A STUDY OF PLASMA VITAMIN D LEVELS AS A RISK FACTOR IN PRIMARY HYPERTENSION: A CASE CONTROL STUDY.

By

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Dissertation submitted to

BLDE (Deemed to be University), VIJAYAPURA

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IN

GENERAL MEDICINE

Under the guidance of

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Dr. SHIVARAJ REDDY.K

LIST OF ABBREVATIONS USED

%	-	Percentage
µg	-	Microgram
ng/ml	-	Nano gram per milli liter
mol/l	-	Mole per liter
ml	-	Milli liter
<	-	Less than
>	-	Greater than
sr	-	Serum
mmHG	-	Millimeters of mercury
IU	-	International unit
UV	-	Ultra violet
AT 1	-	Angiotensin 1 receptor
ARB	-	Angiotensin receptor blocker
ACE	-	Angiotensin converting enzyme
BP	-	Blood pressure
BMI	-	Body mass index
BMD	-	Bone mineral density
Ca	-	Calcium
CVD	-	Cardiovascular disease
CKD	-	Chronic kidney disesse
CT	-	Computed tomography
CSF	-	Cerebrospinal fluid
cAMP	-	Cyclic adenosine monophosphate
CHF	-	Congestive heart failure
DM	-	Diabetes mellitus
DNA	-	Deoxyribonuclic acid
d	-	Margin of error
EH	-	Essential hypertension
ECFV	-	Extra cellular fluid volume
ENaC	-	Benzamil blockable epithelial Na blocker
ESRD	-	End stage renal disease
HTN	-	Hypertension

HPLC	-	High pressure liquid chromatography
HR	-	Hazard ratio
ISI	-	Insulin sensitivity index
IL	-	Interlukin
IML	-	Intermedio lateral
K	-	Potassium
LDL	-	Low density lipoprotein
LC-MS	-	Liquid chromatography coupled with tandem mass spectrometry
MS	-	Metabolic syndrome
metab	-	Metabolism
MED	-	Minimal erythemal dose
MAP	-	Mean arterial pressure
MR	-	Mineralocorticoid receptor
MI	-	Miocardial infarction
MnPO	-	Mean preoptic nucleus
Nacl	-	Sodium chloride
NHANES	-	National health and nutrition examination survey
NO	-	Nitric oxide
OVLT	-	Organum vasculosum laminae terminalis
OR	-	Odds ratio
PTH	-	Parathyroid harmone
pPVN	-	Paraventricular nucleus
PAD	-	Peripheral artery disease
P	-	Prevelance rate
RAS	-	Renin angiotensin system
RIA	-	Radioimmuno assy
RAAS	-	Renin angiotensin-aldosterone system
ROS	-	Reactive oxygen species
ROC	-	Receiver operating characteristic
SBP	-	Systolic blood pressure
SZA	-	Solar zenith angle
SFO	-	Sub fornical angle
TB	-	Tuberculosis

TF	-	Tissue factor
TNF	-	Tumor necrosis factor
T2DM	-	Type 2 Diabetes mellitus
VIT	-	Vitamin
VDR	-	Vitamin D Receptor
Wt	-	Weight
WHI	-	Women health initiative
WHO	-	World health organisation

ABSTRACT

Background and objectives

Hypertension is the third leading killer disease in the world and is responsible for 1 in every 8 deaths. About 1 billion people are affected by hypertension worldwide.(1) There is strong positive and continuous correlation between BP and the risk of cardiovascular disease (myocardial infarction, heart failure), renal disease, stroke and mortality. The present was aimed to find low plasma vitamin D levels are the risk factors for primary hypertension or not.

Methodology

This was a case control study carried out in 100 patients, 50 controls and 50 cases with primary hypertension, aged between 18 to 60 years, from October 2016 to august 2018, admitted in BLDE (Deemed to be university) Shri B.M.Patil hospital.

Results

The number of patients in both the groups was predominantly female (52%) respectively while male patients constituted 48% of the study population. The mean systolic blood pressure (SBP) value in controls was significantly lower as compared to cases (116.8 ± 6.7 vs. 155.0 ± 8.4 mmHg). The mean diastolic blood pressure (DBP) values in controls was significantly lower as compared to cases (74.5 ± 4.8 vs. 87.6 ± 5.2 mmHg). The mean Vitamin D level in controls was higher as compared to cases (21.2± 11.5 vs. 18.0 ± 6.3ng/ml). However there was no significant difference between the groups as per Student t-test ($p>0.05$).

Conclusion and Interpretation

In controls, 8% patients had Vitamin D deficiency while 24% patients had Vitamin D sufficiency. In cases, 10% patients had Vitamin D deficiency while 84% patients had Vitamin D insufficiency. The systolic and diastolic blood pressure values were significantly higher in cases as compared to controls in Vitamin D deficient, insufficient and sufficient patients

Key words

Primary Hypertension, Vitamin D.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

Hypertension is the third leading killer disease in the world and is responsible for 1 in every 8 deaths. About 1 billion people are affected by hypertension worldwide.[1] Hypertension is a major public health problem in India and other countries as well. There is strong positive and continuous correlation between BP and the risk of cardiovascular disease (myocardial infarction, heart failure), renal disease, stroke and mortality. Hyperuricemia predicts mortality in patients with heart failure or coronary heart disease, cerebrovascular events in individuals with diabetes and cardiac ischemia in hypertension.[2]

Hypertension, defined as a systolic blood pressure ≥140 mmHg and/or a diastolic pressure ≥90 mmHg, is one of the most common chronic diseases. The overall hypertension prevalence among the adult population was estimated at 26.4% in 2000;[3] moreover it has been reported that this prevalence increased from 23.9%, in 1994, to 29.0%, in 2008, in the USA;[4] from 25.0%, in 1993, to 43.2%, in 2006, in Mexico;[5] and from 15.3%, in 1995, to 24.5%, in 2005, in Canada[6] among other countries. "From this prevalence, it is evident that hypertension is a very important public health challenge because its complications, including cardiovascular, cerebrovascular, and renal diseases, are major causes of morbidity and mortality. Reducing blood pressure in individuals with hypertension prevents or attenuates these complications.[7],[8]"

"Hypertension is due to specific causes in a small fraction of cases, but in the vast majority of individuals (≈90-95%), its etiology cannot be determined; therefore, the essential hypertension term is employed.[7],[9]" "Essential hypertension is currently understood as a multifactorial disease arising from the combined action of many genetic, environmental, and behavioral factors. Given the multifactorial nature of

blood pressure homeostasis, any change in blood pressure as, for example, one due to a mutation, is likely to be compensated by feedback, complementary action, or change, in some other control mechanisms, in an effort to return blood pressure to normal. It is only when the balance between the factor(s) that tend to increase the blood pressure and those that try to normalize it is sufficiently disturbed, when the compensatory mechanisms fail to counteract the perturbation, that essential hypertension results.[10]" "A century of epidemiological, clinical, and physiological research in humans and animals has provided remarkable insights on the relationships existing between dietary salt (Nacl), renal sodium handling, and blood pressure. The evidence points to a causal link between a chronically high salt intake and the development of hypertension, when the kidneys are unable to excrete the ingested amount of sodium unless blood pressure is increased.[11–13]" In conjunction with this primary causal factor, a number of adjunctive factors, such as obesity, diabetes, aging, emotional stress, sedentary life style, and low K intake, may increase the probability of developing hypertension.[14] Hence, on a similar dietary salt, some individuals develop hypertension while others do not; and the probability to develop hypertension depends on the individual's wt of the hypertension's adjunctive factors.

"Vitamin D is a steroid molecule and lipid soluble vitamin, mainly produced by the skin and absorbed from the gut in diet that regulates the expression of a large number of genes. Its main role is in the control of bone metabolism and calcium and phosphorus homeostasis".

"Vitamin D deficiency has been traditionally associated with poor bone growth and development and development of rickets in children and osteoporosis in adults and in recent year's emphasis has been given to the role of vitamin D in areas beyond those traditionally known". "During the last two decades new research and

data is showing that vitamin D could be a risk factor in many chronic diseases like hypertension, diabetes mellitus, dyslipidemia, CVD, some cancers, auto immune disease and TB".

