency of vitamin D as a trigger for its development.

Young S. Oh did a study [19] on AS and HTN in the year 2018 in which it has been showed AS as important arterial phenotype and an excellent indicator of cardiovascular complications and it is an independent predictor of HTN and CVD.

Lata Mullur et. al. in 2019 observed [20] vitamin D level has a beneficial relationship with BP of various types of cardiac diseases.

Ann Burgaz et al. did a meta-analysis [21] in 2011 to review the association of vitamin D concentration and BP which concluded as developing a inverse relationship of HTN with serum vitamin D level concentration.

The study [22] of Yan Chun Li in 2003 concluded that vitamin D regulate BP by regulating the RAAS and also suggested to use analogues of vitamin D in purpose of prevention or treatment of high BP.

Following studies have been done to show association of serum NO and BP:

Higashino H et al. showed in their study [40] conducted in 2007 that there is a significant higher serum NO level in hypertensive male participants than the normotensive males.

Goch A et al. in their study [41] in 2009 found no endothelium dysfunction in hypertensive patients unless they have a family history of CVD or having predisposed other cardiovascular risk factors.

Bagali S et al. in their study [42] on low oxygen microenvironment and cardiovascular remodeling in 2020 found that treatment with antihypertensive drugs ameliorate endothelial dysfunction and cardiovascular remodeling.

Following studies have been done to show the association of Malondialdehyde (MDA) in AS and HTN:

Ferroni P et al. in their study conducted in 2006 showed that endothelial dysfunction is seen in hypertensive individuals because oxidative stress plays the major role by promoting prothrombic state in vessels. [43]

Hou JS et al. in their study [44] in 2020 shown that serum MDA greater than 80.33 mg/dl is related to increase AS which might lead to future CVD.

Following studies has been conducted to show the relationship of the Anthropometric parameters on arterial stiffness (AS):

Melo E Silva FV et al. conducted a study [45] in 2021 aiming 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.

Kanthe PS et al. in 2015 showed in their study [46] that adiposity is directly proportional to future development of cardiovascular events such as HTN, atherosclerosis etc.

Following studies has been done to show the association of EPO with BP:

Omer Gedikli et al. did a study [47] on Circulating levels of EPO and its relation to AS in patients with HTN in the year 2013 which concluded by founding the level of Serum EPO of hypertensive patients and normotensive patients are comparable.

Vaziri ND in his study [48] in 1999 found that there is a positive correlation between EPO and increase BP.

Khodnapur JP in her study [49] in 2021 found that EPO is responsible for age associated vascular health and an altered level of EPO may contribute towards alteration in the BP.

Following studies has been done to show the association of VEGF with BP:

Emily S. Robinson et al. did a study [50] in the year of 2010 named HTN induced by VEGF Signaling Pathway Inhibition: Mechanisms and Potential Use as

a Biomarker, in which they showed that HTN in VEGF targeted therapies is common and causes significant morbidity but it can be effectively managed.

A study [51] conducted in 2009 by Papaioannou AI et al. suggested a significant positive correlation between serum VEGF and other CVD such as systemic sclerosis and HTN.

Caletti S et al. did a study [52] in 2018 in which they concluded to treat HTN due to VEGF targeted therapies with RAAS inhibitors and calcium channel blocker (CCB).

METHODOLOGY

Source of data: Patients from Department of Medicine, Shri B. M. Patil Medical College, Hospital and Research Centre, B.L.D.E. (Deemed to be University) Vijayapura.

Study Period: 1st July 2021 to 30th June 2022.

Type of study: Prospective case control study.

Study design: A total number of 108 participants have been included in our study, divided into equal numbers in 3 groups. Each group is consisting of 36 participants of both genders as follows:

Group 1: Control group: 36 Participants (18 males and 18 females) "(SBP = <120 mmHg and DBP = <80 mmHg)" [2].

Group 2: Stage 1 Hypertension: 36 Participants (18 males and 18 females) "(SBP = 130-139 mmHg or DBP = 80-89 mmHg)" [2].

Group 3: Stage 2 Hypertension: 36 Participants (18 males and 18 females) "(SBP = ≥140 mmHg or DBP = ≥90 mmHg)" [2].

Methods of collection of data: Institutional ethical clearance (IEC) was obtained (IEC/No-09/2021 Dated 22/01/2021). Voluntary informed written consent was obtained from all the participants. All the anthropometric parameters, physiological parameters, and electrophysiological parameters were recorded in the supine posture after rest for 10 minutes between 9AM to 11AM at room temperature.

After dividing the 108 persons according to the study design mentioned above, the following parameters have been measured.

I. **Anthropometric Parameters**:

a. Height: Height has been measured using a device (BIOCON^{TM}) mounted on the wall and was expressed in centimeters (*cms*).
b. Weight: Weight has been measured using a weighing machine and is expressed in Kilograms (*Kg*).
c. Body Mass Index (BMI): Body Mass Index has been calculated manually from weight in Kilograms (*Kg*) divided by height in meters square (m^2) and was expressed as Kg/m^2.
d. Waist Circumference (WC) in cms (WHO STEPS protocol 2000).
e. Hip Circumference (HC) in *cms* (WHO STEPS protocol 2000).
f. Waist Hip Ratio (WHR) (WHO STEPS protocol 2000).

II. Physiological parameters:

a. Measurement of BP: SBP (mmHg) and DBP (mmHg) was recorded by using mercury sphygmomanometer. [53]

b. Pulse Rate (PR) in beats per minute (bpm) was measured manually.

c. Respiratory Rate (RR) in cycles per minute was measured manually.

d. Temperature (Temp.) in degree ferhenhite was measured using a thermometer.

III. Electrophysiological Parameters:

a. ASI: ASI were recorded in right (R Bra ASI) and left (L Bra ASI) brachial arteries, right (R Ank ASI) and left (L Ank ASI) ankle arteries by using Periscope which is a non-invasive automatic device, work on oscillometric method (Periscope, Genesis Medical Systems, India). [54, 55] The values were calculated by estimating the oscillometric envelopes, obtained from the oscillations in the respective artery.

"ASI = [Systolic side value of cuff pressure at 80% of maximal oscillation amplitude of cuff] − [Diastolic side value of cuff pressure at 80% of maximal oscillation amplitude of cuff]" [54, 55].

b. PWV: PWV was measured by using Periscope and reported as Right Brachial-Ankle PWV (PWV_{b-a} Right) and Left Brachial-Ankle PWV (PWV_{b-a} Left) and Carotid-Femoral PWV (PWV_{c-f}). [54,55]

All recording were done in supine position and operational bias was avoided as this device is fully automated.

IV. Biochemical Parameters:

a. Serum total vitamin D level analysis (Chemiluminesence Assay)
b. Serum Triglyceride (TGL): Serum TGL was estimated by glycerol phosphatase-oxidase (GPO-PAP) method (McGowan MW et al., 1983).
c. Serum Cholesterol (Chol.): Cholesterol was estimated by using cholesterol oxidase-peroxidase (CHOD-PAP) enzymatic method (Allian CC et al., 1974).
d. HDL Cholesterol: It was be estimated by using phosphotungstic acid (PTA) method (Burstein M et al., 1970).
e. Serum Creatinine: It was estimated by using Jaff's Method.
f. Blood Urea: It was estimated by Diacetyl Monoxime (DAM) method.
g. FBS: It was measured by Glucose oxidase-peroxidase (GOD-POD) method.
h. Serum Malondialdehyde (MDA): It was measured by using UV Spectrophotometer at 535 nm.
i. Serum Nitric Oxide (NO): It was measured by using UV spectrophotometer at 535 nm.

V. Molecular Parameters:

a. Quantitative estimation of Serum VEGF and Serum EPO were done by Enzyme-linked immunosorbent assay (ELISA) method (Alon T et al., 1995).

Sample size: With Anticipated correlation coefficient between Vitamin D and PWV - 0.555 [48] at 95% confidence level and 90 power in the study, the sample size worked out is 36 per group.

Total sample size= 36+36+36=108

Formula used is

$$N=\left[\left(\frac{Z_\alpha+Z_0}{C}\right)\right]^2+3$$

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

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

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

Inclusion criteria:

1. Participants with stage 1 and stage 2 HTN of the age group of 35 to 50 years in B.L.D.E. (Deemed to be University), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijaypura have been included in the study group.
2. Control group was normotensive participants of same age group.

Exclusion criteria:

1. HTN along with Diabetes Mellitus, Thyroid disorder or other endocrine diseases.
2. Patients with vascular diseases or with any other chronic diseases.
3. Chronic smokers, Alcoholics & Tobacco chewers.

Statistical Analysis:

The data obtained was entered in a Microsoft Excel sheet and statistical analysis was performed using statistical package for the social sciences (SPSS) (Version 20). Data was presented as Mean ± Standard deviation (SD), frequency, percentages and diagrams. Categorical variables were compared by using Chi-square test. Differences between groups of continuous variables were compared using Mann Whitney U test, Analysis of variance (ANOVA) test, Kruskal Wallis test. Spearman's correlation was used to find correlation between the variables of Anthropometric parameters, Physiological parameters, Electrophysiological parameters, Biochemical parameters and Molecular parameters. $p<0.05$ was considered statistically significant. All statistical tests are performed two tailed.

RESULTS

All the participants (n = 108) examined were age matched. Table 1 shows the comparison of age between different groups of the study which is statistically insignificant by ANOVA test.

Anthropometric Parameters:

Comparison between anthropometric parameters of three groups is shown in Table 2. 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 3). The detailed Post hoc analysis report is depicted in Table 4.

Table 1: Comparison of Age between three groups:

N=108	Control Group (n=36)		Stage I HTN (n=36)		Stage II HTN (n=36)		ANOVA Test	P Value
	Mean	SD	Mean	SD	Mean	SD		
Age (Year)	42.75	5.65	43.11	5.73	44.72	4.79	F=1.35	P=0.26
Statistically insignificant								

Table 2: Comparison of Anthropometric Parameters between three groups:

N=108	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		
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 3: Comparison of Anthropometric Parameters between Genders:

Gender	Male (n=54)		Female (n=54)		Mann-Whitney U Test	P Value
	Mean	SD	Mean	SD		
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 4: Post hoc test of Anthropometric Parameters between three groups:

N=108	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

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 5. We did not find any significant difference of any of the physiological parameters between the genders by using Mann Whitney U test (Table 6). In case of stage I and stage II HTN group participants, MAP (mmHg) shows 100.2±2.684 and 109.7±4.911 respectively (Table 5). The detailed analysis report of the Post hoc test of the Physiological parameters is shown in Table 7.

Table 5: Comparison of Physiological Parameters between three groups:

N=108	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		
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 6: Comparison of Physiological Parameters between Genders:

Gender	Male (n=54)		Female (n=54)		Mann-Whitney U Test	P Value
	Mean	SD	Mean	SD		
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 7: Post hoc test of Physiological Parameters between three groups:

N=108	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

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 8 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 ($P<0.001$). The detailed Post hoc analysis report of the electrophysiological parameters is depicted in Table 10.

Table 8: Comparison of Electrophysiological Parameters between 3 groups:

N=108	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		
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								

There were no significant differences of all these parameters between Male and Females when compared by using Mann-Whitney U test (Table 9).

Table 9: Comparison of Electrophysiological Parameters between Genders:

Gender	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 10: Post hoc test of Electrophysiological Parameters between three groups:

N=108	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

Biochemical Parameters:

After using Kruskal-Wallis test, the report of comparison of all the biochemical parameters between the three groups is shown in Table 11.

Serum 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 ($P<0.001$).

Concentration 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 (Table 11).

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 chol. 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 chol. and serum TGL level concentration as compared to control group participants (Table 11). 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 (Table 11).

Parameters of Oxidative stress (Serum MDA) and Endothelial Dysfunction (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 (Table 11). 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 (Table 11). There were no significant differences of all these biochemical parameters between the Male and the Females of each group (Table 12).

Table 11: Comparison of Biochemical Parameters between three groups:

N=108	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		
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 12: Comparison of Biochemical Parameters between Genders:

Gender	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						

The detailed Post hoc analysis report of the biochemical parameters is depicted in Table 13.

Table 13: Post hoc test of Biochemical Parameters between three groups:

N=108	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

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 14.

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 (Table 14).

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 (Table 14). 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 15).

Table 14: Comparison of Molecular Parameters between three groups:

N=108	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 15: Comparison of Molecular Parameters between Genders:

Gender	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						

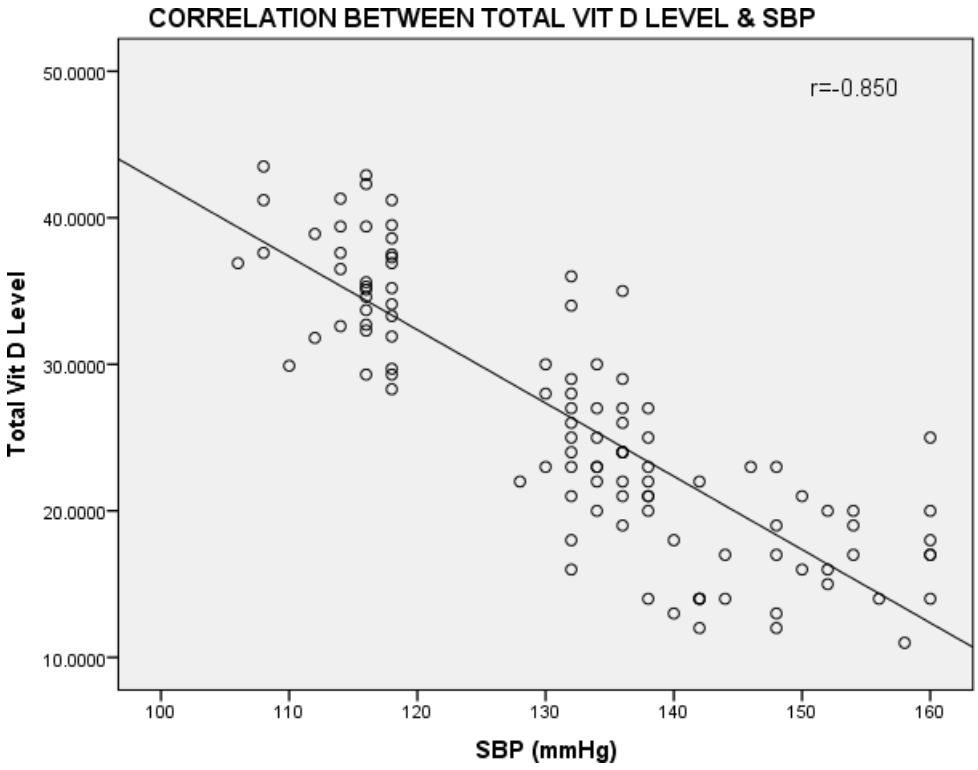
The detailed Post hoc analysis report of the biochemical parameters is depicted in Table 16.

Table 16: Post hoc test of Molecular Parameters between three groups:

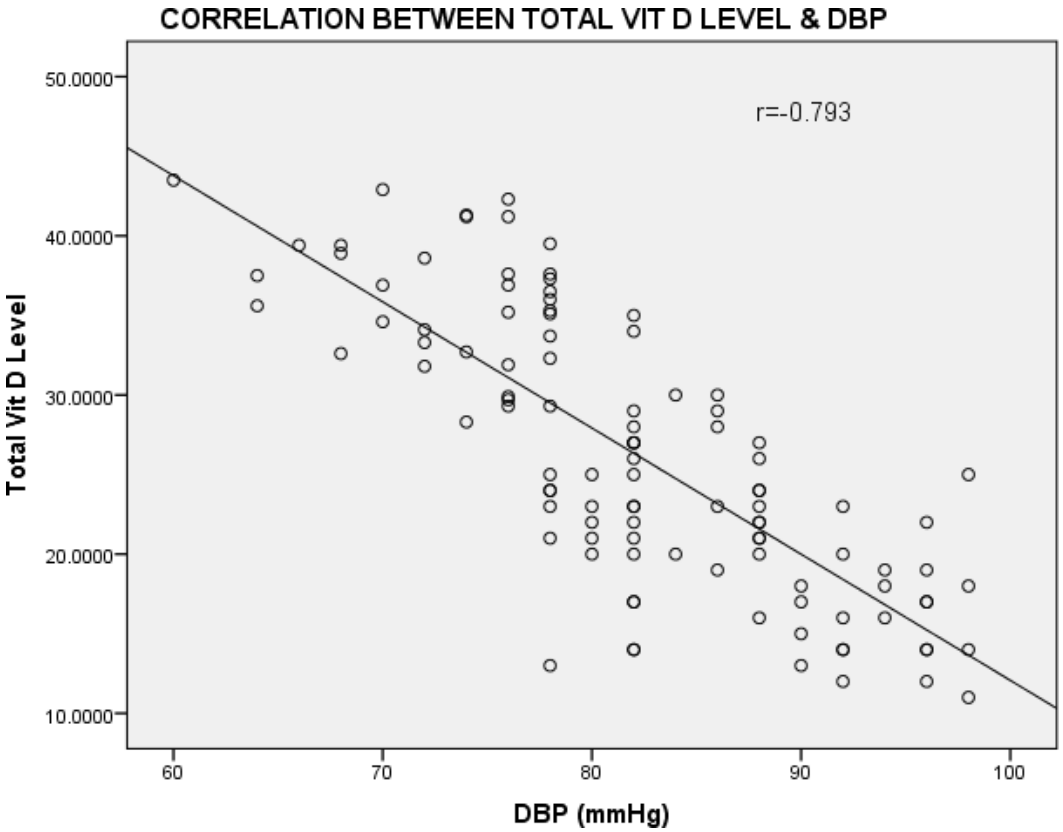
N=108	Control Group (n=36)		Stage I HTN (n=36)		Stage II HTN (n=36)	
	Stage I	Stage II	Control	Stage II	Control	Stage I
EPO (pg/ml)	0.001	0.001	0.001	0.001	0.001	0.001
VEGF (pg/ml)	0.002	0.001	0.002	0.001	0.001	0.001

Correlation between serum vitamin D concentration and Blood Pressure:

Graph 1 depicts the correlation between SBP and serum total vitamin D concentration of all the participants of each group. Our results indicate a negative correlation (r = -0.850) between SBP and serum total vitamin D. Graph 2 depicts correlation between DBP and serum total vitamin D concentration of all the participants of each group. Results indicate a negative correlation (r = -0.793) between DBP and serum total vitamin D.