Vitamin D deficiency, defined as a plasma 25-hydroxyvitamin D3 (25(OH) D) level under 20 ng/mL, is highly prevalent with an incidence of about 30-50% in all over of the world.[15–17] A low level of vitamin D is linked to the increased risk of cardiovascular diseases (CVD) and mortality.[16],[18–20]

Currently, the potential effect of vitamin D on the cardiovascular system has been elucidated. Moreover, the association of vitamin D deficiency and the incidence of thromboembolism is described in various studies.[21–24]

The main suggested mechanisms for anti-thrombotic properties of vitamin D including "up-regulation of thrombomodulin"[23][25][26] and "down-regulation of tissue factor" (TF).[23][26]

Adiposity, lack of physical activity and excessive salt intake are some of the best-known environmental factors associated with hypertension. In recent years, yet another cause has been postulated: vitamin D deficiency.[27–31] Vitamin D is a key player in calcium homeostasis, in maintaining optimal bone metabolism and reducing fracture risk.[32] Several studies indicate that vitamin D also seems to play a protective role against the development of hypertension.[30][33]

"A wealth of observational data has demonstrated relationships between circulating vitamin D metabolite levels and blood pressure (BP). Lower 25-hydroxyvitamin D (25OHD) levels are associated with higher BP levels in cross-sectional studies[34][35]" "and with increased rates of incident hypertension.[36]" "Such observations are underpinned by a number of biologically plausible mechanisms and the fact that vitamin D receptors are found on endothelial cells, smooth muscle cells,

and myocytes.[37]" "Vitamin D has been shown to improve endothelial function in some studies,[38][39]" reduce the production of proinflammatory cytokines,[40]" "reduce activity of the renin-angiotension-aldosterone system, and reduce parathyroid hormone (PTH) levels.[41]" "Parathyroid hormone has been posited as vasculotoxic in its own right. Any or all of these mechanisms therefore potentially mediate an effect of vitamin D on BP levels".

"Intervention studies to date have produced conflicting evidence on the BP-lowering effect of vitamin D. One previous meta-analysis[42]" "based on a number of small trials demonstrated a modest but significant decrease in BP in studies in which the mean BP reading was elevated at baseline; another meta-analysis[43]" "conducted at a similar time did not demonstrate a significant effect of vitamin D supplementation on BP; and a more recent meta-analysis[44]" "showed a small decrease in diastolic BP (DBP) but not systolic BP (SBP). Furthermore, selected subgroups (eg, nonwhite populations and those with very low 25OHD levels) could benefit to a greater extent, potentially making vitamin D part of the therapeutic armamentarium in treating individuals with hypertension".

Studies in India have demonstrated the low levels of vitamin D in the Indian population and hypertension, diabetes, vascular disease show high incidence and prevalence in India.[45]

Hence the present study was done at our hospital to assess the relationship between plasma levels of vitamin D and hypertension in this part of our country. Based on this observatation further prospective studies can be taken up on the role of supplementation of vitamin D3 and reduction in blood pressure in hypertensive patients.

AIMS AND OBJECTIVES

1. To determine the plasma vitamin D levels in Primary hypertensive patients
2. To assess low plasma vitamin D levels are the risk factors for primary hypertension or not.

REVIEW OF LITERATURE

Lipid soluble vitamins are non-polar hydrophobic molecules which are all isoprene derivatives, they generally cannot be synthesized by the body in adequate amounts and therefore must be supplied by diet. They are absorbed efficiently in fat diet and once absorbed are bound to lipoproteins or specific binding proteins and transported to the target organs. The important lipid soluble vitamins are vitamin A, vitamin D, vitamin E and vitamin K Vitamin D is in reality a steroid pro hormone. It is represented by steroids that occur in animal's plants and yeast which by various metabolic changes in the body give rise to a hormone known as calcitriol which plays a central role in calcium and phosphorous metabolism.

Historical Aspects

Vitamin D

Cod liver oil was discovered by American researchers Elmer McColloum and Marguerite Davis in 1914[46] later called as vit A. Cod liver oil dint develop rickets when fed to dogs was noticed by British doctor Edward mellanby and concluded vit A. Elmer McCollum in 1922 tested modified cod liver which cured sick dogs, and called it as vit D as it was 4th vitamin to be named.

Alfred Fabian Hess in 1925 showed 7-dehydrocholestrol is irradiated with light and a fat soluble vitamin is produced and stated ''light equals vitamin D''.[47] In 1928 Adolf Windaus got Noble price for his work on sterols and vitamins connection.[48] A paper was published by Otto Rosenheim and Harold king in 1932 on structures of sterols and bile acids.[49] Isolation and characterization of vitamin D was led by Robert Benedict Bourdillon,Otto Rosenheim, Harold King, Kenneth Callow.[50] In the 1930s, Windaus clarified further the chemical structure of vitamin D.[51]

In 1923, American biochemist Harry Steenbock at the University of Wisconsin demonstrated that irradiation by ultraviolet light increased the vitamin D content of foods and other organic materials. After irradiating rodent food, Steenbock discovered the rodents were cured of rickets. A vitamin D deficiency is a known cause of rickets.

In the liver, vitamin D was found to be converted to calcifediol. Calcifediol is then converted by the kidneys to calcitriol, the biologically active form of vitamin D. Calcitriol circulates as a hormone in the blood, regulating the concentration of calcium and phosphate in the bloodstream and promoting the healthy growth and remodeling of bone. The vitamin D metabolites, calcifediol and calcitriol, were identified by competing teams led by Michael F. Holick in the laboratory of Hector DeLuca and by Tony Norman and colleagues.(52)

CHEMISTRY OF VITAMIN D

Vitamin D is a secosteroid molecule. It is not classified as an essential nutrient as it is produced in abundance by the skin on exposure to sunlight. It functions as a hormone in the body to play a key role in the overall metabolism of the bony skeleton. Its structure is similar to that of cholesterol. It differs from cholesterol by presence of double bonds between C-7, C-8 and C-19 and an open ring structure. The two forms of vitamin D utilized in the human body D2 and D3 begin with four intact rings. The whole body half- life of vitamin D3 molecule is 62days. This estimate is based on studies of radioactively labeled vitamin D3.

SYNTHESIS OF VITAMIN D

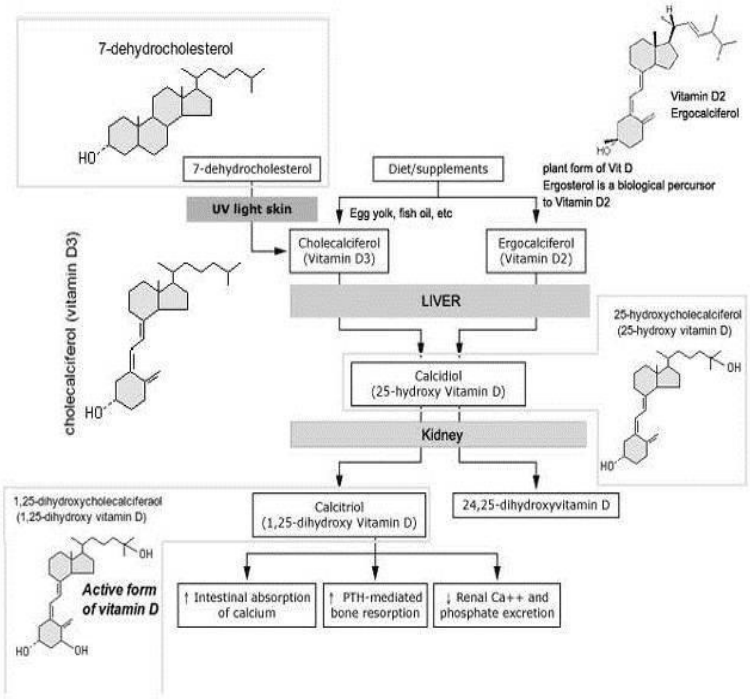
Ergosterol occurs in plants and invertebrate animals and 7-dehydrocholesterol occurs in vertebrate animals. Ergosterol differs from 7-dehydrocholesterol only in its side chain which is unsaturated and contains an extra methyl group. Vitamin D is

generated from the pro-vitamin Dehydrocholesterol by the action of sunlight. Ultraviolet B (UV-B 290-310nm) irradiation cleaves the B ring of both compounds by breaking the bond between C-9 and C-10 of B ring following which a double bond is formed between C-10 and C-19. In plants ergocalciferol (vitamin D2) is formed and in animals cholecaliciferol (vitamin D3) is formed from sunlight and vitamins D2 and D3 are of equal potency.

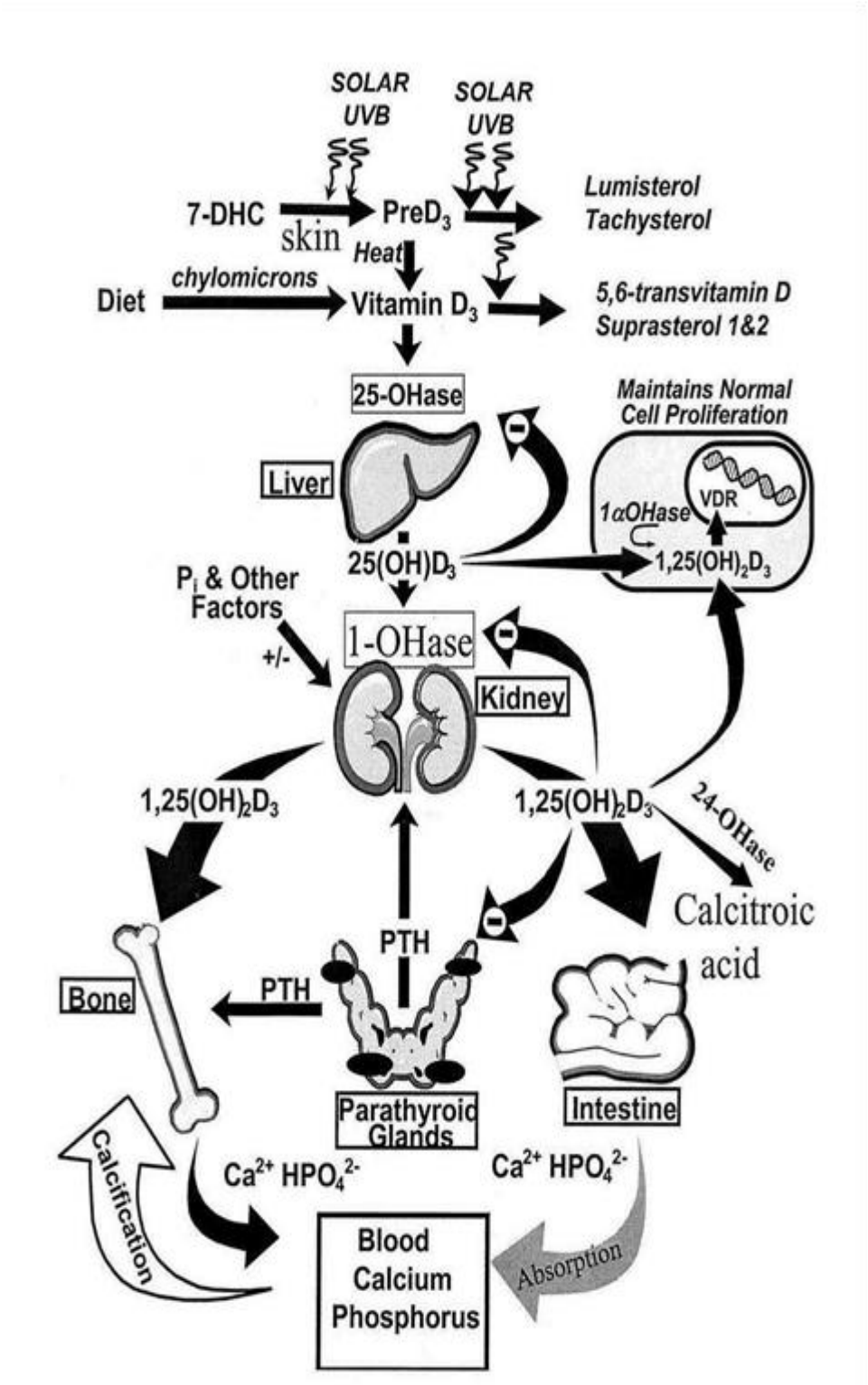
Dietary vitamin D2 or D3 absorbed from the gut in micelles and transported in to the lymphatic after incorporation into chylomicrons and then enters into systemic circulation where it is bound to vitamin D binding protein (glycoprotein). This complex is taken up by the liver where vitamin D3 is hydroxylated on the 25 position by vitamin D3-25-hydroxylase, this enzyme present in the endoplasmic reticulum of hepatocytes.

25-hydroxyvitamin D3 (calcidiol) is the major circulatory form and storage form. It is stored in the liver and to an lesser extent in skeletal muscle and adipose tissue. A significant fraction of 25-hydroxy vitamin D3 undergoes enterohepatic circulation and disturbances of this process can lead to deficiency. This form is biologically inactive. "It is the most important and reliable indicator of vitamin D sufficiency".

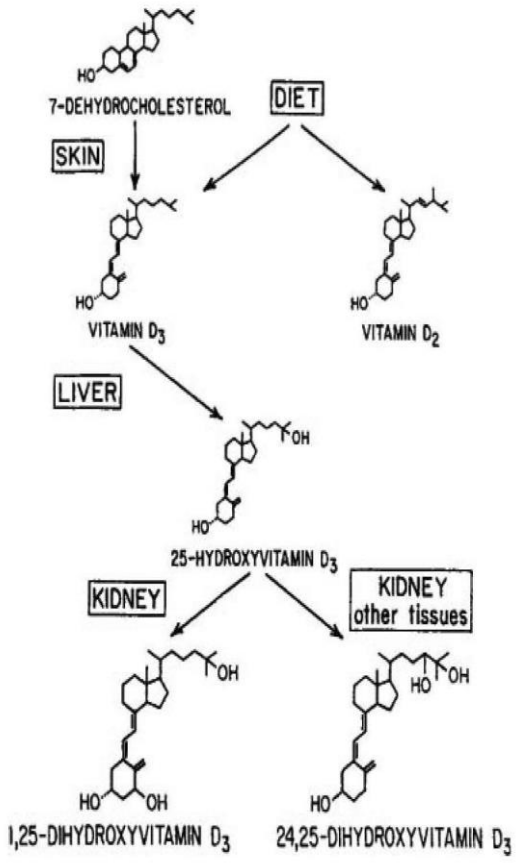
The circulating form in the renal tubules undergoes hydroxylation at position 1 by 25- hydroxyvitamin D3-1-hydroxylase to form 1alpha,25-dihdroxyvitamin D3 (calcitriol). This enzyme is a mitochondrial enzyme present in the renal cells. This step is considered to be a rate limiting step. This second hydroxylation can also occur in bone and placenta to a lesser extent. Calitriol is the most potent vitamin D metabolite.



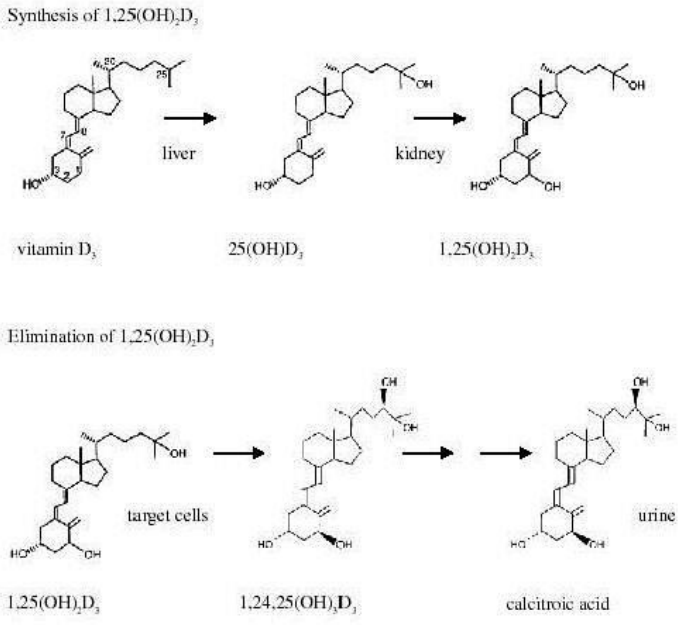
VITAMIND CYCLE- 1



VITAMIN D CYCLE- 2



VITAMIN D CYCLE- 3



SYNTHESIS AND ELIMINATION OF VITAMIN D

CLASSIFICATION AND FORMS OF VITAMIN D

"All though 5 forms of vitamin D are known (D1 to D5) D2 and D3 are the most studied forms. The two major forms are vitamin D2 or ergocalciferol, and vitamin D3 Or cholecalciferol, vitamin D without a subscript refers to either D2 or D3 or both".