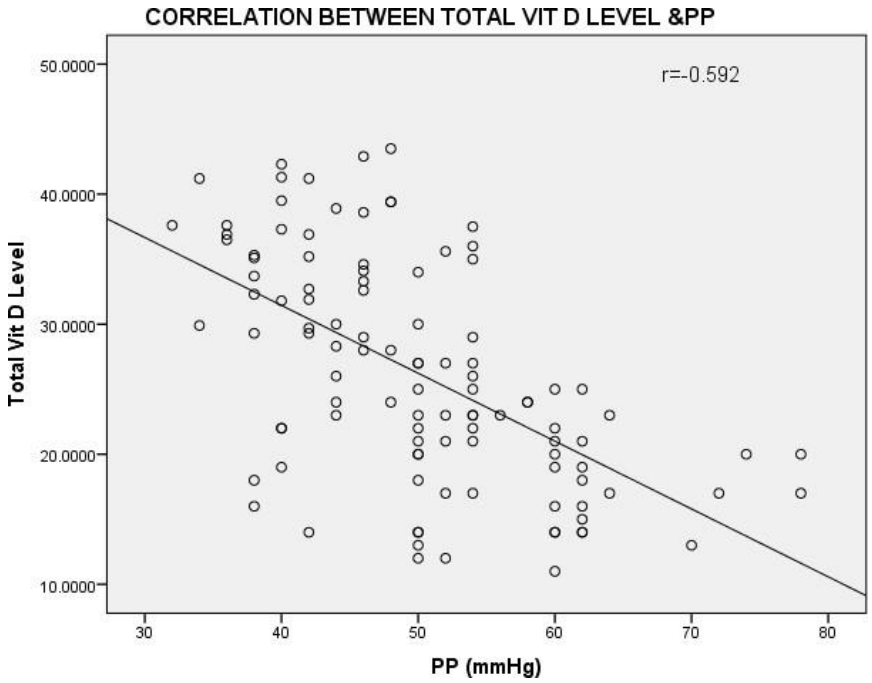


Graph 1: Correlation between serum vitamin D and SBP

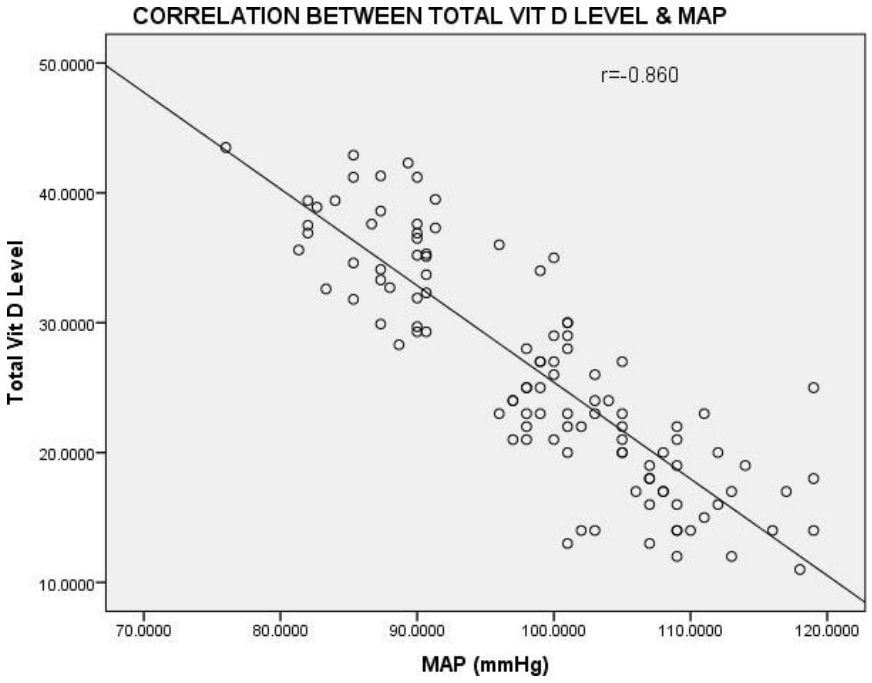


Graph 2: Correlation between serum vitamin D and DBP

Graph 3 depicts the correlation between PP and serum total vitamin D concentration of all the participants of each group. Our results indicate a negative correlation (r = -0.592) between PP and serum total vitamin D. Graph 4 depicts correlation between MAP and serum total vitamin D concentration of all the participants of each group. Results indicate a negative correlation (r = -0.860) between MAP and serum total vitamin D.



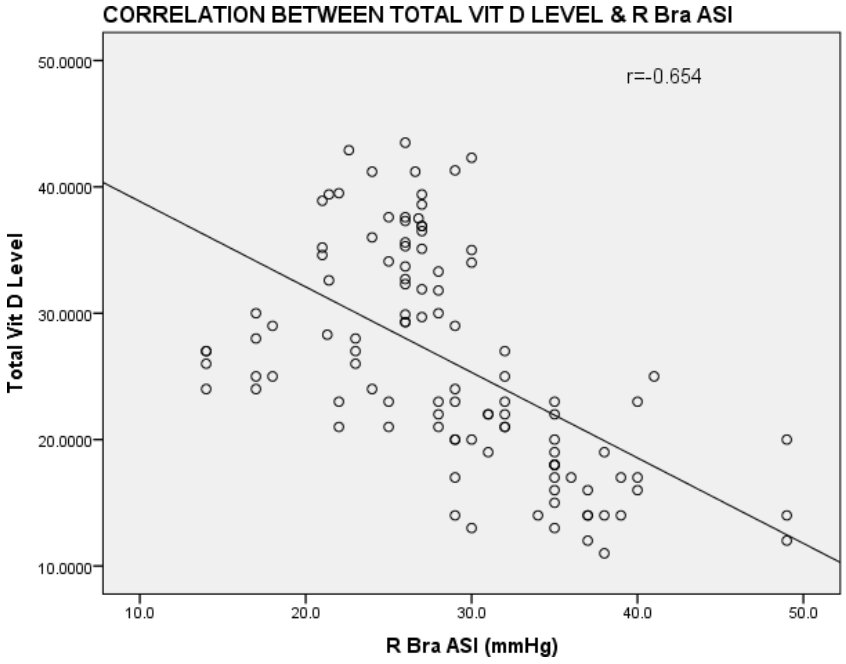
Graph 3: Correlation between serum vitamin D and PP



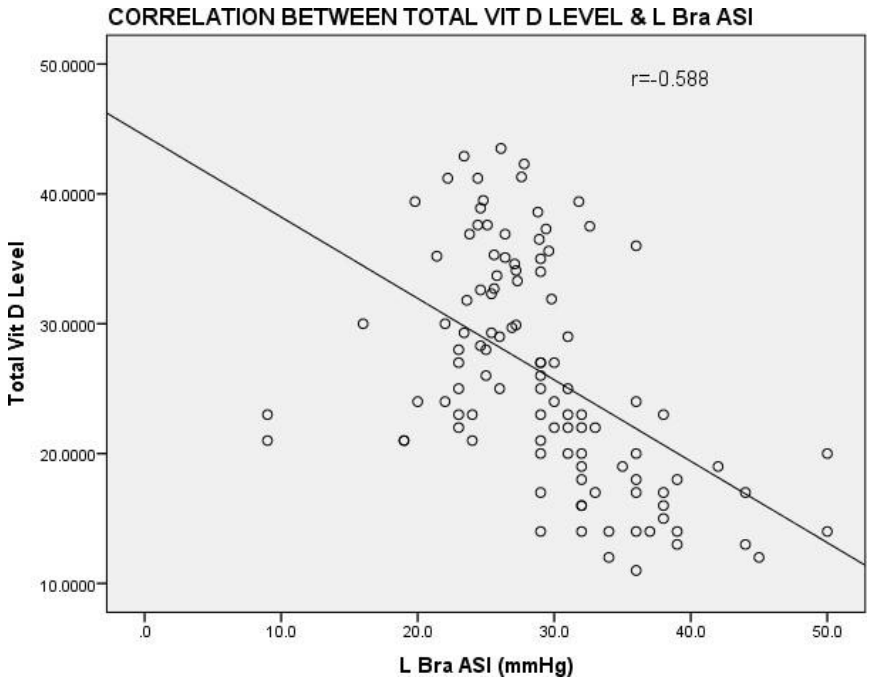
Graph 4: Correlation between serum vitamin D and MAP

Correlation between serum vitamin D concentration and AS:

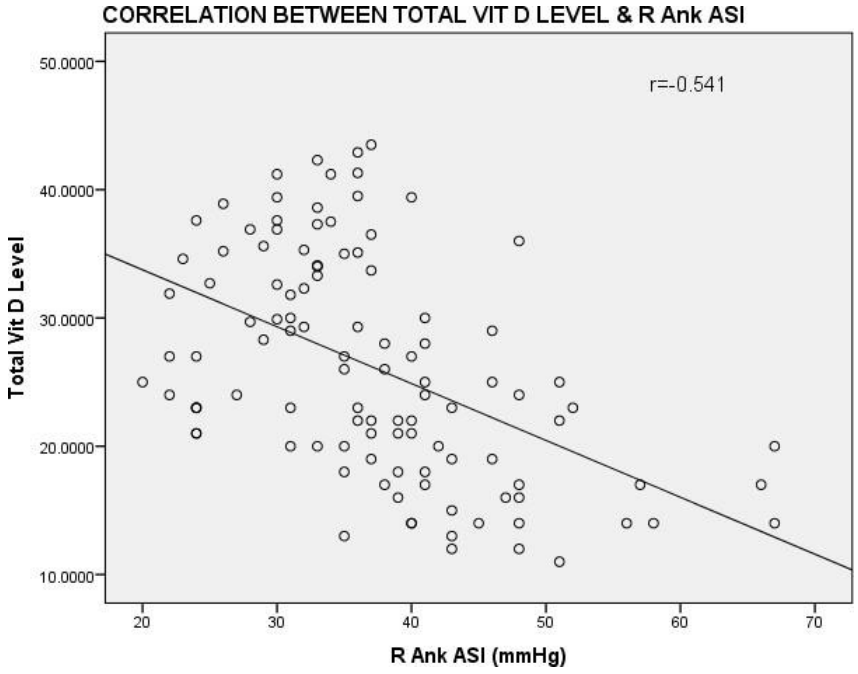
Graph 5 depicts correlation between R Bra ASI and serum total vitamin D concentration of all the participants of each group. Results indicate a negative correlation (r = -0.654) between R Bra ASI and serum total vitamin D. Graph 6 depicts the correlation between L Bra ASI and serum total vitamin D concentration of all the participants of each group. Our results are indicating a negative correlation (r = -0.588) between L Bra ASI and serum total vitamin D. Graph 7 depicts the correlation between R Ank ASI and serum total vitamin D concentration of all the participants of each group. Our results indicate a negative correlation (r = -0.541) between R Ank ASI and serum total vitamin D.



Graph 5: Correlation between serum vitamin D and R Bra ASI

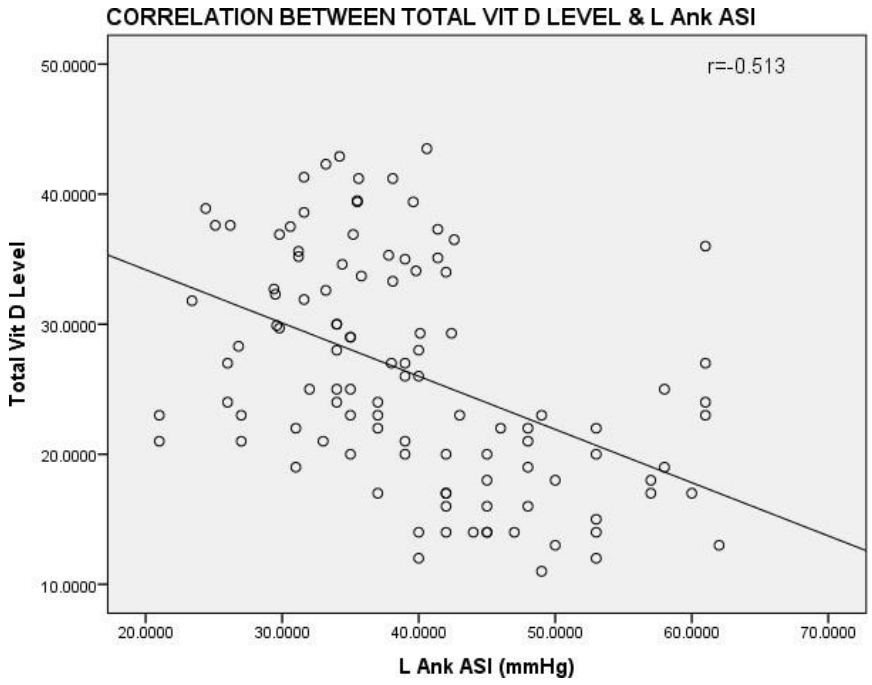


Graph 6: Correlation between serum vitamin D and L Bra ASI

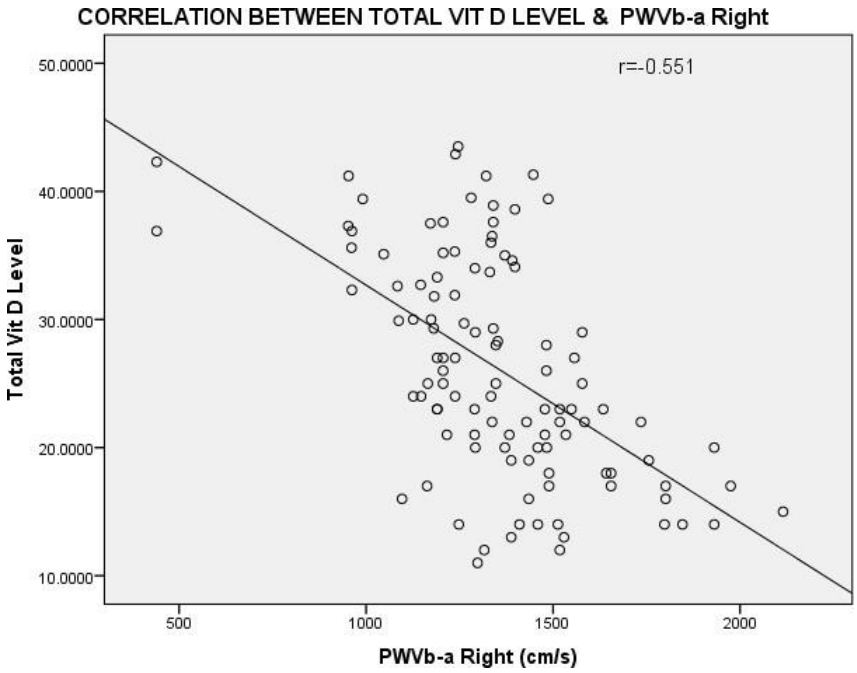


Graph 7: Correlation between serum vitamin D and R Ank ASI

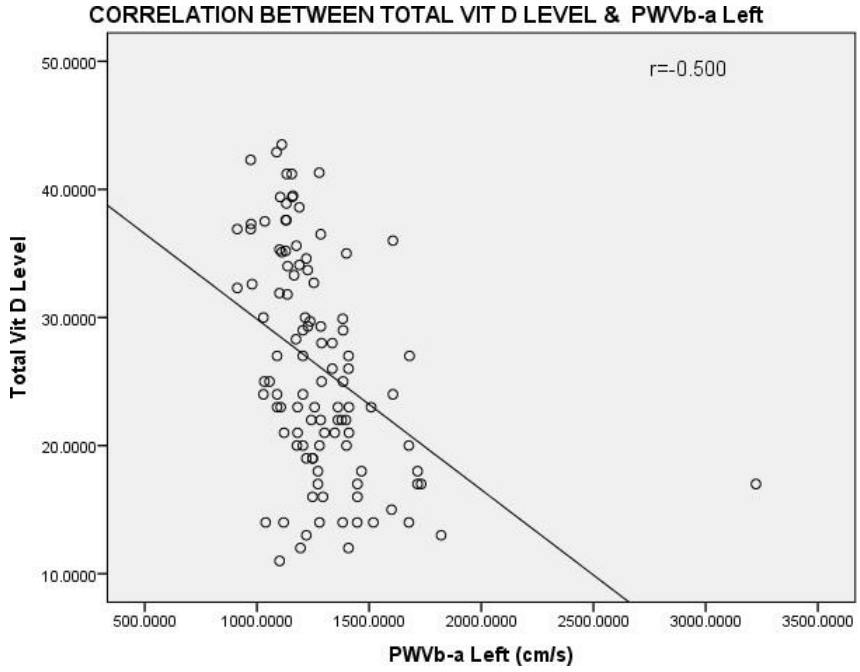
Graph 8 depicts correlation between L Ank ASI and serum total vitamin D concentration of all the participants of each group. Results indicate a negative correlation (r = -0.513) between L Ank ASI and serum total vitamin D. Graph 9 depicts correlation between PWV_{b-a} Right and serum total vitamin D concentration of all the participants of each group. Our results are indicating a negative correlation (r = -0.551) between PWV_{b-a} Right and serum total vitamin D. Graph 10 depicts correlation between PWV_{b-a} Left and serum total vitamin D concentration of all the participants of each group. Results indicate a negative correlation (r = -0.500) between PWV_{b-a} Left and serum total vitamin D.



Graph 8: Correlation between serum vitamin D and L Ank ASI

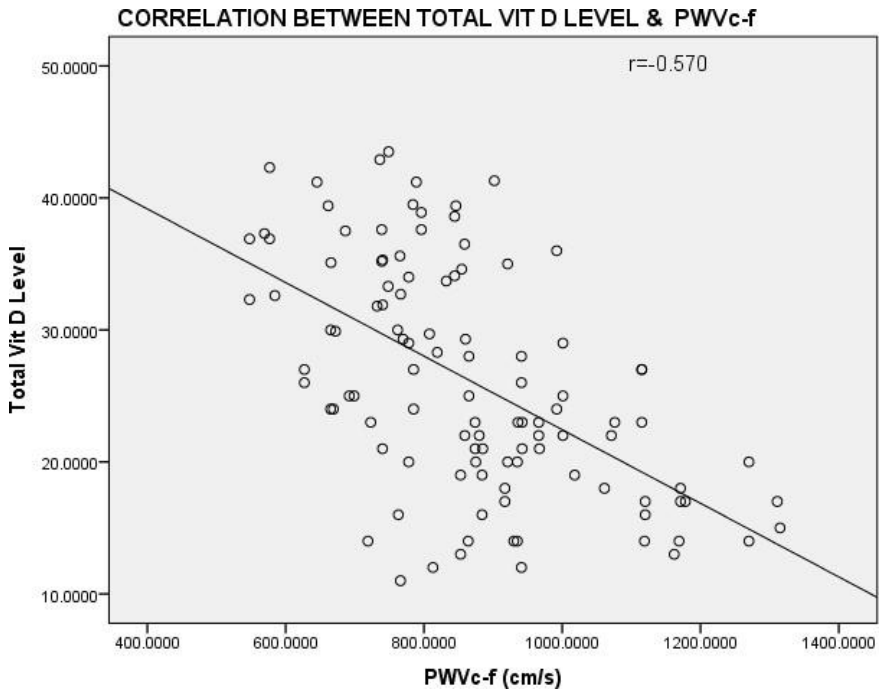


Graph 9: Correlation between serum vitamin D and PWV_{b-a} Right



Graph 10: Correlation between serum vitamin D and PWV_{b-a} Left

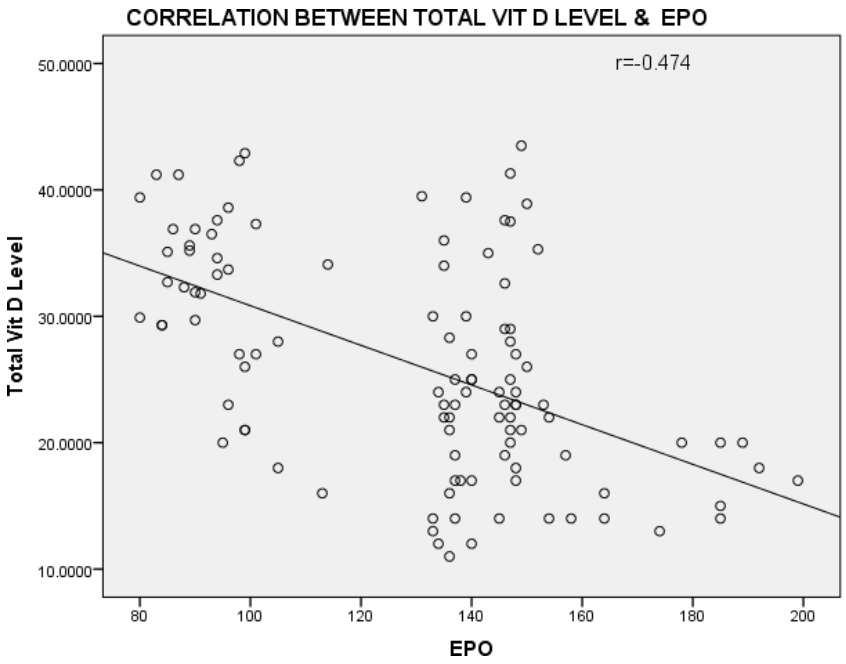
Graph 11 depicts correlation between (PWV_{c-f} and serum total vitamin D concentration of all the participants of each group. Results indicate a negative correlation (r = -0.570) between PWV_{c-f} and serum total vitamin D.



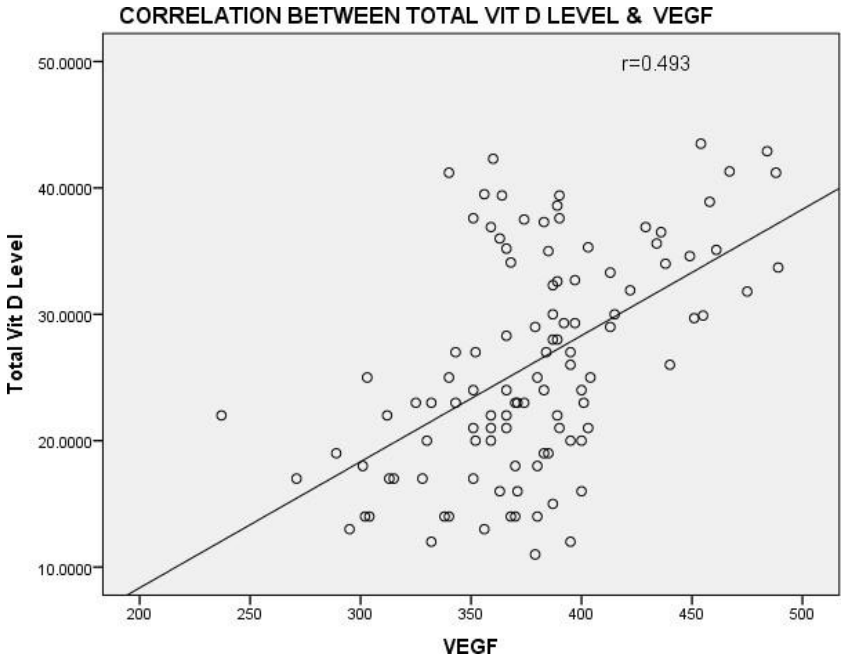
Graph 11: Correlation between serum vitamin D and PWV_{c-f}

Correlation between vitamin D concentration and Molecular Parameters:

Graph 12 depicts correlation between serum EPO and serum total vitamin D concentration of all the participants of each group. Results indicate a negative correlation (r = -0.474) between EPO and serum total vitamin D.



Graph 12: Correlation between serum vitamin D and EPO

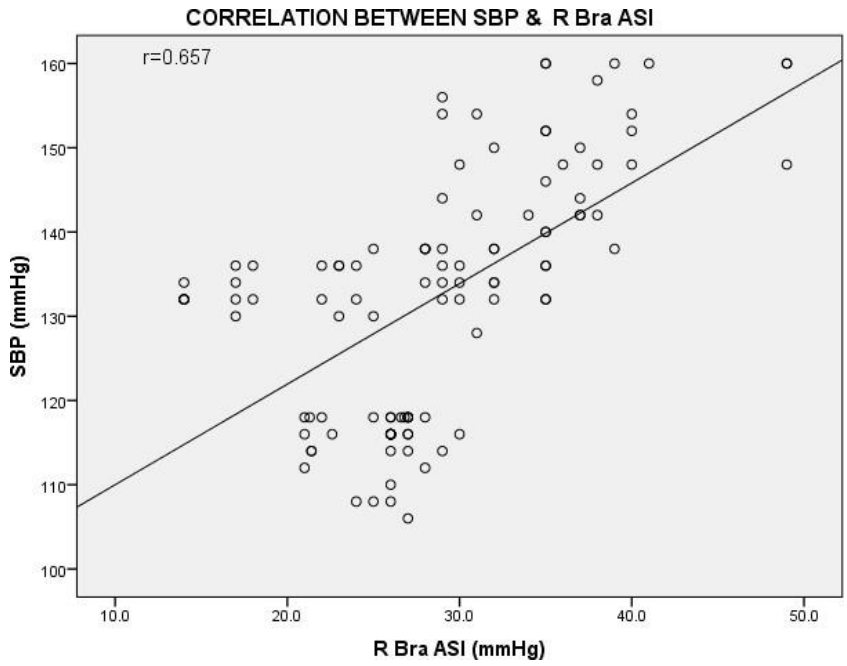


Graph 13: Correlation between serum vitamin D and VEGF

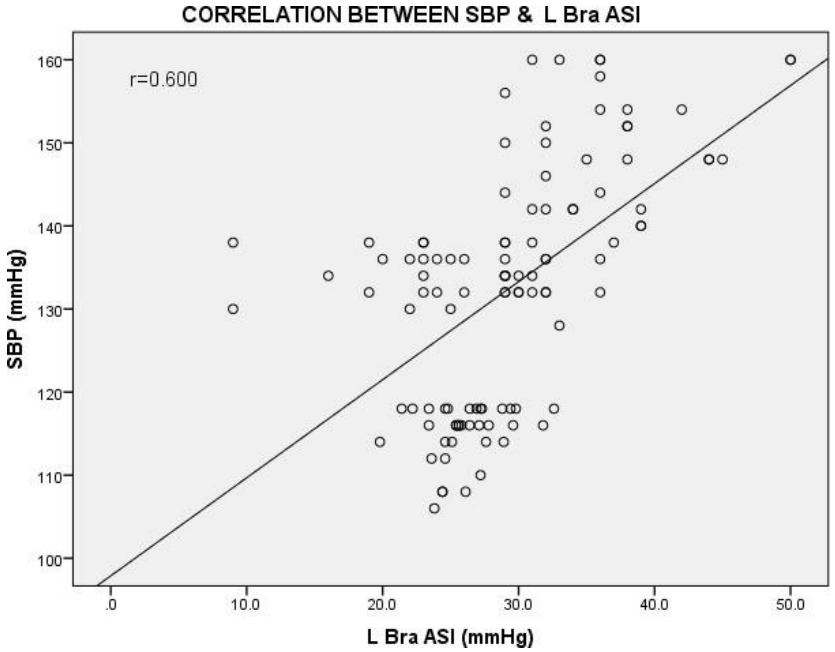
Graph 13 depicts correlation between serum VEGF and serum total vitamin D concentration of all the participants of each group. Results indicate a positive correlation (r = 0.493) between VEGF and serum total vitamin D.

Correlation between SBP and AS:

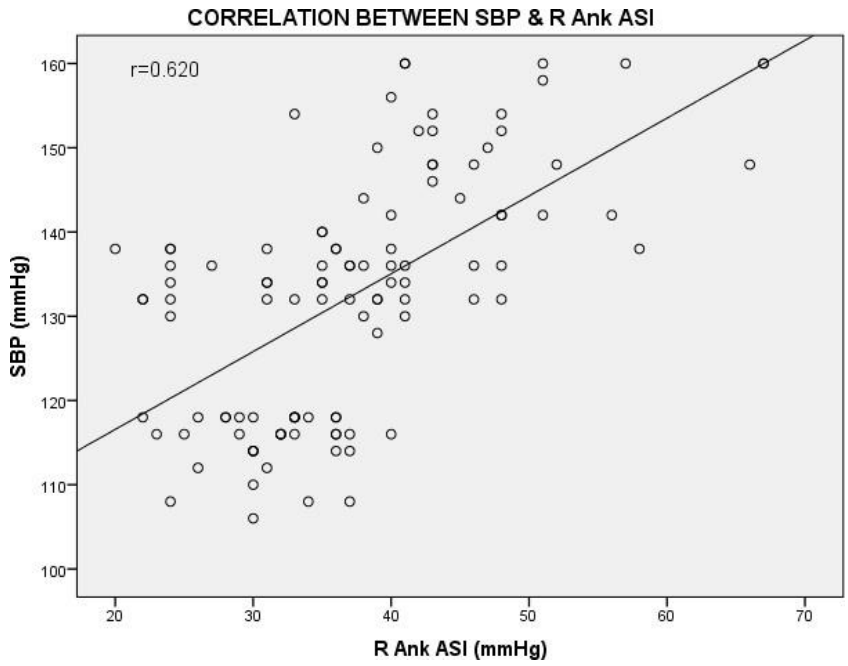
Graph 14 depicts correlation between SBP and R Bra ASI of all the participants of each group. Results indicate a positive correlation (r = 0.657) between R Bra ASI and SBP. Graph 15 depicts correlation between SBP and L Bra ASI of all the participants of each group. Results indicate a positive correlation (r = 0.600) between L Bra ASI and SBP. Graph 16 depicts correlation between SBP and R Ank ASI of all the participants of each group. Results indicate a positive correlation (r = 0.620) between R Ank ASI and SBP. Graph 17 depicts correlation between SBP and L Ank ASI of all the participants of each group. Results indicate a positive correlation (r = 0.606) between L Ank ASI and SBP. Graph 18 depicts correlation between SBP and PWV_{b-a} Right of all the participants of each group. Results indicate a positive correlation (r = 0.528) between PWV_{b-a} Right and SBP.



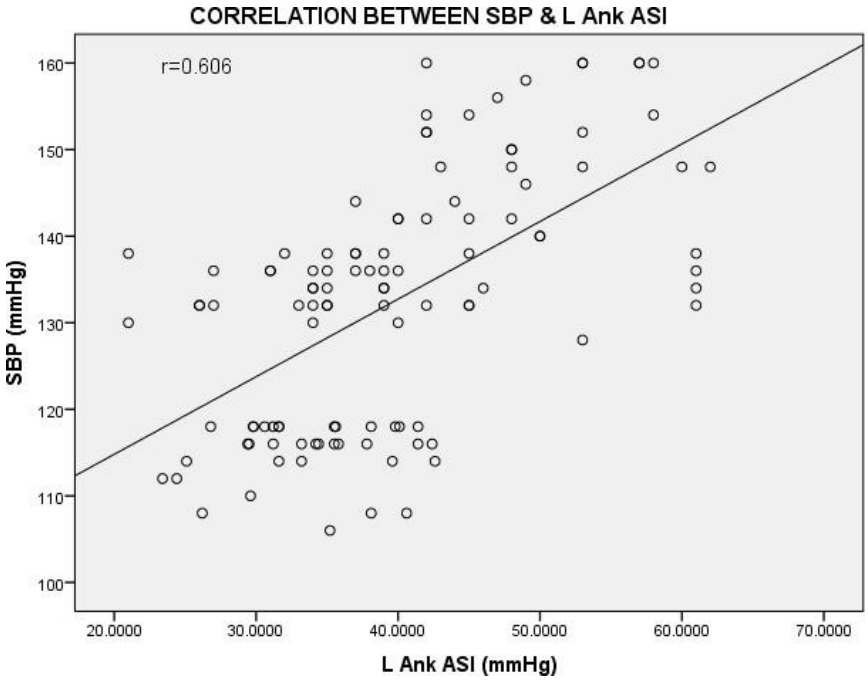
Graph 14: Correlation between SBP and R Bra ASI