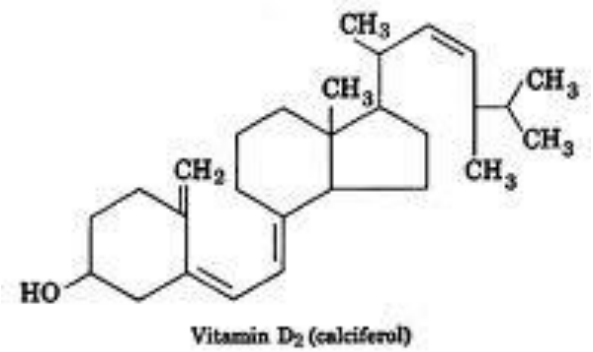
"These are known collectively as calciferol. Vitamin D2 was chemically characterized in 1932". "In 1936, the chemical structure of vitamin D3 was established and proven to result from the ultraviolet radiation of 7-dehydrocholesterol".

"Chemically, the various forms of vitamin D are secosteroids; i.e., steroids in which one of the bonds in the steroid rings is broken". "The structural difference between vitamin D2 and vitamin D3 is in their side chains. The side chain of D2 contains a double bond between carbons 22 and 23, and a methly group on carbon". "Vitamin D3 (cholecalciferol) is produced by ultraviolet radiation (UV) of its precursor 7- dehydrocholesterol. This molecule occurs" naturally in the skin of animals and in milk. Vitamin D3 can be made by exposure of the skin to UV, or by exposing milk directly to UV (one commercial method)".

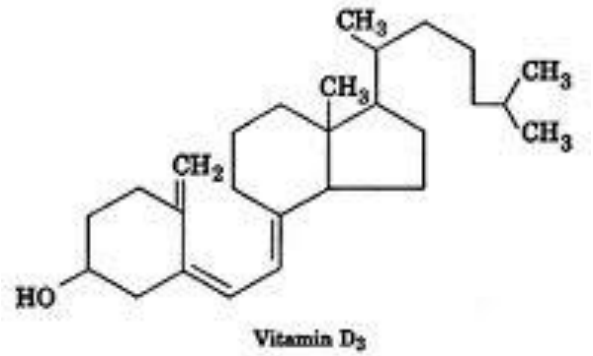
"Vitamin D2 is a derivative of ergosterol, a membrane sterol named for the ergot fungus", "which is produced by some organisms of phytoplankton, invertebrates, yeasts and higher fungi such as mushrooms. The vitamin ergocaliferol (D2) is produced in all of these organisms from ergosterol", "in response to UV irradiation. However, like all forms of vitamin D" it cannot be produced without UV irradiation. "D2 is not produced by land plants or vertebrates, because they lack the precursor ergosterol".

TYPES OF VITAMIN D

CLASS	**COMPOSITION CHEMICAL**	IMPORTANCE
D1	'Combination of ergocaliciferol and lumisterol'	
D2	'Ergocalciferol: made from fungus ergosterol or pre-vitamin D2'	'Made by invertebrates, and plants in response, to Ultraviolet radiation. Not made by vertebrates'
D3	'Cholecalciferol: made from 7-dehydrocholesterol or pre vitamin D3'	'Made in skin as a response to ultraviolet B radiation, after reacting with 7-dehydrocholesterol'
D4	'Dihydroergocalciferol: vitamin D2 without 22,23 double bond'	'Ineffective form of vitamin D'
D5	'Sitocalciferol: made from 7-dehydrositosterol'	'anti-tumor properties'

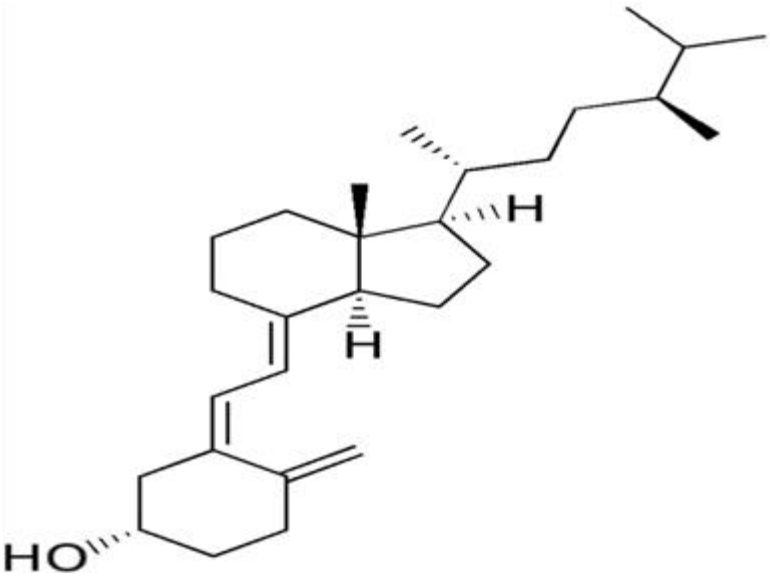


Vitamin D_2 (calciferol)

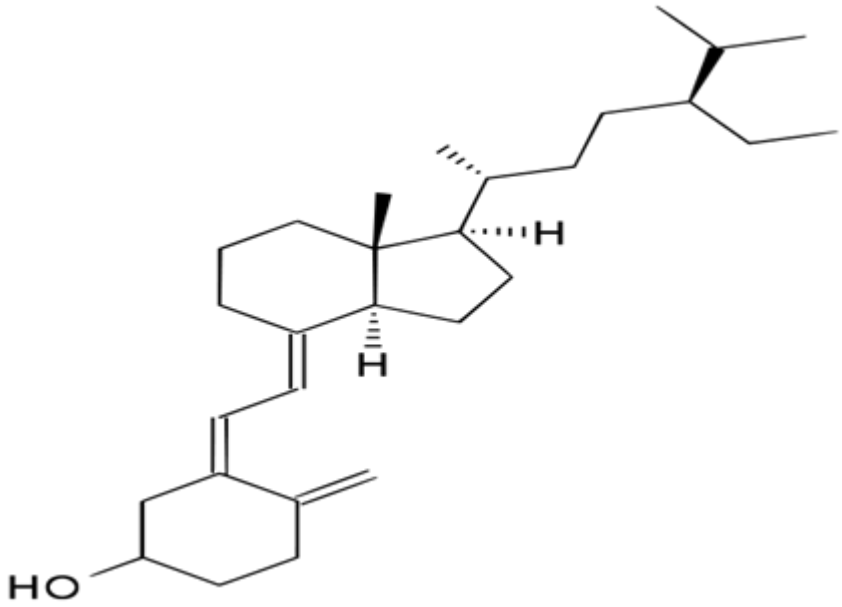


Vitamin D_3

STRUCTURE OF VITAMIN D2 AND D3



STRUCTURE OF VITAMIN D4



STRUCTURE OF VITAMIN D5

SOURCES OF VITAMIN D

Vitamin D3 which is synthesized cutaneously by light exposure is the major source for humans.(80-90%). 'Total body sun exposure to 1 minimal erythema dose while bathing suit provides equivalent of 250-500 ug (10000-20000 IU) of vitamin D per day [3].' When compared to skin formation dietary supply of vitamin D is minor. Fortified foods gives 5-10ug of vitamin D dialy and ordinary food gives 2.5ug vitamin D per day. Oily fish such as salmon. Mackerel, herring and sardines are rich sources of vitamin D. Other sources include egg yolk, butter, liver and fish liver oil. Fortified milk and Juices available in developed countries provide ~100 units per 8-oz serving.[53]

IMPORTANT VITAMIN D SOURCES

Source	Type	Weight in Gm	Weight in oz	Vitamin D IU	Vitamin D in IU/gm
catfish		85	3	425	5
Salmon	Cooked	100	3.5	360	3.6
Mackerel	Cooked	100	3.5	345	3.45
Sardines	Drained	50	1.75	250	5
Tuna	Canned	100	3.5	235	2.35
Eel	Cooked	100	3.5	200	2
Whole egg	Boiled	60		20	0.33
Beef liver	Cooked	100	3.5	15	15
Fish liver oil		1Tbs (15ml)		1360	90.6IU/ml

VITAMIN D RECEPTORS

Vitamin D receptor (VDR) is binded by active form of Vitamin D (1,25[OH]2 vitamin D).The VDR are intracellular and intra-nuclear receptors. The vitamin D receptor complex is translocted across the nuclear membrane into an intra-nuclear site. Together with several factors and activator, complex attaches to vitamin D-responsive elements in deoxyribonucleic acid (DNA) and alters gene expression[(5)'] and therefore vitamin D is now considered as a gene transcription factor. It regulates transcription of >200 proteins. The presence of various VDR polymorphisms and their abnormalities have been implicated in role of vitamin D in chronic disease.