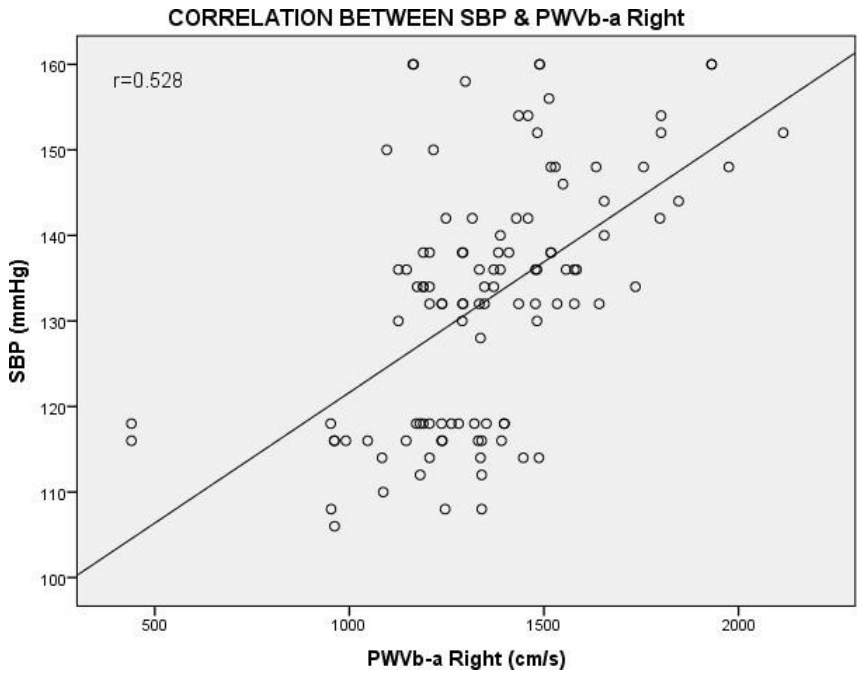
Graph 15: Correlation between SBP and L Bra ASI



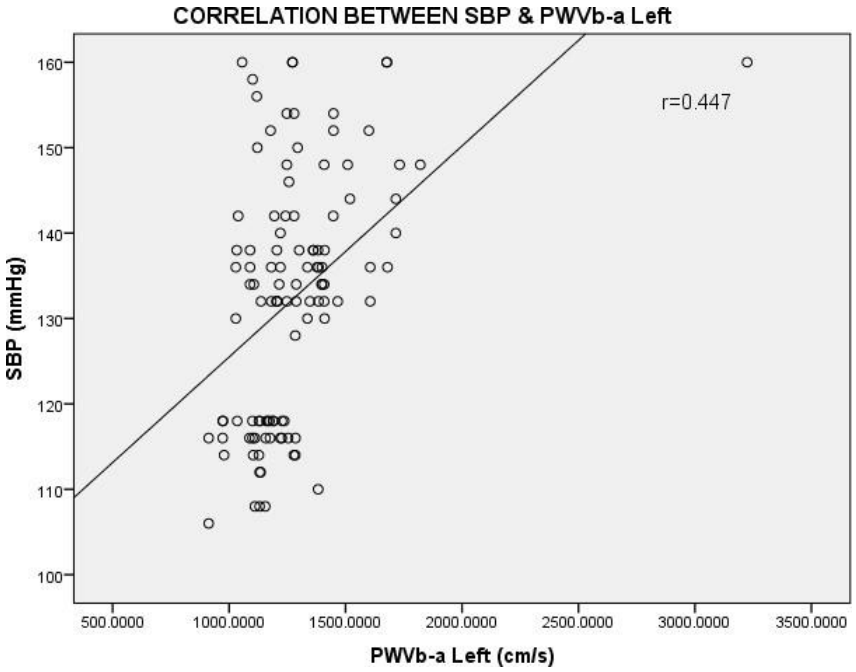
Graph 16: Correlation between SBP and R Ank ASI



Graph 17: Correlation between SBP and L Ank ASI

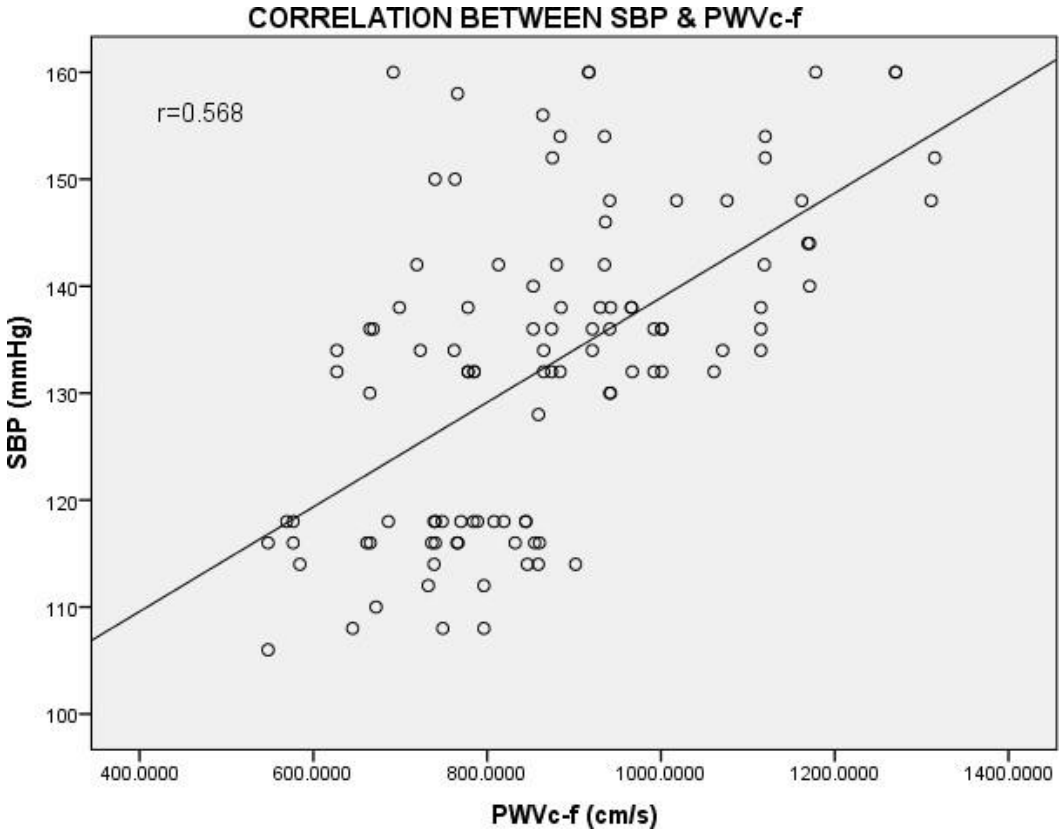


Graph 18: Correlation between SBP and PWV_{b-a} Right



Graph 19: Correlation between SBP and PWV_{b-a} Left

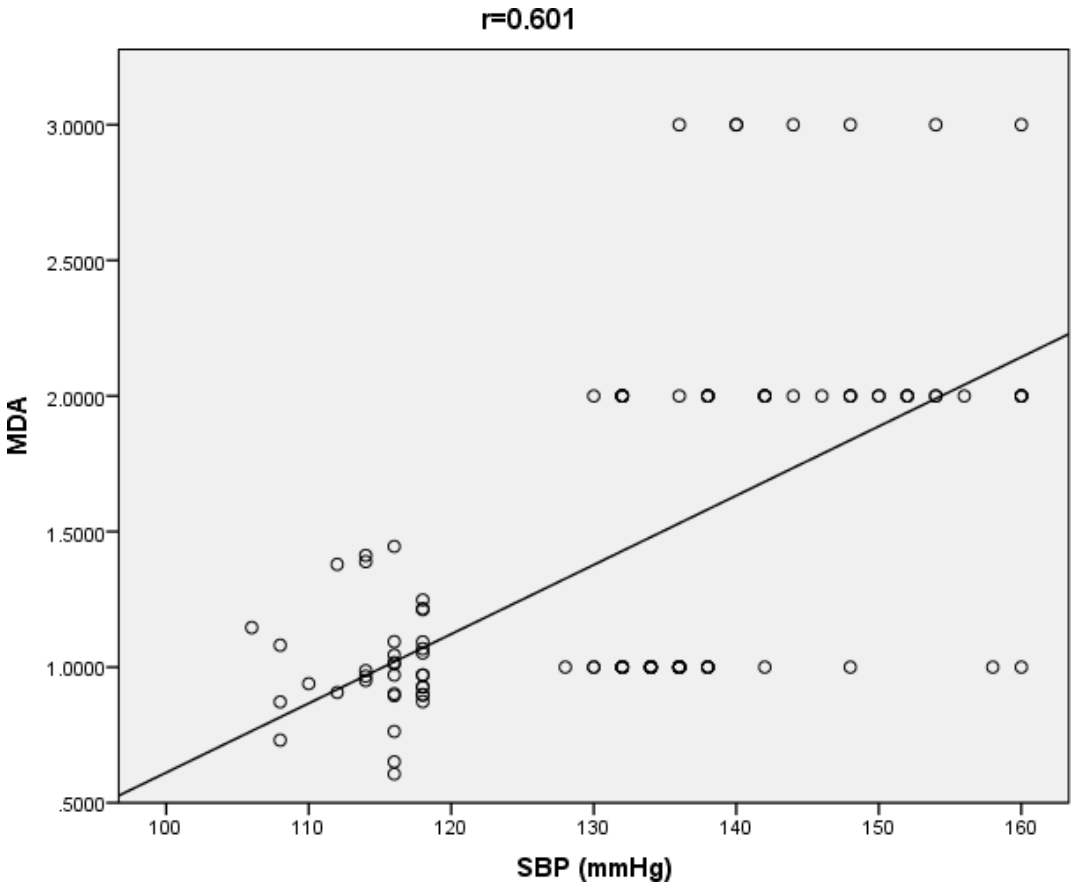
Graph 19 depicts correlation between SBP and PWV_{b-a} Left of all the participants of each group. Results indicate a positive correlation (r = 0.447) between PWV_{b-a} Left and SBP. Graph 20 depicts correlation between SBP and PWV_{c-f} of all the participants of each group. Results indicate a positive correlation (r = 0.568) between PWV_{c-f} and SBP.



Graph 20: Correlation between SBP and PWV_{c-f}

Correlation between SBP and oxidative stress (Malondialdehyde; MDA):

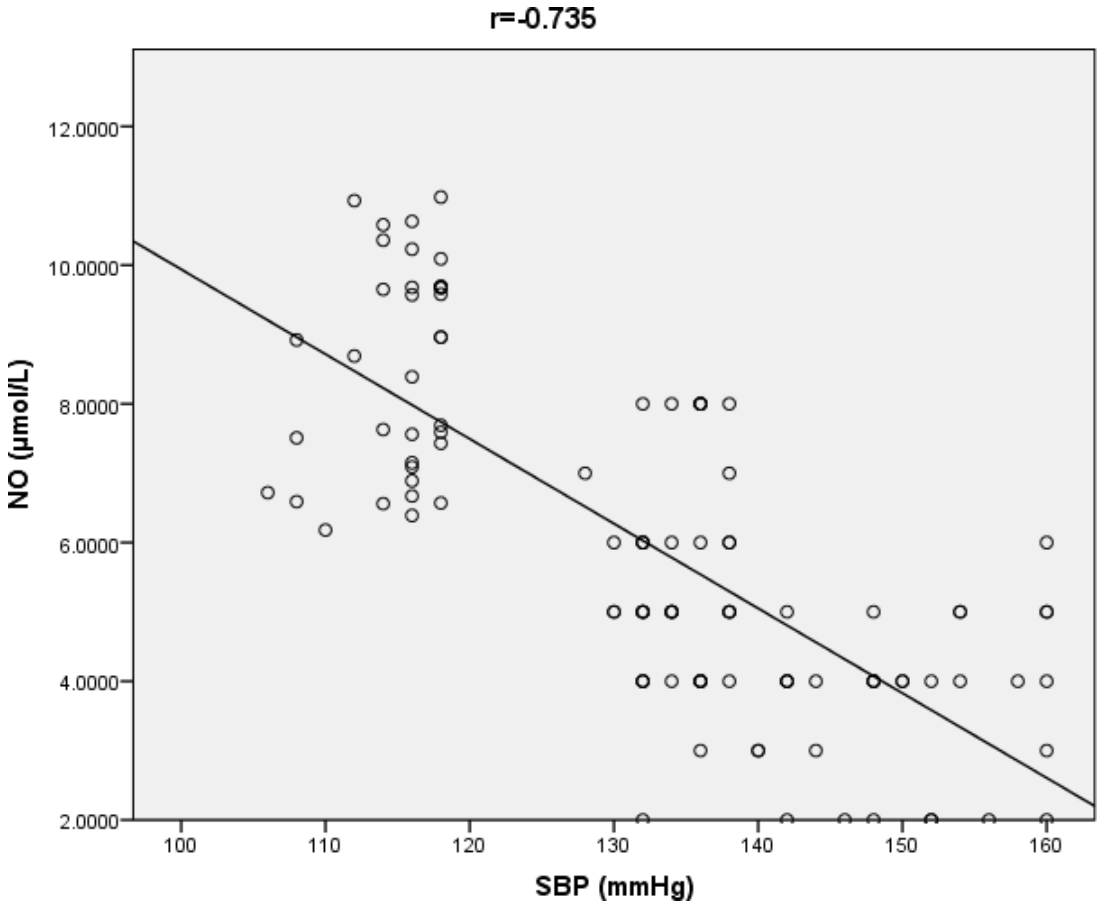
Graph 21 depicts correlation between SBP and serum MDA of all the participants of each group. Results indicate a positive correlation (r = 0.601) between serum MDA and SBP.



Graph 21: Correlation between SBP and serum MDA

Correlation between SBP and the endothelial dysfunction (Nitric Oxide; NO):

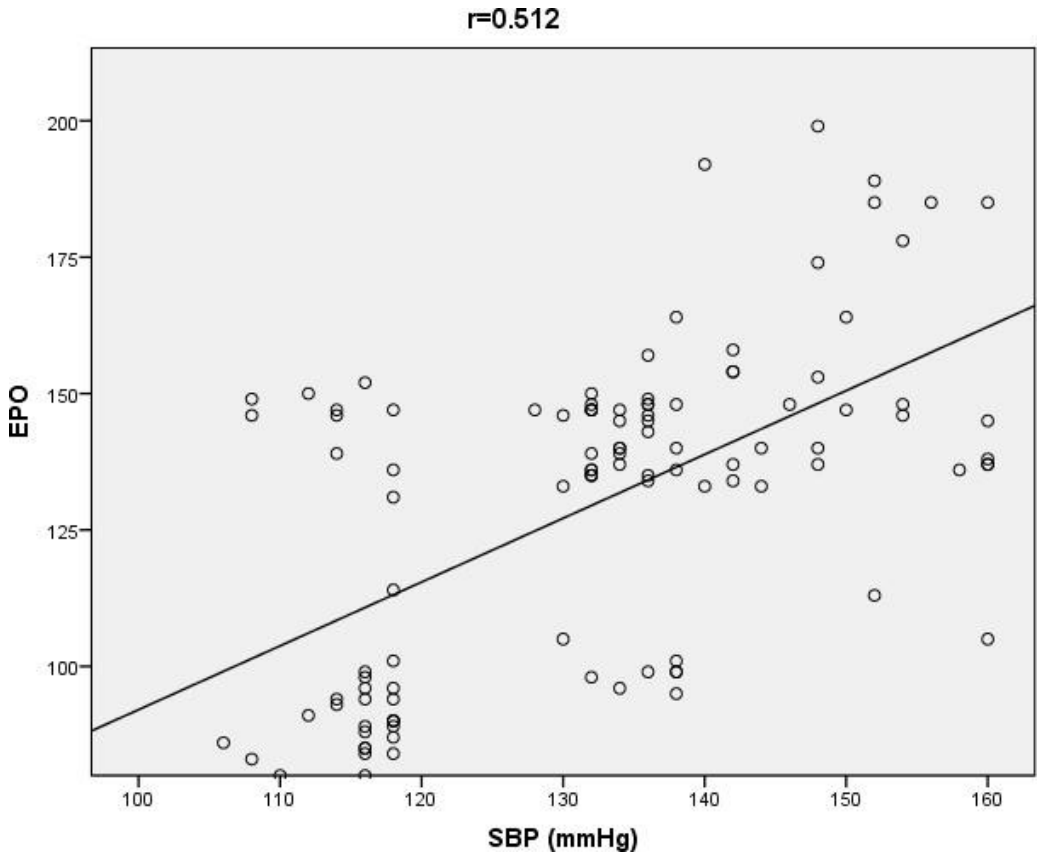
Graph 22 depicts correlation between SBP and serum NO of all the participants of each group. Results indicate a negative correlation (r = -0.735) between NO and SBP.



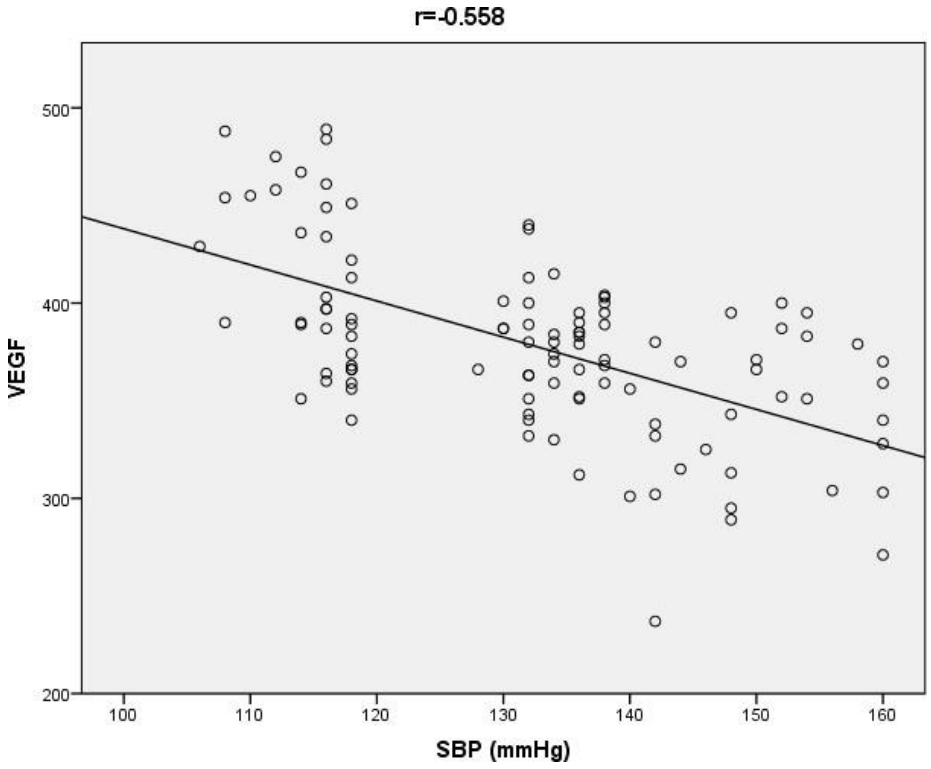
Graph 22: Correlation between SBP and serum NO

Correlation between Systolic Blood Pressure and Molecular Parameters:

Graph 23 depicts correlation between SBP and Serum EPO concentration of all the participants of each group. Results indicate a positive correlation (r = 0.512) between EPO and SBP. Graph 24 depicts correlation between SBP and Serum VEGF of all the participants of each group. Results indicate a negative correlation (r = -0.558) between VEGF and SBP.



Graph 23: Correlation between SBP and serum EPO



Graph 24: Correlation between SBP and serum VEGF

Correlation between Diastolic BP and AS:

Graph 25 depicts correlation between DBP and R Bra ASI of all the participants of each group. Results indicate a positive correlation (r = 0.578) between R Bra ASI and DBP. Graph 26 depicts correlation between DBP and L Bra ASI of all the participants of each group. Results indicate a positive correlation (r = 0.496) between L Bra ASI and DBP. Graph 27 depicts correlation between DBP and R Ank ASI of all the participants of each group. Results indicate a positive correlation (r = 0.570) between R Ank ASI and DBP. Graph 28 depicts correlation

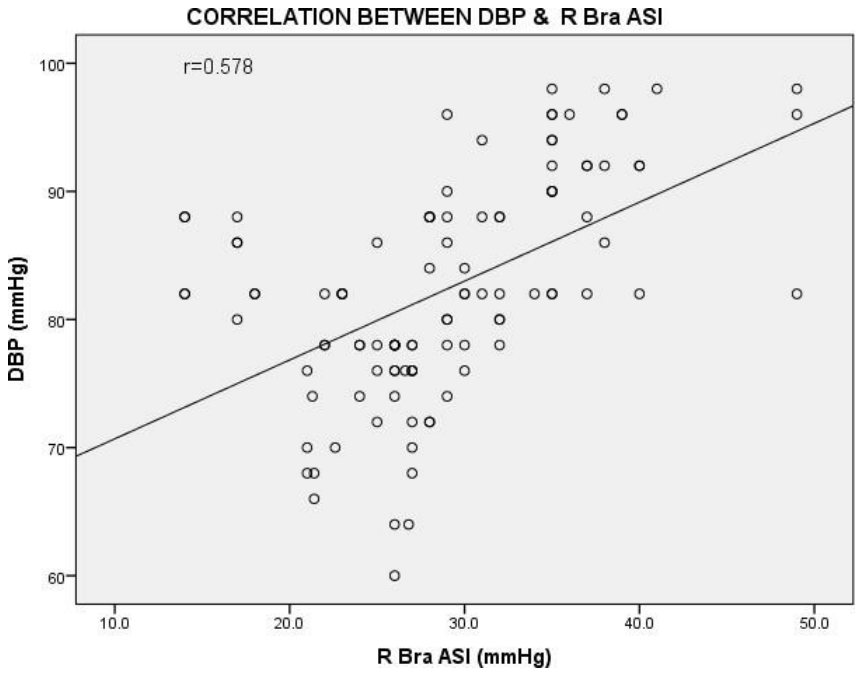
between DBP and L Ank ASI of all the participants of each group. Results indicate a positive correlation (r = 0.486) between L Ank ASI and DBP. Graph 29 depicts correlation between DBP and PWV_{b-a} Right of all the participants of each group. Results indicate a positive correlation (r = 0.447) between PWV_{b-a} Right and DBP. Graph 30 depicts correlation between DBP and PWV_{b-a} Left of all the participants of each group. Results indicate a positive correlation (r = 0.424) between PWV_{b-a} Left and DBP. Graph 31 depicts correlation between DBP and PWV_{c-f} of all the participants of each group. Results indicate a positive correlation (r = 0.478) between PWV_{c-f} and DBP.

Correlation between DBP and Oxidative Stress (Malondialdehyde; MDA):

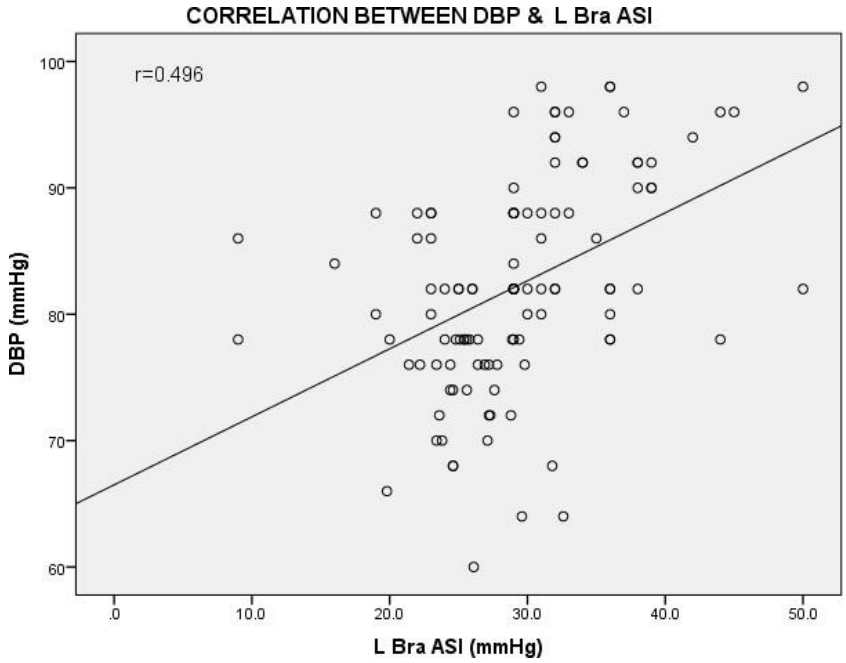
Graph 32 depicts correlation between DBP and serum MDA of all the participants of each group. Results indicate a positive correlation (r = 0.565) between serum MDA and DBP.

Correlation between DBP and endothelial dysfunction (Nitric Oxide; NO):

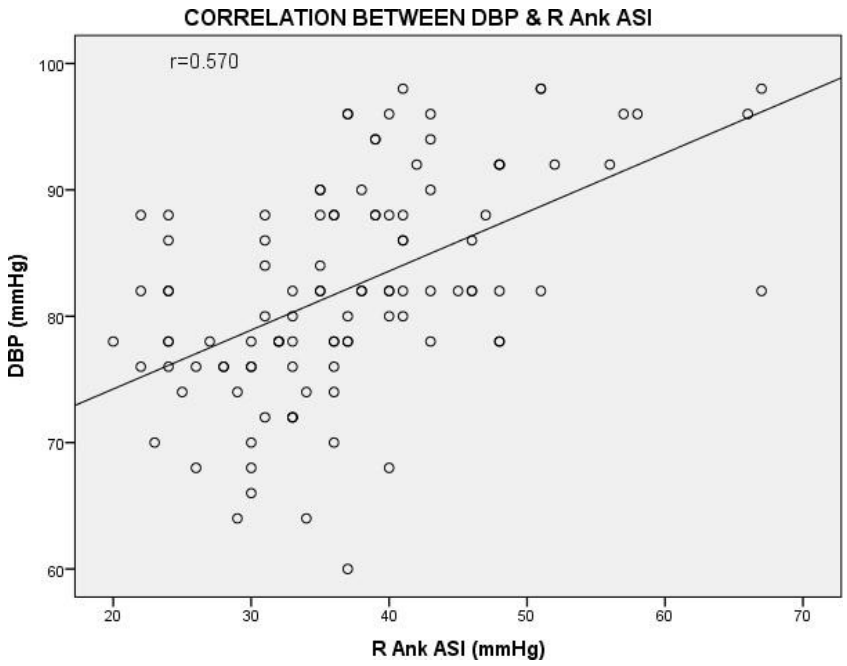
Graph 33 depicts correlation between DBP and serum NO of all the participants of each group. Results indicate a negative correlation (r = -0.711) between NO and DBP.



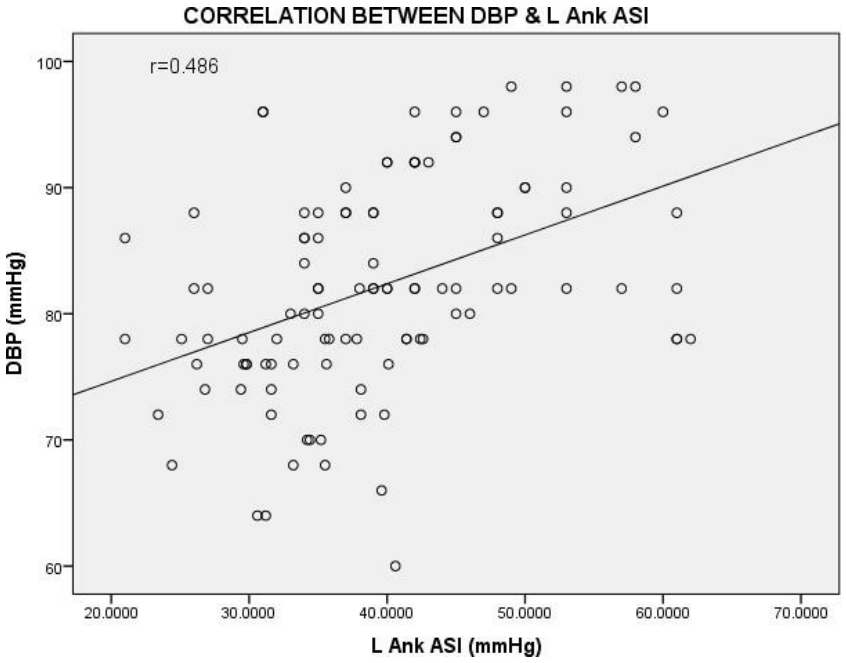
Graph 25: Correlation between DBP and R Bra ASI



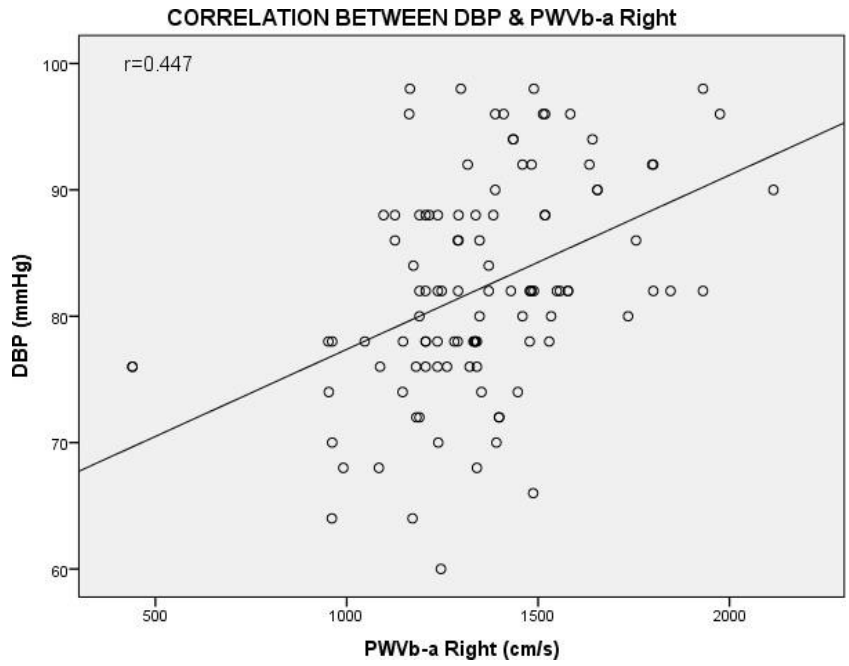
Graph 26: Correlation between DBP and L Bra ASI



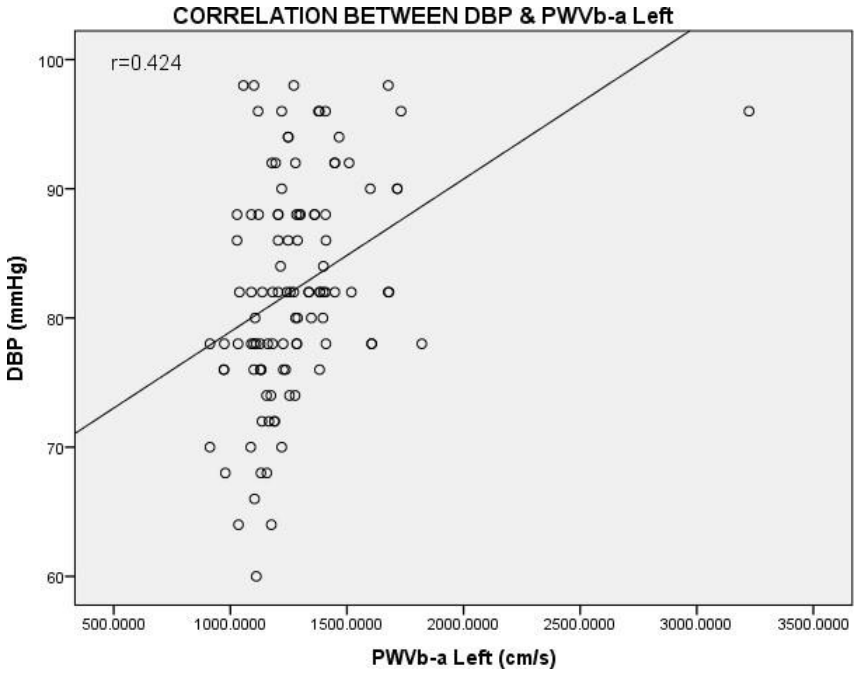
Graph 27: Correlation between DBP and R Ank ASI



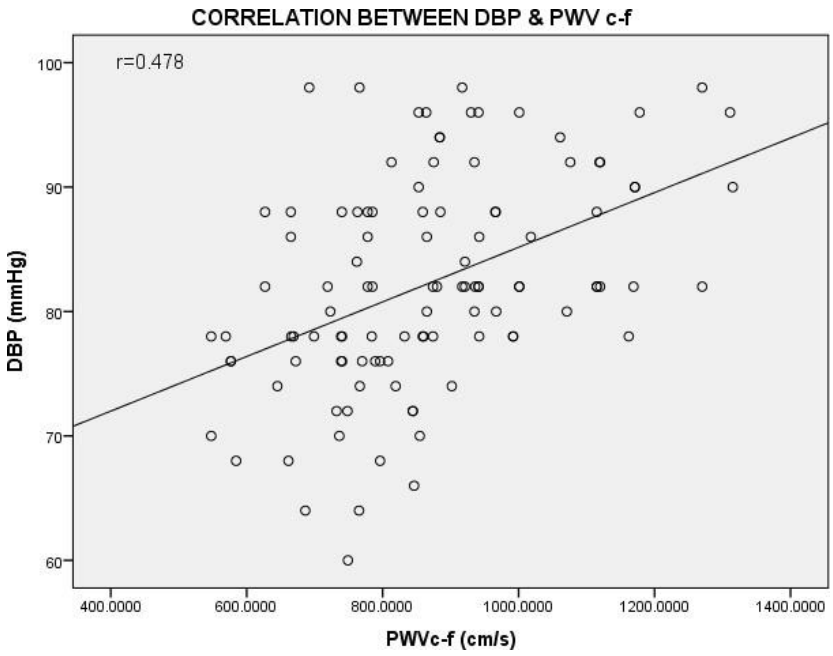
Graph 28: Correlation between DBP and L Ank ASI