The American academy of pediatrics increased the recommended minimum daily requirement of vitamin D to 400IU for all infants and children including adolescents in 2008. The national osteoporosis foundation recommends vitamin D 400IU-800IU daily for adults under the age of 50years, and 800-1000IIU daily for adults older than 50years. The North American menopause society

recommends 700-800 IU daily for women at risk of deficiency due to low sun exposure. Guidelines from the osteoporosis society of Canada recommended vitamin D 400 IU daily per day for people up to the age of 50years and 800IU per day for people over 50 years.

Vitamin D: Metabolism

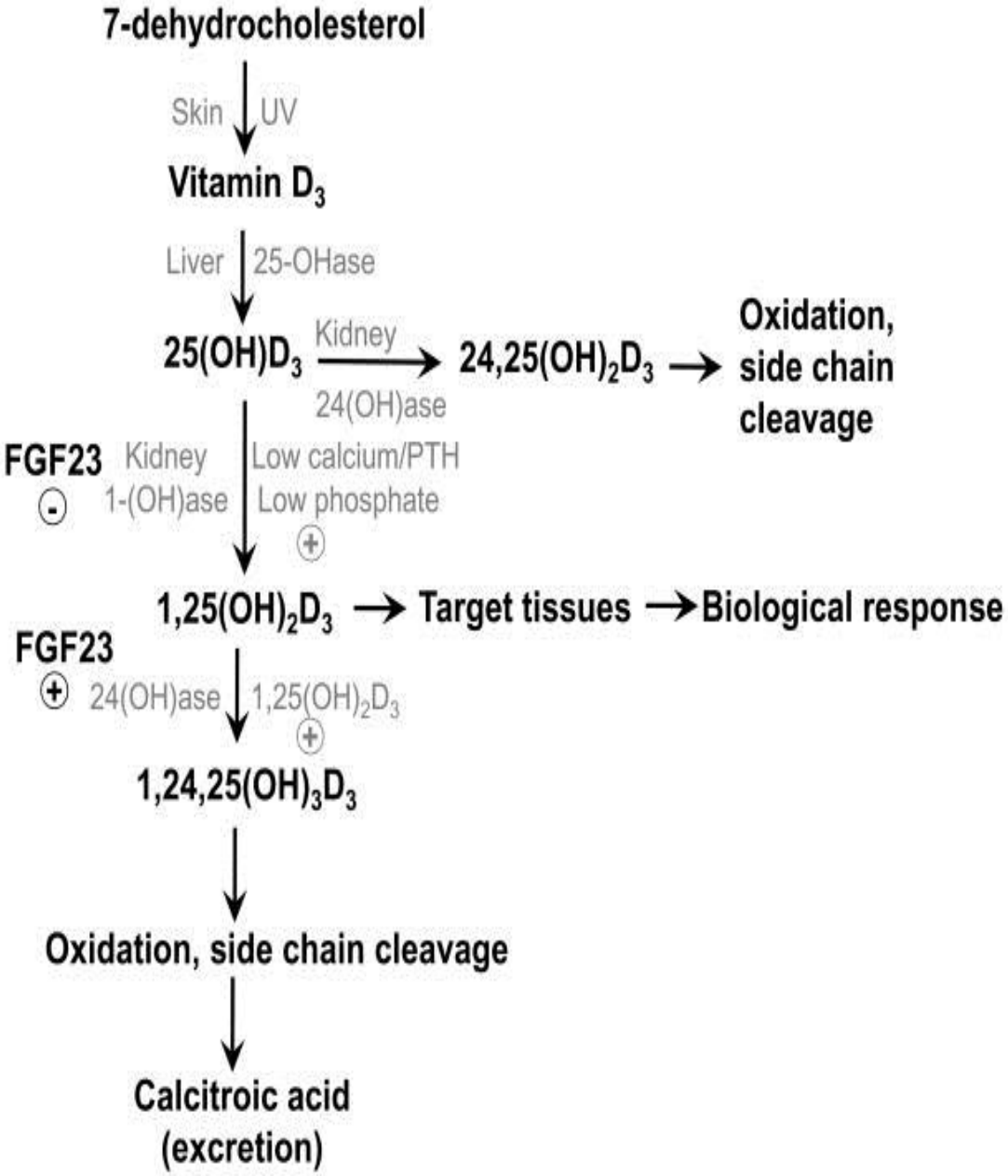
SYNTHESIS OF 1,25(OH)2D3 FROM VITAMIN D3

Vitamin D3 is obtained from diet and from skin by 7-dehydrocholestrol by ultraviolet irradiation.it depends on intensity of uv irradiation and season and latitude varies it.[54] Conversion of vitamin D3 from 7-dehydrocholestrol is prevented by sunscreen lotion and cloths.[55],[56]

Active form of vitamin D can prevent growth of cancer cells and immune mediated disorders.so vitamin d must be converted into active form. Vitamin D binding protein (DBP) transports vitamin d to liver in the blood. In liver 25-hydroxyvitamin D3 is formed by hydroxylation of vitamin D which uses vitamin D 25 hydroxylases such as CYP2R1,CYP2D11,CYP2D25.

25(OH)D3 is transported to kidney by vitamin D binding protein DBP. Internalization of 25(OH)D3 is caused by magalin a member of LDL receptor superfamily.[57]

Active form of vitamin D , 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) is formed in the proximal renal tubule when 25(OH)D3 is hydroxylated in carbon 1 of A ring. Along with rickets reproductive , and immune defects are seen mice.



Vitamin D metabolic pathway

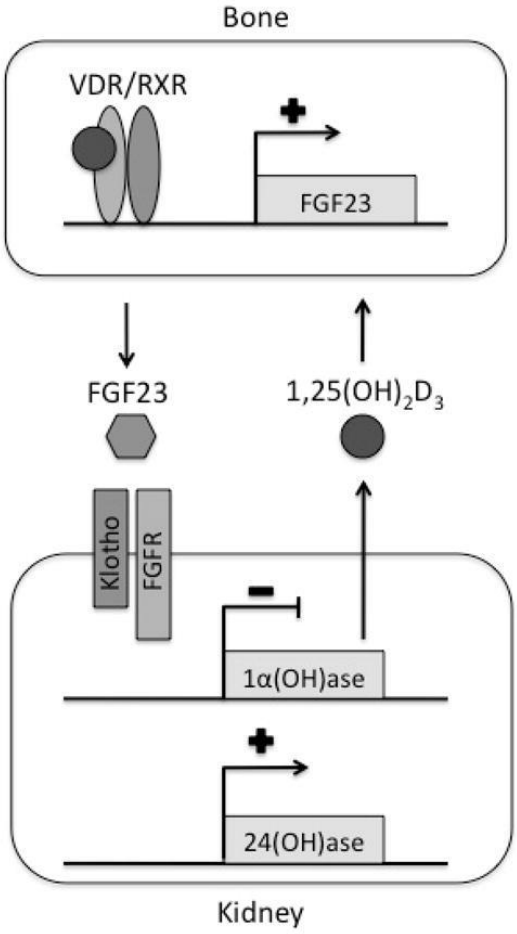
METABOLISM AND ACTION OF VITAMIN D BY VITAMIN D BINDING PROTEIN(DBP).