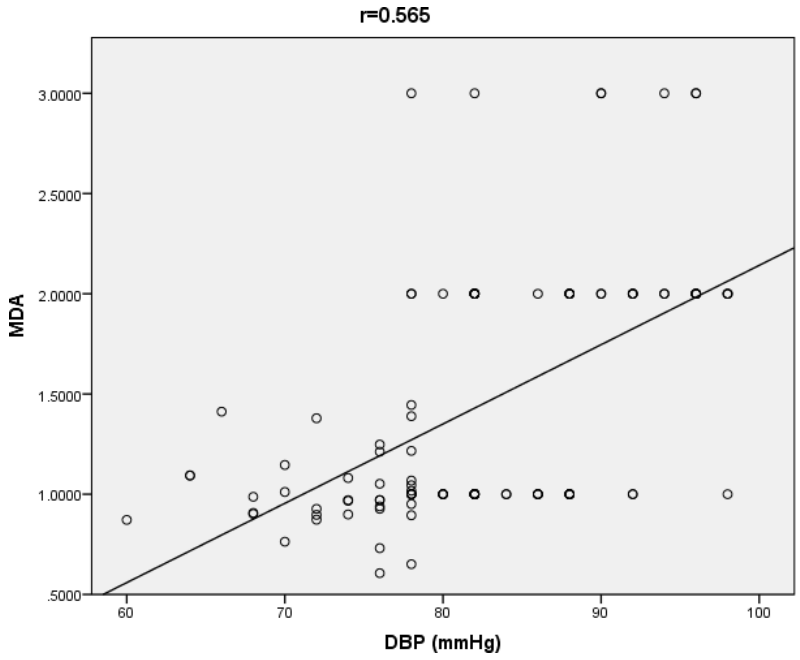
Graph 29: Correlation between DBP and PWV_{b-a} Right



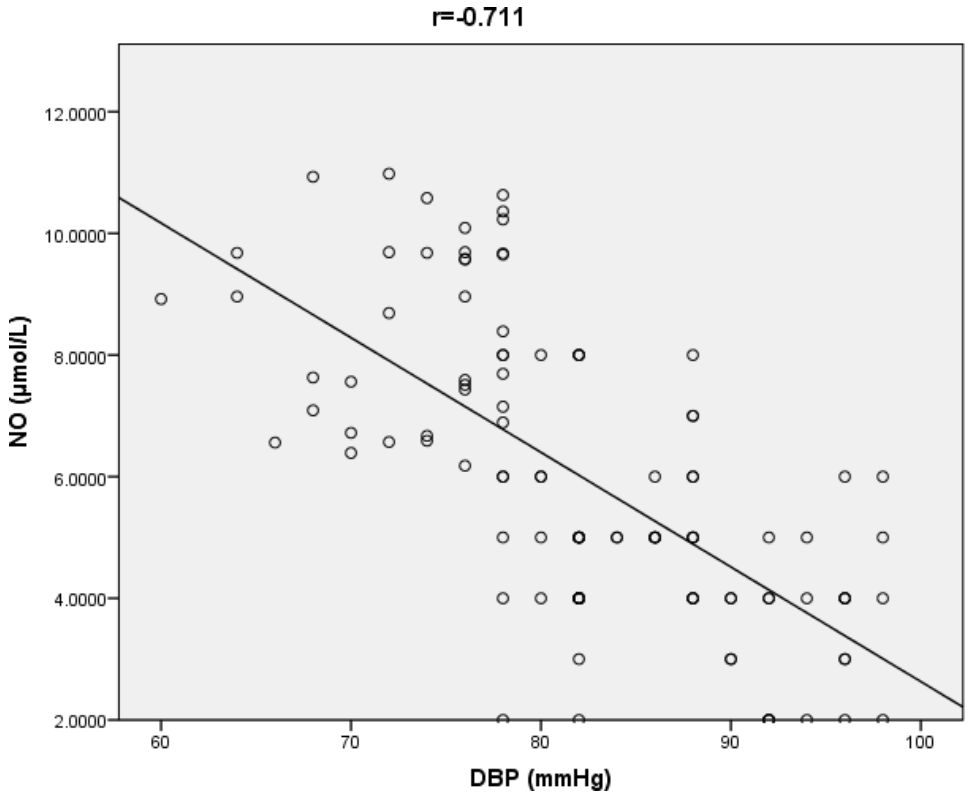
Graph 30: Correlation between DBP and PWV_{b-a} Left



Graph 31: Correlation between DBP and PWV_{c-f}



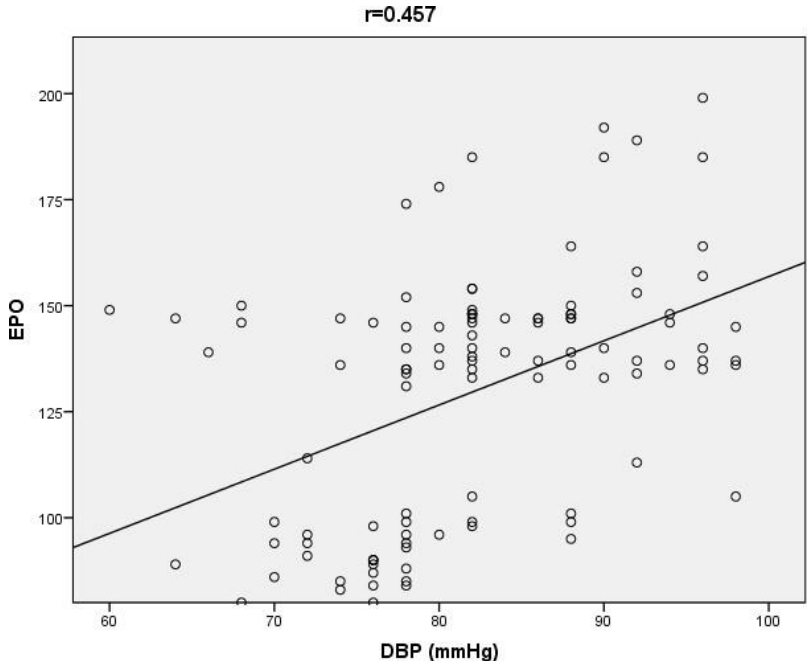
Graph 32: Correlation between DBP and MDA



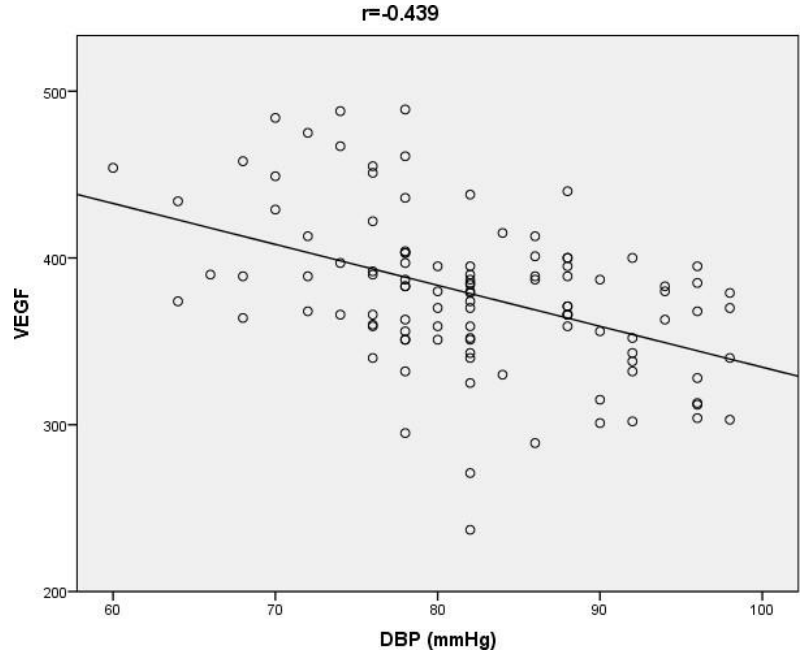
Graph 33: Correlation between DBP and NO

Correlation between DBP and Molecular Parameters:

Graph 34 depicts correlation between DBP and Serum EPO concentration of all the participants of each group. Results indicate a positive correlation (r = 0.457) between EPO and DBP. Graph 35 depicts correlation between DBP and Serum VEGF of all the participants of each group. Results indicate a negative correlation (r = -0.439) between VEGF and DBP.



Graph 34: Correlation between DBP and EPO



Graph 35: Correlation between DBP and VEGF

Correlation between MAP and Oxidative Stress (Malondialdehyde; MDA):

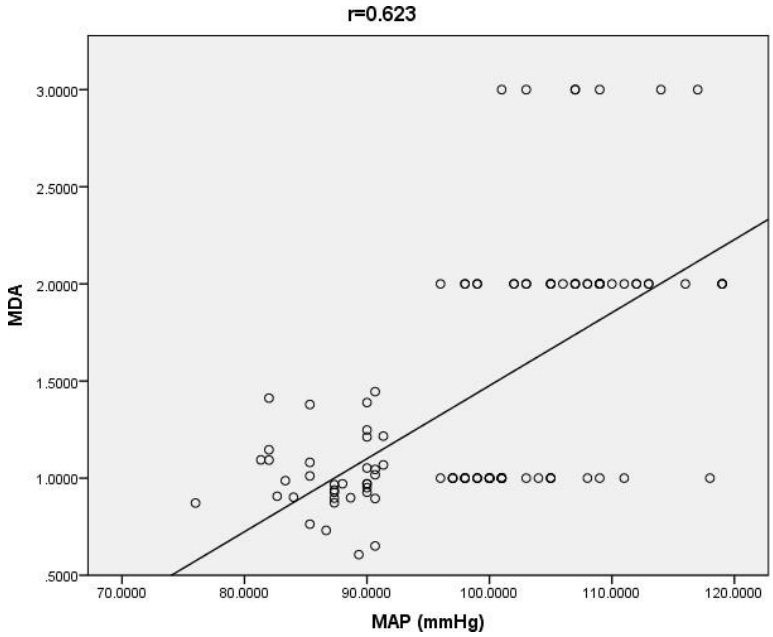
Graph 36 depicts correlation between MAP and serum MDA of all the participants of each group. Results indicate a positive correlation (r = 0.623) between serum MDA and MAP.

Correlation between MAP and endothelial dysfunction (Nitric Oxide; NO):

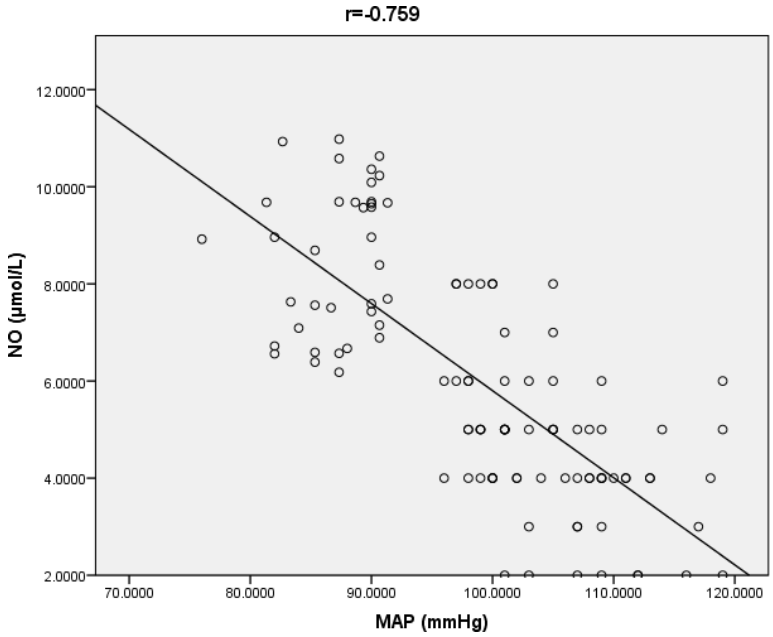
Graph 37 depicts correlation between MAP and serum NO of all the participants of each group. Results indicate a negative correlation (r = -0.759) between NO and MAP.

Correlation between MAP and Molecular Parameters:

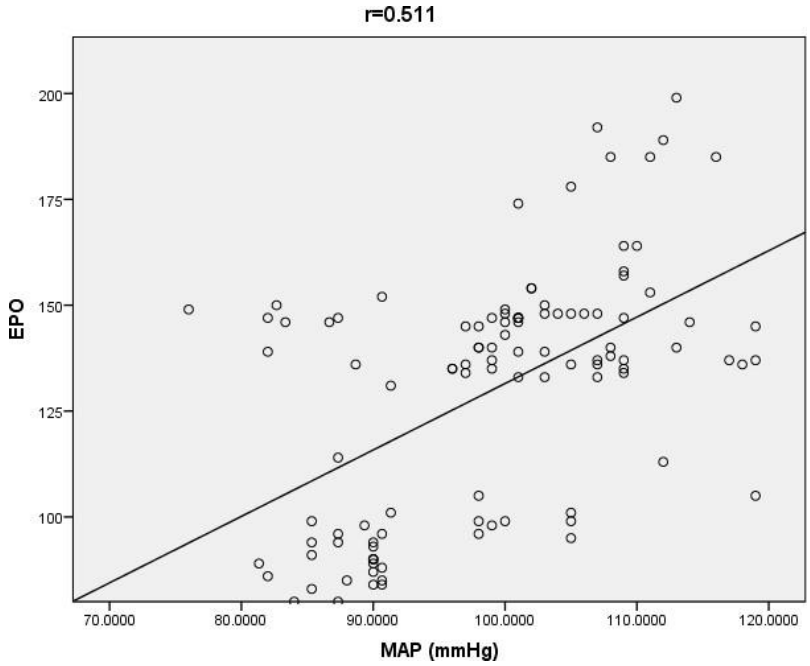
Graph 38 depicts correlation between MAP and Serum EPO concentration of all the participants of each group. Results indicate a positive correlation (r = 0.511) between EPO and MAP. Graph 39 depicts correlation between DBP and Serum VEGF of all the participants of each group. Results indicate a negative correlation (r = -0.501) between VEGF and MAP.



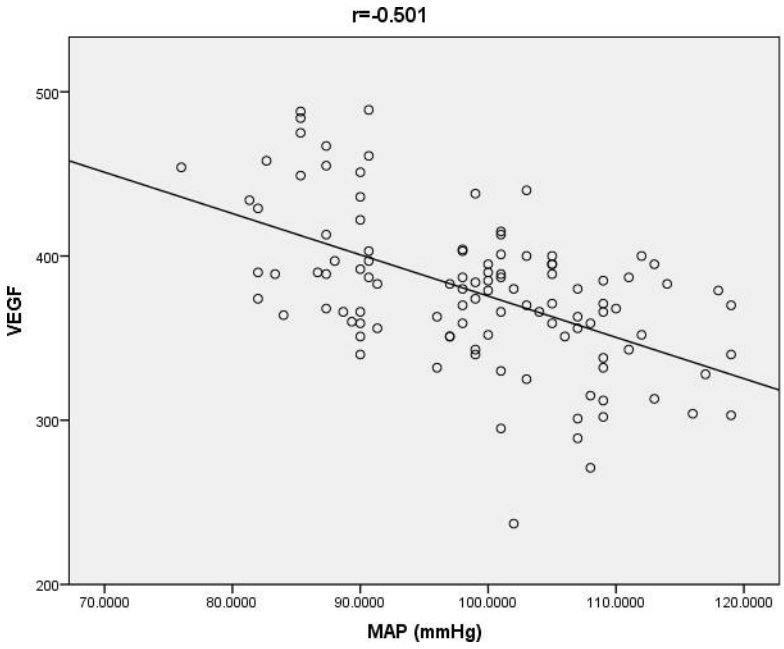
Graph 36: Correlation between MAP and MDA



Graph 37: Correlation between MAP and NO



Graph 38: Correlation between MAP and EPO



Graph 39: Correlation between MAP and VEGF

DISCUSSION

As all the samples from control, stage I HTN and stage II HTN groups showed no significant difference between each other, hence, in this study, all the subjects were found age matched (Table 1).

Comparison of Anthropometric parameters:

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 blood pressure (Table 2, 4). From our observations on physical anthropometry in stage I and stage II hypertensive patients as compared to the normal controls did not show any gender biasness (Table 3). Although in case of female WHR, average is showing above the normal which might be one of the reasons for inducing increase BP. [46]

Comparison of Physiological parameters:

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 (Table 5, 7). Results also indicate that there is no gender biasness on physiological parameters among the study groups (Table 6).

Comparison of Electrophysiological parameters:

Results of ASI of Right and Left brachial, Right and Left ankle of stage II HTN group were higher as compared to control group (Table 8, 10). 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 vascular disintegrity.

Melo E Silva FV et al. conducted a study [45] 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, dementia 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, 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 [46] 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. [60] Further, these dearrangements will lead to HTN. Our results found ASI and PWV clearly indicate altered pathophysiology of cardiovascular system. [49] 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 (Table 9).

Comparison of Biochemical Parameters:

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. [58] 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. [58] 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. [57] 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. [56]

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. [57] 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. [58]

Oxidative and nitrosative 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. [59]

Comparison of Molecular Parameters:

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. [61] 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. [48] 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. [62, 63] 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. [64] 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. [64] Our results of lower level of NO and VEGF support these findings. Another possible reason of VEGF signaling NO synthesis is VEGF receptor (VEGFR). [65] 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.

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.