DBP null mice is important for circulating 1,25(OH)2D3 and does not affect the pool of 1,25(OH)2D3 which is used for vitamin D target proteins synthesis. so biologically active form of 1,25(OH)2D3 is not always reflected by direct measurement of 1,25(OH)2D3.[58]

This may be, in part, why 25(OH)D3, which is also more stable than 1,25(OH)2D3, is used to assess clinical vitamin D status. 1,25(OH)2D3 in tissues have the ability to concentrate vitamin d receptor where serum calcium levels in DBP null mice is maintained. Calcium homeostasis maintenance is done by transcriptional regulation of genes.[59]

24-HYDROXYLASE (24(OH)ase)

1,25(OH)2D3 is the preferred substrate for 24(OH)ase. Catabolism of 1,25(OH)2D3 by 24(OH)ase is first time provided by 24(OH)ase null mutant mice. 1,25(OH)2D3 is not getting cleared from bloodstream by 24(OH)ase null mice mutant even after acute and chronic treatment by 1,25(OH)2D3.[60]



"Vitmin D regulation and hydroxylases by FGF23-Klotho. 1,25(OH)2D3 binds to VDR". "The ligand-bound VDR forms a heterodimer with nuclear retinoid X receptor (RXR) resulting in increased the expression of FGF23 in osteocytes. Secreted FGF23 activates FGFR bound by klotho in renal tubular cells". "FGF signaling suppresses expression of 1α(OH)ase and induces 24(OH)ase thereby inhibiting synthesis and promoting catabolism of 1,25(OH)2D3". "Thus, the FGF23-Klotho results in decreased levels of 1,25(OH)2D3.

Adapted from Kuro-o M (2008) Endocrine FGFs and Klothos": "emerging concepts. Trends Endocrinol Metab 19:239–245"

VITAMIN D AND DISEASE

Vitamin D deficiency is now a globally recognized pandemic. Observational studies have indicated that vitamin D concentration <50nmol/L or 20ng/ml, is an indicator of vitamin deficiency, levels of 51-74nmol/L or 21-29ng/ml is an indicator of insufficiency and concentrations of >75nmol/L or 30ng/ml is considered to be sufficient. These levels are applicable to both children and adults, however no comparative studies are available to quantify the deficiency in children.

Research has highlighted the fact that there is a widespread prevalence of vitamin D deficiency in both sexes in all age group populations in India. There is high prevalence of osteoporosis and osteopenia in the country. A rough estimate would be 3 quarters of adult Indians have vitamin D deficiency.

The main cause of vitamin D deficiency is lack of exposure to sunlight, use of sunscreen, melanin pigmentation, winter, latitude, Mal-absorption and use of medications like glucocorticoids, rifampicin, anti-seizure medication, retroviral therapy, St. John's wort. Vitamin D deficiency in children classically causes rickets and growth retardation. In adults vitamin D deficiency will precipitate and exacerbate both osteopenia and osteoporosis. It has also been associated with proximal myopathy.

“Recent studies have shown association between vitamin D deficiency” and hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, cerebrovascular disease, metabolic syndrome(ms), chronic kidney disease(CKD), cancer, infections and tuberculosis and auto-immune disorders.

VITAMIN D DEFICIENCY IN CHILDREN

Vitamin D deficiency in children causes poor mineralization of the collagen matrix in young children's bones leading to growth retardation and bone deformity known as rickets. The long bones and weight bearing bones are commonly affected. There is a disruption in chondrocyte maturation at the epiphyseal plates, leading to widening of the ends of the long bone and costochondral junctions.

Rickets presents within 1year with abnormal softening of cranial bones – craniotabes, frontal and parietal bossing – hot cross bun appearance, delayed closure of anterior fontanelle, delayed dentation and defective enamel, costochondral junctions are enlarged and beaded – rachitic rosary, pigeon chest, horizontal groove along the attachment of diaphragm due to contraction of the muscle pulling on the softened bony cage, spinal abnormalities, widened epiphysis of wrists and ankles, bending of long bones.

"Pregnant women in India have been shown to have 84% prevalence of vitamin D deficiency" which correlated significantly with serum vitamin D level status of new born". "Mothers with suboptimal vitamin D status have offspring's with reduced intrauterine and postnatal skeletal development. Brooke et al.[61]" "first reported reduced incidence of low birth weight babies in vitamin D supplemented Asian mothers". "Vitamin D and calcium supplementation of pregnant mothers is associated with increased skeletal growth and bone mass in offspring's".

"Marya et al[62] reported higher body weight, crown heel length, head circumference and mid arm circumference in offspring's of mothers who received vitamin D in third trimester of pregnancy to those who did not receive vitamin D".

"Apgar score at birth was higher in newborns of mothers with adequate vitamin D and calcium intake than in newborns whose mothers had inadequate intake".

"Mannion et al.[63] showed that each additional microgram of vitamin D was associated with an 11gm increase in birth weight. Morley et al.[64]" "showed that gestation length was 0.7 week shorter and knee-heel length was 4.3mm smaller in infants of mothers with vitamin D level <11.2/ml at 28-32 weeks when compared to babies whose mothers who had higher vitamin D concentrations".

Marwaha et al[65] showed lower forearm bone density and mean serum vitamin D levels in school children from low socio-economic region as compared to children from high socio- economic region. Shatrugna et al.[66] from national institute of nutrition, Hyderabad, studied the effect of micro-nutrient supplementation in schoolchildren of 6-16 years of age. "After14 months of supplementation, the increments in height, weight", "whole body bone mineral content, and bone mineral density (BMD) at the neck of femur were significantly greater in the supplemented than a placebo group".

VITAMIN DEFICIENCY IN ADULTS

"Vitamin D deficiency in Asian Indians have been shown to be associated with higher serum PTH and lower serum calcium. Such effects would promote bone resorption and precipitate osteomalacia and osteoporosis in predisposed individuals. Shatrugna et al.[67]" "studied the prevalence of osteoporosis in 289 middle aged women from Hyderabad". "The prevalence of osteoporosis at femoral neck was around 29%".

"The T scores in BMD of Indian women studied at all the skeletal sites were much lower than the values reported from developed countries".

Several investigators from the west have reported a significantly lower hip BMD in subjects with low serum vitamin D levels and vitamin D supplementation has led to beneficial effects on hip BMD. "Overall results of various studies in urban and rural India" "indicate that widely prevalent vitamin D deficiency and associated increase in serum PTH and low BMD".

VITAMIN D AND HYPERTENSION

Essential hypertension is a major and significant risk factor for cardiovascular disease. Various pathways like endothelial cell function, renin angiotensin pathway and proliferation of vascular smooth muscle cells are influenced by Vitamin D. Studies have shown pivotal "role of vitamin D in regulation of" hypertension due to increased calcium in intracellular by which renin activity is decreased, renin promoter gene is suppressed by calcitrol which causes changes in endothelial dysfunction.[68]

"Cyclic AMP is long known to be a major intracellular signal that stimulates renin production in the" juxtaglomerular cells. "Intracellular cAMP is thought to be critically involved in the stimulation of renin expression by sympathetic nerve activity" or by low tubular sodium chloride concentration. "It is hypothesized that by targeting the cAMP signaling pathway", 1, 25-dihydroxy "vitamin D may function as a gatekeeper to counterbalance the other renin stimulating factors and prevent the detrimental overproduction of renin".

Vitamin D regulation of renin expression was independent of calcium metabolism and vitamin D markedly suppressed renin transcription by VDR mediated mechanism in cell cultures. The stimulatory effect of vitamin D deficiency on RAS may induce BP independent of angiotensin mediated inflammatory responses and vascular growth

Vitamin D may also exert its effects on vascular structure and function independent of the RAS. It has been shown to mediate endothelium-dependent vasodilation by increasing expression of vascular endothelial growth factor that can upregulate nitric oxide synthase and produce vasodilation by prostacyclin production and by reducing endothelium- dependent vascular smooth muscle cell contractions by reducing influx of calcium in to the endothelial cells. Short term exposure to parathormone can produce vasodilation, but long term exposure is associated with elevation of BP. Parathormone may induce hypertension by increasing intracellular calcium concentration, blunting endothelial function and stimulating vascular growth.

In a study by Li et al[69] people with hypertension had a good response to RAS antagonists as RAS is inhibited by vitamin D which was lacking in mice. The inhibition of 1,25(OH)vitamin D synthesis led to an increase in renin expression, whereas, 1, 25(OH) vitamin D injections led to renin suppression. Resnick et al[68] 25 years ago showed an inverse association between low vitamin D levels and plasma renin activity and hypertension in patients.

In a recent study by Tomaschitz et al[70] there is inverse relation between both 1,25(OH)D and 25(OH)D with plasma renin and angiotensin 2 by cohort for coronary angiography. A evaluation was done on patients on their medications and sodium diet including anti-hypertensive drugs which showed increased renal vascular RAS activity and angiotensin 2 concentrations due to deficiency of 25(OH)D.

Epidemiological observations like incidence of hypertension increasing with higher latitude, higher recordings of blood pressure in winter months and racial and ethnic differences in 25(OH) vitamin D levels led to much larger interest and studies in correlation between vitamin D and hypertension. It has been shown

that for each 100 north or south of the equator BP increases by 2.5mm of Hg and prevalence of hypertension increases by 2.5%. Initial small retrospective observational studies have shown a significant inverse correlation between vitamin D and systolic blood pressure. Duprez et al[71] in a small study in Belgium conducted on 25 hypertensive patients demonstrated that vitamin D levels inversely correlated with systolic blood pressure, "diastolic blood pressure and calf vascular resistance".

When compared to vitamin D sufficient population (>75nmol/L) there is increased risk of hypertension by 6.13 in men and 2.67 in women in lower serum 25(OH)vitamin D levels of 15 ng/ml in a study conducted by Forman et al involving 613 men from health professionals follow up study and 1198 women from nurses health study. The authors concluded that plasma 25(OH) vitamin D levels were inversely associated with a risk of incident hypertension. This study was followed up by another study by forman et al which was "prospective study in 1448 women demonstrated a 2.21 fold increase in incident hypertension in hypovitaminosis D group versus control groups".[72]

A cross-sectional study by Jorde et al[73] "conducted on 4125 subjects showed a significant association between hypovitaminosis D and hypertension" however when the study was extended into a prospective study did not show any increase in incidence among the case and control groups.

Sugden et al.[36] tested whether a single large dose of vitamin D can improve endothelial function in patients with diabetes mellitus, who also had low serum 25(OH) vitamin D levels. Brachial artery flow mediated vasodilation is improved by 2.3% due to supplementation of Vitamin D. Independent of this effect, supplementation also significantly reduced systolic blood pressure by 13mm of Hg ($p=0.02$) when compared to placebo.

In a randomized controlled trial conducted by Pfeifer et al on "148 elderly German women, demonstrated that a modest amounts of vitamin D (400IU) with calcium given over 8 week period significantly reduced systolic blood pressure (SBP) by" 9%.[74] The study found that compared with calcium, supplementation with vitamin D and calcium resulted in an increase in serum 25(OH) vitamin D of 72%, decrease in serum parathyroid hormone by 17%, a decrease in SBP by 9.3% and decrease in heart rate by 5.4%

Krause et al[75] conducted a study in 18 patients with stage 1 hypertension where he randomized them to ultraviolet A exposure to skin which does not produce vitamin D and to ultraviolet B which produces vitamin D, the study demonstrated that systolic and diastolic blood pressure significantly decreased after 6 weeks of therapy in those subjects receiving ultraviolet B therapy suggesting that cutaneously produced vitamin D resulted in lowering blood pressure.

However the largest trial to date the women`s health initiative (WHI) done on a population of non-hypertensive at baseline failed to show any significant impact of a small dose of vitamin D (400IU) with calcium 1000mg/day on systolic blood pressure or diastolic blood pressure after a mean follow up of 7 years in post - menopausal women. However, the authors said the lack of effect may have been due to the low doses of vitamin D used and poor adherence (59%) to the medication.

VITAMIN D AND DIABETES MELLITUS

Diabetes mellitus is a worldwide pandemic with high prevalence in both urban and rural India. It is estimated that by 2030 Indians would bear the maximum brunt of the disease worldwide. Vitamin D is thought to play some role in insulin secretions as 1,24(OH) vitamin D receptors have been identified in pancreatic beta cells, suggesting that vitamin D may play a role in insulin secretion and subsequent control of blood

glucose levels and insulin sensitivity. The possible mechanisms involved in the role of vitamin D and its deficiency on glucose homeostasis[76] are immunomodulatory effects by reducing tumor necrosis factor-alpha, interleukin-10 and parathyroid hormone, decreased insulin receptor expression, leading to increased peripheral resistance of insulin, its effect of intracellular calcium levels leading to decreased insulin secretion and pancreatic beta cell dysfunction.

The presence of specific vitamin D receptors on pancreatic beta cells, the expression of 1-alpha-hydroxylase enzyme on pancreatic beta cells, which catalyzes the conversion of 25(OH) vitamin D to 1, 25(OH) vitamin D may play a role in insulin secretion. Basal insulin secretion rate was not altered in VDR- knock out mice but insulin secretion rate after challenge with glucose diet was impaired in vitamin D deficiency. Vitamin D affects the intracellular calcium levels which is an important stimulus for insulin secretion. The presence of vitamin D responsive element in the human insulin gene promoter in beta cells may play a role in reducing beta cell dysfunction and aging. The presence of vitamin D receptors in skeletal muscle and adipose tissue may play a role in expression of insulin receptors via the peroxisome proliferative-activated receptors and insulin responsiveness for glucose transport.

Knekt et al.[77] conducted two nested case-control studies to analyze the relation of serum vitamin D with T2DM incidence. This analysis found an inverse association between age-adjusted serum vitamin D levels and incidence of type 2 diabetes mellitus in pooled population of individuals. Men had higher serum vitamin D concentrations than women in a pooled cohort and showed a reduced risk of diabetes mellitus in their highest vitamin D quartile. This study reinforced the perception that high vitamin D status provides protection against type 2 diabetes

mellitus Supplementation of vitamin D has been linked with prevention of increase of glycaemia and insulin resistance in healthy subjects without type 2 diabetes mellitus.

VITAMIN D AND CARDIOVASCULAR DISEASE

Coronary heart disease continues to be the number one cause of mortality worldwide. The estimated prevalence of coronary heart disease in India is high around 8-10% and is much higher in urban India when compared to rural India.. "Endothelial dysfunction is a hallmark of many vascular diseases including cardiovascular disease. Vitamin D deficiency may cause endothelial dysfunction by itself ,or by its significant correlation with its risk factors like hypertension, diabetes mellitus and dyslipidemia.

"Vitamin D deficiency is also related to increased activity of renin-angiotensin- aldosterone system, cardiac contractility, vascular tone, cardiac collagen content and cardiac tissue maturation and chronic sub-acute inflammation with increase in pro- inflammatory cytokines, loss of suppression of anti-inflammatory cytokine IL-10 and loss of suppression of foam cell formation thereby, promoting atherosclerosis". Low vitamin D level is associated with loss of inhibition by VDR mediated G1a matrix protein suppression which results in vascular calcification. Raised PTH levels is associated with calcification of myocardium and blood vessels.

Vitamin D sufficient state has shown decrease endothelial dysfunction and "decrease vascular calcification and calcification of coronary arteries inversely correlated to vitamin D levels". "Earlier observations in the 1980s and 1990s found geographic and seasonal differences in mortality form ischemic heart disease". "The initial suggestion of vitamin D as risk factor came from a study in United Kingdom

showing that mortality from Ischemic heart disease was inversely proportional to the Hours of sunlight".

In a laboratory study conducted by Tarcin O et al.[39] 1,25(OH)vitamin D suppressed foam cell formation by reducing acetylated or oxidized low density lipoprotein (LDL) cholesterol uptake in diabetic subjects. The deletion of vitamin D receptor in macrophages from diabetic patients accelerated foam cell formation induced by modified LDL and prevented oxidized LDL-derived cholesterol uptake. This showed that reduced vitamin D receptor signaling is a potential mechanism leading to accelerated cardiovascular disease in diabetic patients.

VITAMIN D AND RENAL DISEASE

Low levels of vitamin D were associated with albuminuria in a cross-sectional study of NHANES. Vitamin D supplementation in some studies showed a decrease in proteinuria. The selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes mellitus (VITAL) study has shown that the addition of vitamin D receptor activator – paricalcitol to therapy in patients with diabetic nephropathy reduces. Albuminuria without the risk of hypercalcaemia or hypophosphatemia and calcification of diabetic vessels.[78]

VITAMIN D AND TOXICITY

Oral supplementation of Vitamin D for prolonged massive doses causes Vitamin D toxicity. In healthy adults, sustained intake of more than 1250 micrograms/day (50,000 IU) can produce overt toxicity after several months; those with certain medical conditions such as primary hyperthyroidism are far more sensitive to vitamin D and develop hypercalcemia in response to any increase in vitamin D nutrition, while maternal hypercalcemia during pregnancy may increase

fetal sensitivity to effects of vitamin D and lead to a syndrome of mental retardation and facial deformities.