SUMMARY

- Most of the current population of hypertensive patients may remain undiagnosed and don't seek medical attention. The debate regarding the role of vitamin D in control of BP is still ongoing. Many studies showed increase prevalence of hypertension during winter and in the areas where the exposure to the sunlight is reduced.
- Our study was undertaken to understand the role of vitamin D and its impact on stage I and stage II hypertension.
- We found a beneficial role of vitamin D in prevention of hypertension and other cardiovascular diseases. Optimal vitamin D concentration is helpful in delaying arterial stiffness. Arterial stiffness is positively correlated with hypertension. Oxidative stress, endothelial functions get altered if arteries become stiffer. Oxygen sensing proteins such as EPO positively and VEGF negatively influence blood pressure and arterial stiffness.
- All persons who are suspected for future cardiovascular risks may regularly screened for their vascular status and serum vitamin D concentration.
- Altered anthropometric parameters may be considered as risk factors for altered vascular status and should be screened for future cardiovascular pathophysiological events.

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ANNEXURE-I

CONSENT FORM

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____, S/O D/O W/O _____, aged ___years, ordinarily resident of _____ do hereby state/declare that Dr _____ of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled _____ under the guidance of Dr _____requesting my participation in the study.

Doctor has also informed me that during conduct of this procedure adverse result may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of Patient:

Signature of Doctor:

Witness: 1.

2.

ANNEXURE-II

CLINICAL PROFORMA

NAME :
AGE: GENDER:
RELIGION :
OCCUPATION :
RESIDENCE :
OP/IP No.:
D.O.A:
D.O.D:

Presenting Complaints :
Past history :
Personal history :
Treatment history :
General physical examination :
VITALS: PR: RR: WC: HC:
BP: TEMPERATURE: BMI:
SYSTEMIC EXAMINATION:
Cardiovascular system:
Other Systems:
Clinical Diagnosis:

INVESTIGATIONS:

Parameter	Group-1	Group-2	Group-3
Total Vitamin D level			
Serum lipid profile			
Serum Creatinine			
Blood Urea			
Fasting Blood Sugar			
MDA			
NO			
EPO			
VEGF			
PWV			
ASI			

ANNEXURE-III

ETHICAL CLEARANCE CERTIFICATE

IEC/NO-09/
Date-22/01

B.L.D.E. (DEEMED TO BE UNIVERSITY)

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Influence of Vitamin D on Arterial Stiffness in Hypertensive patients with special reference to Oxygen sensing protein expression.

Name of PG student: Dr Amrit Podder, Department of Physiology

Name of Guide/Co-investigator: Dr Sumangala Patil, Professor & HOD of Physiology

Co - Guide : Dr Sharan Badiger, Professor & HOD of Medicine

DR .S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

ANNEXURE-IV

MASTER DATA

1	Name	Age (Yrs)	Gender (M/F)	BMI (kg/m2)	WC (cm)	HC (cm)	Waist:Hip Ratio (WHR	Pulse (bpm)	RR (cpm)	Temp. (F)	SBP (mm Hg)	DBP (mm Hg)	PP (mm Hg)	MAP (mm Hg)	R Bra ASI (m	L Bra ASI (m	R Ank ASI (m	L Ank ASI (m	PWV b-a Right (cm/s	PWV b-a Left (cm/s	PWV c-f (cm/s)	Group	Total Vit D Lev	Serum Creat.	Blood Urea	FBS	Serum TGL.	Serum Cholesterol	HDL Cholesterol	MDA	NO (µmol/L)	EPO	VEGF
2	Jyoti Khodna	38	F	22.5	86	88	0.977	76	13	97	116	78	38	90.67	27	26	36	41	1047	1111	666	Control	35	0.6	20	68	137	208	40	1.017	7.15	85.5	461.3
3	Shailaja Patil	50	F	24.7	84	89	0.944	74	12	98	118	76	42	90	26	23	36	40	1181	1227	770	Control	29	0.8	25	68	150	199	49	0.971	10.1	83.5	392.2
4	Shrilaxmi Bag	39	F	24.5	86	83	1.036	76	13	97	106	70	36	82	27	24	30	35	962.3	912.4	548	Control	37	0.6	12	74	160	204	64	1.146	6.72	86.3	429.1
5	Gouher Banu	46	F	22.9	83	86	0.965	80	13	97	116	68	48	84	27	32	40	36	991	1157	662	Control	39	0.7	23	60	100	79	48	0.902	7.09	80.4	363.5
6	Prachi Kulkar	35	F	23	86	91	0.945	88	14	98	116	74	42	88	26	26	25	29	1146	1254	766	Control	33	0.7	26	65	106	160	50	0.971	6.67	84.6	397.2
7	Neleema Dor	50	F	23.1	81	83	0.976	84	12	98	110	76	34	87.33	26	27	30	30	1087	1382	672	Control	30	0.6	17	77	108	167	42	0.939	6.18	80.1	455.5
8	Ashok Pattar	41	M	24.6	85	88	0.966	72	13	97	118	76	42	90	27	27	28	30	1262	1237	808	Control	30	0.7	29	65	97	115	41	1.052	9.58	89.5	451.1
9	Praveen Gan	39	M	23.8	84	88	0.955	78	13	98	116	78	38	90.67	26	26	32	38	1237	1101	740	Control	35	0.6	28	71	151	202	73	1.445	8.39	152	402.7
10	Ranjeeta Imn	43	F	20.3	85	86	0.988	74	13	97	118	72	46	87.33	28	27	33	38	1190	1166	748	Control	33	0.6	26	72	157	209	62	0.927	6.57	94.2	413.2
11	Sudhir Bagali	35	M	21.1	84	85	0.988	67	14	97	108	74	34	85.33	24	24	34	38	953.4	1155	645	Control	41	0.6	27	69	100	179	55	1.081	6.59	83.3	487.6
12	M P Dodamar	37	M	21.1	81	84	0.964	64	12	97	108	60	48	76	26	26	37	41	1246	1111	749	Control	44	0.8	34	62	93	118	49	0.872	8.92	149	453.7
13	Chandrika Do	36	F	23.9	85	87	0.977	74	12	97	116	76	40	89.33	30	28	33	33	439.9	972.2	577	Control	42	0.8	29	65	94	152	58	0.606	9.57	97.9	360.3
14	Sona Tejaswi	47	F	23.3	85	89	0.955	62	14	98	118	76	42	90	21	21	26	31	1206	1128	739	Control	35	0.9	32	73	154	208	64	0.927	7.59	89.2	365.6
15	Aarathi Neell	50	F	21.1	86	84	1.024	74	13	97	118	72	46	87.33	25	27	33	40	1398	1189	844	Control	34	0.6	24	71	157	198	61	0.897	9.69	114	367.8
16	Samragyi Nee	49	F	23.2	84	87	0.966	70	14	98	112	68	44	82.67	21	25	26	24	1340	1131	796	Control	39	0.9	23	75	103	158	56	0.907	10.9	150	457.6
17	Lata Mullur	49	F	23.6	84	83	1.012	88	15	98	114	74	40	87.33	29	28	36	32	1447	1278	902	Control	41	0.8	29	94	91	180	46	0.967	10.6	147	466.9
18	Amrit Podder	35	M	23.8	84	84	1	68	13	97	118	78	40	91.33	26	29	33	41	952.5	974	569	Control	37	0.8	29	63	90	146	54	1.216	7.69	101	383.2
19	Manchala Pri	45	F	23.7	86	89	0.966	78	13	97	116	64	52	81.33	26	30	29	31	961.3	1176	765	Control	36	0.9	31	65	148	94	61	1.094	9.68	89.2	434.3
20	Jayashree N	38	F	20.3	86	90	0.956	72	15	97	118	76	42	90	27	30	22	32	1237	1101	740	Control	32	0.7	29	69	114	174	52	1.212	8.96	89.9	421.9
21	Shanta Hirem	35	F	23.7	85	88	0.966	70	12	97	114	78	36	90	27	29	37	43	1337	1284	859	Control	37	0.7	24	65	98	152	56	1.389	9.65	92.9	435.8
22	Ganapati V P	46	M	23.1	85	88	0.966	78	13	98	118	78	40	91.33	22	25	36	36	1281	1160	784	Control	40	0.8	27	63	103	168	63	1.068	9.67	131	356.4
23	Ram Singhan	42	M	23.4	83	83	1	75	14	98	116	78	38	90.67	26	25	32	42	1340	1285	860	Control	29	0.9	29	69	107	167	59	0.895	6.89	84.2	396.6
24	Ravi Siddanth	38	M	23.5	81	84	0.964	70	12	97	112	72	40	85.33	28	24	31	23	1182	1136	732	Control	32	0.8	23	78	109	153	65	1.379	8.69	91.4	474.9
25	Yashpal Srika	42	M	23.1	84	89	0.944	80	15	97	116	70	46	85.33	21	27	23	34	1391	1220	855	Control	35	0.6	31	77	108	159	61	0.763	7.56	94	449
26	Anand Ingale	38	M	21.2	82	81	1.012	70	14	97	116	78	38	90.67	26	26	37	36	1331	1226	832	Control	34	0.6	28	83	114	203	62	0.651	10.6	95.6	489.3
27	Suryakanth	36	M	22.9	83	83	1	78	12	98	114	68	46	83.33	21	25	30	33	1084	978.4	584	Control	33	0.5	29	87	151	209	61	0.987	7.63	146	388.8
28	Govindagoud	50	M	23.2	84	89	0.944	75	12	97	114	66	48	82	21	20	30	40	1487	1104	846	Control	39	0.9	35	87	108	143	48	1.412	6.56	139	390
29	B S Patil	46	M	24	83	86	0.965	62	14	98	118	76	42	90	27	22	30	36	1321	1133	789	Control	41	0.6	24	61	103	128	50	0.971	7.43	87.4	340.1
30	Chandrasekh	43	M	21.6	86	81	1.062	95	15	97	118	74	44	88.67	21	25	29	27	1352	1174	819	Control	28	0.8	27	79	154	213	62	0.899	9.68	136	365.9
31	Rajasekhar A	50	M	23.9	85	81	1.049	75	15	97	116	70	46	85.33	23	23	36	34	1239	1088	736	Control	43	0.7	27	68	155	211	47	1.011	6.39	99.2	483.8
32	Meghu B Cha	35	M	24	85	89	0.955	81	12	98	118	64	54	82	27	33	34	31	1172	1035	686	Control	38	0.5	32	79	126	210	48	1.093	8.96	147	373.7
33	Sridevi Singh	46	F	23.9	83	86	0.965	84	12	98	116	78	38	90.67	26	25	32	30	962.3	912.4	548	Control	32	0.6	31	91	151	212	52	1.045	10.2	88.4	386.9
34	Jayashree Ma	50	F	23.5	86	83	1.036	72	14	97	118	76	42	90	27	26	28	30	439.9	972.2	577	Control	37	0.7	23	69	97	157	59	1.248	9.69	89.5	359.2
35	Shreya Nirwa	40	F	23.9	83	86	0.965	78	14	98	114	78	36	90	26	25	30	25	1206	1128	739	Control	38	0.7	25	67	94	169	57	0.951	10.4	94	350.7
36	G M Mathapa	50	M	23	86	91	0.945	74	14	98	118	72	46	87.33	27	29	33	32	1398	1189	844	Control	39	0.9	24	82	93	152	53	0.873	11	95.6	388.8
37	Janeshab H B	50	M	23.1	81	83	0.976	67	12	98	108	76	32	86.67	25	24	24	26	1340	1131	796	Control	38	0.7	25	79	98	160	61	0.731	7.51	146	390
38	Indira Hunde	48	F	23.6	89	88	1.011	88	12	98	160	96	64	117.3	39	33	57	42	1163	3225	1178	Stage II	17	0.7	22	72	132	209	37	2.706	2.89	137	327.9
39	Sushila Bhim	50	F	25.6	92	95	0.968	78	14	97	148	78	70	101.3	30	44	43	62	1529	1821	1162	Stage II	13	0.8	20	89	214	262	68	2.692	2.36	174	294.6
40	Jayashree Ma	50	F	27.5	101	104	0.971	74	13	97	144	82	62	102.7	37	36	45	44	1846	1519	1169	Stage II	14	0.9	32	72	201	261	41	2.697	2.69	133	369.6
41	Shreya Nirwa	40	F	27.8	105	107	0.981	78	12	98	148	86	62	106.7	38	35	46	48	1756	1248	1019	Stage II	19	0.6	18	87	198	257	43	1.611	4.86	137	288.7
42	Gayatri Math	50	F	27.6	103	98	1.051	75	14	98	142	92	50	108.7	38	39	56	42	1798	1447	1119	Stage II	14	0.8	31	85	187	231	45	2.012	2.32	158	301.7
43	U C Nuchi	49	M	28	107	104	1.029	92	15	97	138	96	42	110	39	37	58	45	1411	1382	930	Stage II	14	0.9	33	86	136	236	45	1.929	4.36	164	368.2
44	Jaya H B	50	F	25.9	89	83	1.072	83	14	98	146	82	64	103.3	35	32	43	49	1549	1257	936	Stage II	23	0.9	35	77	154	194	31	2.026	2.31	148	325.1
45	Raju Rathod	50	M	23.1	92	87	1.057	82	12	97	142	82	60	102	31	31	51	48	1429	1242	880	Stage II	22	0.7	33	95	245	190	42	1.959	3.56	154	237.2
46	Pallavi Kanth	40	F	29.1	94	89	1.056	76	14	97	150	88	62	108.7	37	32	47	48	1096	1295	763	Stage II	16	0.7	18	99	126	212	58	2.021	3.65	164	370.7
47	Santosh Ram	40	M	25.9	98	97	1.01	66	15	97	160	98	62	118.7	41	31	51	58	1165	1056	692	Stage II	25	0.9	34	85	124	148	49	2.021	5.89	137	302.7

48	Basavaraj An	42	M	28	100	104	0.962	70	14	97	148	92	56	110.7	40	38	52	43	1634	1509	1076	Stage II	23	0.7	18	81	114	216	44	1.394	3.68	153	342.6
49	Pratyusha Ka	35	F	28.4	100	99	1.01	92	12	98	142	82	60	102	34	32	40	45	1248	1039	719	Stage II	14	0.6	34	86	87	242	52	2.021	3.69	154	380.3
50	Kashi Badige	50	F	26.6	100	103	0.971	82	14	97	160	82	78	108	35	36	41	57	1489	1272	917	Stage II	17	0.7	35	75	161	169	54	1.427	5.34	138	271.3
51	Santosh K Ra	39	M	29.1	99	97	1.021	74	12	98	142	92	50	108.7	37	34	48	40	1316	1195	813	Stage II	12	0.9	36	75	182	210	54	1.441	3.96	134	331.6
52	M S Patil	35	M	34.1	104	105	0.99	73	14	97	158	98	60	118	38	36	51	49	1299	1101	767	Stage II	11	0.9	33	99	123	181	49	1.319	4.39	136	379.4
53	Shrishil I Aga	50	M	32.3	104	103	1.01	70	12	97	152	90	62	110.7	35	38	43	53	2115	1600	1315	Stage II	15	0.6	31	83	136	168	47	1.922	3.64	185	387.1
54	Bindu Madha	45	M	23.8	93	89	1.045	94	13	98	160	98	62	118.7	49	50	67	53	1931	1677	1270	Stage II	14	0.6	22	93	175	236	43	2.021	2.12	145	340.5
55	Mallappa Hac	50	M	24	95	91	1.044	88	14	98	148	96	52	113.3	36	44	66	60	1975	1732	1311	Stage II	17	0.7	38	99	126	214	41	2.031	3.54	199	313.5
56	Renuka Patil	38	F	35.7	102	101	1.01	82	15	97	154	80	74	104.7	29	36	33	45	1459	1279	935	Stage II	20	0.6	34	87	106	196	45	2.099	5.34	178	395.3
57	Mahadev Pat	47	M	34.6	93	92	1.011	88	12	98	148	96	52	113.3	49	45	43	53	1518	1408	941	Stage II	12	0.9	33	81	109	147	31	2.027	3.86	140	395.4
58	Sumitra Bala	47	F	32	101	106	0.953	82	13	98	132	94	38	106.7	35	32	39	45	1642	1466	1061	Stage II	18	0.7	33	86	67	103	39	2.029	3.68	148	379.8
59	Sumedha Hip	48	F	27	103	109	0.945	104	12	97	136	96	40	109.3	35	32	37	31	1584	1378	1001	Stage II	22	0.7	26	81	140	128	37	2.978	3.45	135	312
60	Padmah Yara	42	F	27.6	92	98	0.939	87	12	97	154	94	60	114	31	42	43	58	1435	1248	884	Stage II	19	0.6	30	79	122	166	41	2.729	5.36	146	382.6
61	Sabitri Inamd	45	F	25.6	91	99	0.919	87	14	98	140	90	50	106.7	35	39	35	50	1388	1220	853	Stage II	13	0.6	19	77	124	183	43	2.941	3.21	133	355.6
62	G I Kori	45	M	25	98	99	0.99	80	14	98	152	92	60	112	35	32	42	42	1483	1178	875	Stage II	20	0.7	33	84	257	108	43	1.974	2.36	189	351.8
63	Mohan B K	43	M	24.4	89	91	0.978	84	15	97	150	88	62	108.7	32	29	39	48	1216	1121	740	Stage II	21	1	33	90	104	193	56	2.095	3.69	147	365.8
64	S M Patil	47	M	30.8	102	98	1.041	81	13	98	154	82	72	106	40	38	48	42	1801	1448	1120	Stage II	17	0.6	26	99	212	144	46	1.742	3.51	148	351.1
65	Sumangala P	48	F	30.4	97	93	1.043	82	12	97	144	90	54	108	29	29	38	37	1655	1716	1171	Stage II	17	0.7	30	87	200	140	65	1.591	3.65	140	315.1
66	Umesh Kalad	46	M	27.7	104	100	1.04	79	12	97	156	96	60	116	29	29	40	47	1513	1119	864	Stage II	14	0.6	19	73	143	272	62	1.679	2.25	185	303.6
67	Ravi B Patil	39	M	29.1	99	97	1.021	74	14	97	142	92	50	108.7	37	34	48	40	1459	1279	935	Stage II	14	0.8	22	91	143	172	60	1.719	5.34	137	337.9
68	Santosh Nan	45	M	23.8	93	89	1.045	94	13	97	160	98	62	118.7	35	36	41	57	1489	1272	917	Stage II	18	1.1	35	91	75	194	49	1.788	5.21	105	370.5
69	Sankarappa B	50	M	26.6	100	103	0.971	82	13	98	160	82	78	108	49	50	67	53	1931	1677	1270	Stage II	20	0.8	38	76	85	228	47	1.693	4.36	185	358.6
70	Vimala Sures	46	F	32.4	95	105	0.905	78	13	97	132	94	38	106.7	35	32	39	45	1435	1248	884	Stage II	16	1.1	31	79	128	216	41	1.581	2.36	136	362.7
71	Jayashree Bir	36	F	26.1	83	94	0.883	82	12	98	136	96	40	109.3	35	32	37	31	1388	1220	853	Stage II	19	1.2	36	81	162	268	35	1.686	5.94	157	384.6
72	Sanjukta Pati	40	F	27.8	105	107	0.981	87	14	98	140	90	50	106.7	35	39	35	50	1655	1716	1171	Stage II	18	1.1	34	83	184	194	32	2.587	3.21	192	301.2

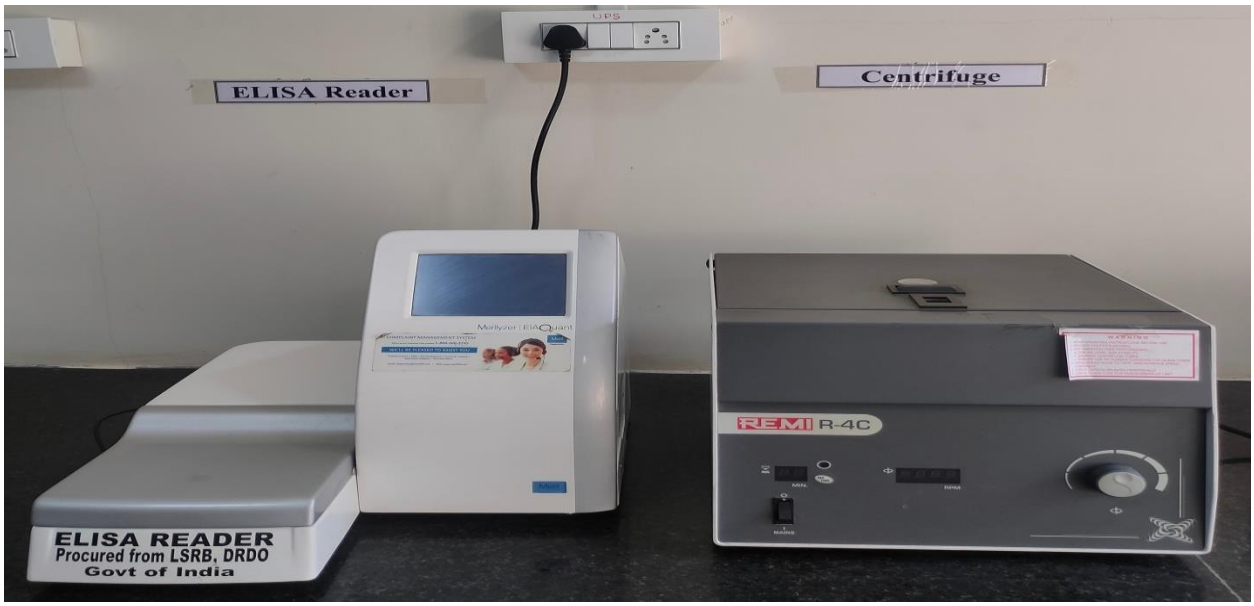
73	L H Pail	45	M	25	98	99	0.99	80	14	97	152	92	60	112	40	38	48	42	1801	1448	1120	Stage II	16	1.1	39	67	164	146	39	2.013	2.25	113	399.7
74	Sana K Patel	35	F	26.1	99	103	0.961	76	14	97	136	82	54	100	23	23	40	38	1557	1680	1115	Stage I	27	0.5	24	76	109	147	52	1.064	7.65	148	351.8
75	Shantaveerya	35	M	28.7	98	96	1.021	70	14	98	128	88	40	101.3	31	33	39	53	1337	1284	859	Stage I	22	0.6	16	77	67	103	39	0.722	6.65	147	365.8
76	Praveen Kho	49	M	24.6	92	88	1.045	67	15	98	136	78	58	97.33	29	20	27	37	1147	1091	669	Stage I	24	1	31	76	140	128	42	0.986	7.89	145	351.1
77	Mallapa Sidd	40	M	21.1	81	78	1.038	78	12	97	134	84	50	100.7	28	16	31	34	1174	1215	762	Stage I	30	0.7	32	79	122	166	55	0.637	5.36	139	415.1
78	Vikas Desai	43	M	23.7	96	94	1.021	76	14	97	138	78	60	98	32	29	20	32	1206	1033	699	Stage I	25	0.8	36	70	140	183	49	1.686	5.69	140	403.6
79	Shrinivas Rail	35	M	22.1	81	79	1.025	80	14	98	132	82	50	98.67	30	29	33	42	1291	1137	778	Stage I	34	1.1	34	92	88	108	34	1.581	7.69	135	437.9
80	Santosh Hipp	35	M	21.5	83	82	1.012	74	15	98	134	80	54	98	29	31	31	35	1190	1106	723	Stage I	23	0.7	24	84	150	146	31	1.188	4.46	95.6	370.5
81	S M Biradar	48	M	24.6	88	92	0.957	60	13	97	138	88	50	104.7	28	19	40	39	1383	1301	885	Stage I	21	0.9	29	99	237	227	37	1.147	4.89	98.6	358.6
82	Rekha S Udgi	46	F	27.4	101	93	1.086	70	12	98	132	78	54	96	24	36	48	61	1334	1606	992	Stage I	36	0.6	22	60	81	157	37	1.078	5.87	135	362.7
83	Huchappa Mu	49	M	27.7	93	90	1.033	76	14	98	136	82	54	100	30	29	35	39	1371	1399	921	Stage I	35	1	18	98	224	254	65	1.071	4.01	143	384.6
84	V G Warad	50	M	24.4	92	95	0.968	72	14	98	130	86	44	100.7	25	9.4	24	21	1290	1410	942	Stage I	23	0.7	22	60	201	157	49	1.075	4.56	146	401.2
85	Murgesh Mat	42	M	21.6	83	94	0.883	72	15	97	132	88	44	102.7	14	30	22	26	1238	1205	785	Stage I	24	0.7	26	80	201	224	37	1.067	4.89	139	399.7
86	Anand Amba	49	M	28.3	95	104	0.913	64	13	97	132	86	46	101.3	17	23	41	34	1347	1288	865	Stage I	28	1.1	32	86	118	190	40	1.491	5.27	147	389.3
87	Balaraj Birad	50	M	28	100	104	0.962	64	14	98	138	88	50	104.7	28	23	36	37	1518	1361	967	Stage I	22	0.8	33	81	114	127	43	1.693	5.63	136	388.8
88	Manjunath K	47	M	26.7	91	101	0.901	82	14	98	136	82	54	100	22	24	24	27	1478	1181	874	Stage I	21	1.1	36	75	112	134	49	1.446	4.21	149	390
89	Vimala Sures	46	F	22.4	95	105	0.905	78	15	97	132	82	50	98.67	18	26	46	35	1578	1384	1001	Stage I	25	0.7	24	97	167	205	47	1.062	4.96	147	340.1
90	Jayashree Bir	36	F	26.1	83	94	0.883	82	13	98	136	88	48	104	17	22	41	34	1126	1029	665	Stage I	24	0.8	21	81	154	201	45	1.064	4.36	148	365.9
91	Nikita R	38	F	24.6	86	88	0.977	78	12	98	134	82	52	99.33	14	29	35	39	1206	1408	627	Stage I	27	0.6	34	81	84	195	49	1.085	5.21	140	383.8
92	Supriya Bhos	35	F	23.7	88	91	0.967	86	12	98	138	88	50	104.7	29	31	31	35	1292	1205	779	Stage I	20	0.5	25	78	92	189	50	1.084	5.36	95	399.9
93	Vijaya Sorgar	36	F	24.6	91	98	0.929	76	14	97	134	82	52	99.33	32	29	24	61	1190	1091	1115	Stage I	23	0.7	30	84	79	179	53	1.066	4.56	137	373.7
94	Santosh Patil	38	M	42.7	129	127	1.016	82	13	97	130	82	48	98	23	25	38	40	1482	1336	941	Stage I	28	0.8	23	76	186	216	45	1.788	4.89	105	386.9
95	Sanjay Wavar	47	M	30.8	102	98	1.041	75	12	98	134	80	54	98	32	30	40	46	1735	1397	1071	Stage I	22	0.8	29	61	107	161	37	0.604	5.89	145	359.2
96	D G Gannur	49	M	27.1	94	99	0.949	72	14	98	132	80	52	97.33	32	19	37	33	1534	1347	967	Stage I	21	0.6	34	85	73	143	57	0.911	5.69	136	350.7
97	Daya Pujari	47	F	24.6	92	88	1.045	67	15	97	136	78	58	97.33	24	36	48	61	1334	1606	992	Stage I	24	0.8	33	86	257	137	59	0.998	7.89	134	382.7

98	Vithoba Neel	50	F	21.1	81	78	1.038	78	12	98	134	84	50	100.7	30	29	35	39	1371	1399	921	Stage I	20	0.7	33	85	104	193	55	1.034	5.46	147	329.8
99	Manjula Nee	49	F	23.7	96	94	1.021	76	12	97	138	78	60	98	25	9.4	24	21	1290	1410	942	Stage I	21	0.8	26	95	212	144	45	1.088	4.56	98.7	402.7
100	Shantaveery	35	M	22.1	81	79	1.025	80	14	97	132	82	50	98.67	14	30	22	26	1238	1205	785	Stage I	27	0.7	30	99	200	140	47	1.719	4.23	98.2	342.6
101	Gayatri V Par	46	F	21.5	83	82	1.012	74	14	98	134	80	54	98	17	23	41	34	1347	1288	865	Stage I	25	0.7	19	85	143	272	43	0.798	7.87	140	380.3
102	Rani Singhan	42	F	24.6	88	92	0.957	60	12	98	138	88	50	104.7	28	23	36	37	1518	1361	967	Stage I	23	0.8	23	81	143	172	61	1.679	7.54	148	371.3
103	Roma Siddan	38	M	27.4	101	93	1.086	70	12	97	132	78	54	96	22	24	24	27	1478	1181	874	Stage I	23	0.9	21	86	75	183	59	1.596	4.32	135	331.6
104	Yashi Srikant	42	F	27.7	93	90	1.033	76	14	97	136	82	54	100	18	26	46	35	1578	1384	1001	Stage I	29	0.9	33	85	85	108	54	0.715	4.21	146	379.4
105	Supriya Patil	36	F	24.4	92	95	0.968	72	15	98	130	86	44	100.7	17	22	41	34	1126	1029	665	Stage I	30	0.8	33	75	79	146	53	1.089	5.67	133	387.1
106	Manjula Nare	50	F	25.6	83	94	0.883	72	12	98	132	88	44	102.7	14	29	35	39	1206	1408	627	Stage I	26	0.6	26	99	186	227	51	1.742	5.69	150	440.5
107	Sabitri Patil	46	F	28.3	95	104	0.913	64	12	97	132	86	46	101.3	29	31	31	35	1292	1205	779	Stage I	29	0.8	30	95	107	157	57	1.125	5.32	147	413.5
108	Chandra Bhui	43	F	28	100	104	0.962	64	12	98	138	88	50	104.7	32	29	24	61	1190	1091	1115	Stage I	27	0.9	19	93	73	254	45	1.171	7.21	101	395.3
109	Rajashree An	50	F	27.7	91	101	0.901	82	12	97	136	82	54	100	23	25	38	40	1482	1336	941	Stage I	26	0.7	24	67	257	157	43	1.111	7.56	99.2	395.4
110																																	

ANNEXURE-V

PHOTOGRAPHS

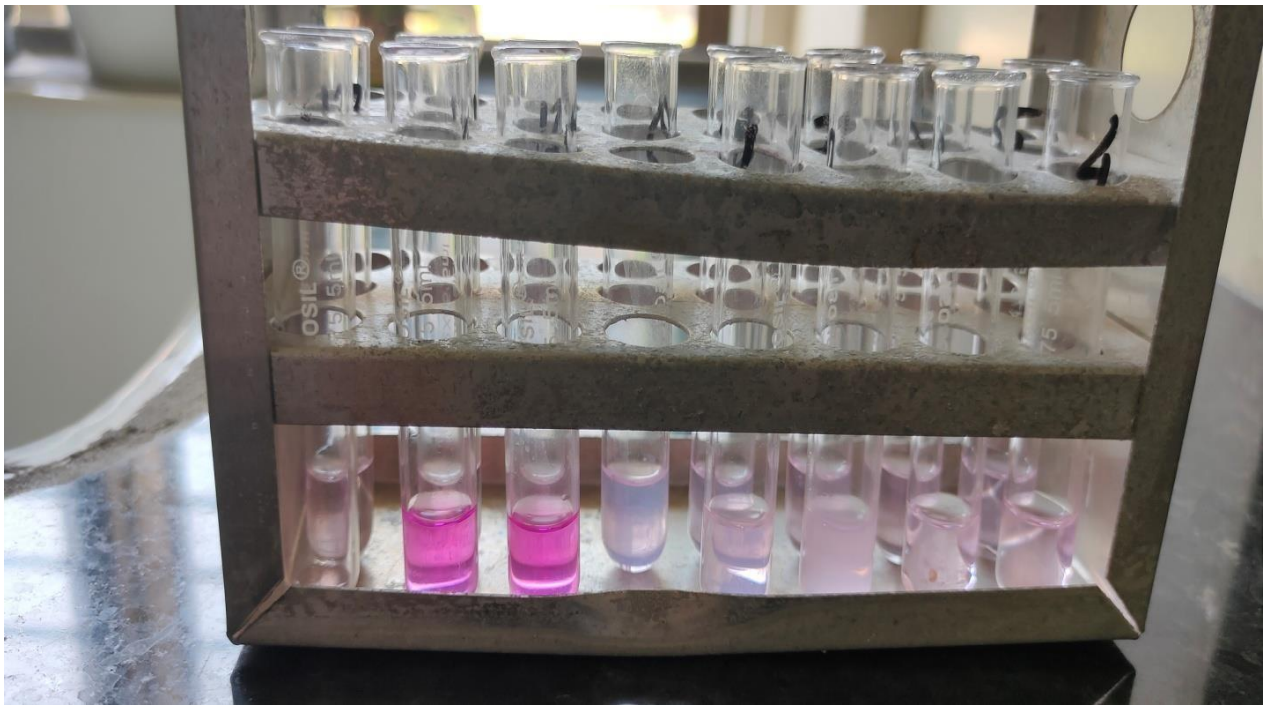
Photograph 1: UV-Spectrophotometer



Photograph 2: ELISA Reader and Centrifuge Machine

Photograph 3: The Biochemical Analysis of Nitric Oxide

Photograph 4: The Molecular Analysis of the samples



Photograph 5: The Process of MDA Analysis

ANNEXURE-VI

SIMILARITY CHECK CERTIFICATE

ORIGINALITY REPORT

5%	1%	4%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Vitamin D, 2010. Publication	2%
2	M. Iftekhar Ullah, Gabriel I. Uwaifo, William C. Nicholas, Christian A. Koch. "Does Vitamin D Deficiency Cause Hypertension? Current Evidence from Clinical Studies and Potential Mechanisms", International Journal of Endocrinology, 2010 Publication	1%
3	Thomas R Gildea, Stacey DaCosta Byfield, D Kyle Hogarth, David S Wilson, Curtis C Quinn. "A retrospective analysis of delays in the diagnosis of lung cancer and associated costs", ClinicoEconomics and Outcomes Research, 2017 Publication	1%
4	Submitted to Manipal University Student Paper	1%
5	cgmj.cgu.edu.tw Internet Source	1%