For infants (birth to 12 months), the tolerable upper limit (maximum amount that can be tolerated without harm) is set at 25 micrograms/day (1000 IU). 1000 micrograms (40,000 IU) per day in infants can produce toxicity within one month. After being commissioned by the Canadian and American governments, the Institute of Medicine (IOM) as of 30 November 2010, has increased the tolerable upper limit (UL) to 2500 IU per day for ages 1–3 years, 3000 IU per day for ages 4–8 years and 4000 IU per day for ages 9–71+ years (including pregnant or lactating women).

Vitamin D overdose causes hypercalcemia, and the main symptoms of vitamin D overdose are those of hypercalcemia: anorexia, nausea, and vomiting can occur, frequently followed by polyuria, polydipsia, weakness, insomnia, nervousness, pruritus, and, renal failure, proteinuria, urinary casts, azotemia, and metastatic calcification (especially in the kidneys). Vitamin D toxicity is treated by discontinuing vitamin D supplementation and restricting calcium intake. Kidney damage may be irreversible.

In recent years study conducted by alposy n et al among people who have white coat hypertension, sustained hypertension and normotensive found that lower vitamin D is among sustained 'hypertensive patients' as 'compared to' white coat and normotensive Study conducted by macreol et al[79] among people who were newly diagnosed and correlation with vitamin d and arterial stiffness resulting in essential hypertension found inverse correlation between arterial stiffness and vitamin d levels KE L[27] et all per formed meta-analysis of many studies available from early 2014 for relationship between vitamin D and hypertension and found inverse

correlation but commented large multi-centered associations were still required for better analysis.

Chen s et al[80] found that vitamin D repletion exerts a clinically significant antihypertensive effect in vitamin D-deficient EH patients. In a population-based study of 1,441 Peruvian adolescents aged 13-15 years, 1,074 (75%) provided a serum blood sample for 25OHD analysis and BP measurements. Relationships between 25OHD and BP metrics were assessed using multiple linear regressions, adjusted for anthropometrics and sociodemographic factors found that elevated systolic and diastolic BP was associated with low vitamin D levels. Tomianketall[81]

TESTS FOR VITAMIN D

Vitamin D status refers to the estimation of the vitamin stored in our body. The 25-hydroxyvitamin D is widely acknowledged as the best indicator/determinant of the nutritional status of this fat-soluble vitamin. The 25(OH) D directly relates to the total body storage of vitamin D, whereas 1, 25(OH) vitamin D, although active metabolite, correlates to the disorder involving vitamin D endocrine system. Apart from this, estimation of parathromone, serum calcium, and serum phosphorus can also be assessed for a better understanding of the bone status. In the past, the measurement of serum vitamin D was challenging and limited by methodological differences due to Highly lipophilic nature of 25(OH) D which binds strongly with proteins and exists in two structurally different forms, D2 and D3. The methods employed for the measurements of 25(OH) D showed large inconsistency and variability between different laboratories. The rising clinical demand for the assessment of vitamin D has increased the need for a simple, high-throughput methods for measuring 25(OH)D. A

period of evolution was seen in the development of techniques for estimating vitamin D, which is broadly classified into two classes:

- Immunoassay techniques - Radioimmunoassay (RIA) and chemiluminescence immunoassay (CLIA)
- Chromatographic techniques - High pressure liquid chromatography (HPLC) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

The chromatographic methods are also known as fractionated or direct detection that quantify 25-OH D3 and 25-OH D2 independently and add both values to get the total 25-OH D.

Radioimmunoassay (RIA)

RIA, the serum samples containing 25(OH) vitamin D are incubated with a fixed amount 25 OH vitamin D tracer labeled with radioactive iodine (125I). This radio-labeled vitamin D tracer competes with 25 (OH) vitamin D from the samples for a limited number of binding sites on antibodies, which are coated on tubes. The amount of vitamin D in the sample can be estimated from the radiation emitted by the tracer, which is inversely proportional to the amount of vitamin D in the samples.

Chemiluminescence immunoassay (CLIA)

CLIA is a quantitative immunoassay method used for the determination of Total 25 (OH) D in serum or plasma on a fully automated platform. It is a highly sensitive technology in which a specific antibody to vitamin D is used for coating magnetic particles (solid phase) and vitamin D is linked to an isoluminol derivative. During incubation, 25- hydroxyvitamin D dissociates from its binding proteins and competes with labeled vitamin D for binding sites on the antibody. After

incubation, the unbound material is removed with a wash cycle. Subsequently, starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units and is inversely proportional to the concentration of 25-hydroxyvitamin D present in the samples.

It offers an array of advantages as:

- Small sample size
- Highly specific, sensitive, and high reproducible method
- Cost effectiveness

But there are two major drawbacks of the immunoassay test. First, due to its lipophilic nature and low concentration of the 25 (OH) D, the test is difficult to use in the blood spot analysis. Second, if individuals are interested in separate measurements of the 25 (OH) D3, this may not be possible with the DiaSorin system as it simply gives the value of the total 25(OH) D. These limitations were circumvented by the use of newer chromatographic techniques like tandem mass spectrometry (LC-MS/MS) method.

Chromatographic techniques –

High Pressure Liquid Chromatography (HPLC) The HPLC separation works on an isocratic method at 30¬degreeC with a normal phase column. Prior to the separation method, sample preparation is done to get rid of high molecular weight substances by precipitation and solid phase extraction. The chromatograms obtained are detected by a UV detector. The obtained results are Quantified by the delivered serum calibrator and calculated by the external standard method by integration of the peak area. The standard ethanol solution is used for the recognition of peaks.

The main advantages of employing HPLC for determination of 25 (OH) D3 are that it is very specific and a reliable method for determination of the parameter without radioactive substances and that it allows simultaneous handling of many analytes in one test. The larger sample size requirement and high equipment costs, needed for a preparative chromatography and high level of expertise requirement, are some of the major demerits of HPLC which overshadow its uses.

Liquid Chromatography-Mass Chromatography LC-MS/MS

LC-MS/MS is the best technique available for the correct quantification of 25 (OH) D3 and 25 (OH) D2 as it overcomes most of the problems associated with protein-binding assays. In this technique, sample derivatization is not required, run time is short, and an internal standard is used which usually compensates for any matrix-related and instrumental effects, thus making LC-MS/MS a favorable technique for vitamin D estimation. Liquid chromatography-Mass chromatography is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry.

"In a non-disease state, vitamin D levels are influenced by season, skin pigmentation, atmospheric pollution", cloud cover, "geographical location (altitude and latitude, time of the day, indoor living, dress code) etc". Therefore when interpreting "vitamin D levels, it should be done in the background of all these factors. The most important of these are the solar zenith angle, UV index, minimal erythema dose and global positioning system".

Important of these are the solar zenith angle, UV index, minimal erythema dose and global positioning system.

"Solar zenith angle (SZA) is the angular distance between an object in the sky (such as the sun) and an object directly overhead. The zenith angle varies from the

time of the day, the season and geographic locations", "UV index is the calculated prediction of the amount of skin damaging UV radiation that will reach a specific location (1m2) during a solar noon hour. It is derived from the combination of latitude, year of the day, total ozone overhead, elevation above sea level and amount of cloud cover". "Minimal erythemal dose (MED) is the amount of skin exposure which causes barely perceptible skin burn (erythema) which appears within 24hours of a previously unexposed skin. Skin types of various races are categorized based on skin pigmentation, eye color, hair color, reaction to sun (freckles, burns, peels", blister, and tan). Based on these are 6 skin types, Indians come under type 5 category.

The geographical location can "sought from the global positioning system which indicates longitude and latitude of the place of study. The quantum of UV-B rays (290-310nm) received by an individual determines the amount of vitamin D synthesized in the" skin. "Most UV-B rays are transmitted between 10-00AM to 2-00PM. Cloud retains 10% of the rays, snow absorbs 20% and reflects the rest, the rays increase by 10% per kilometer above sea level, and shades reduce the rays by 50%, only 10% of the outdoor rays will be experienced indoors, sand reflects 25% of the rays and up to 40% of the rays is present at about half a meter depth of water".

"In the clinical chemistry departments, the normal range for most of the analytes measured is derived from 95% of the population. It is unreliable to establish such a reference range for vitamin D since" it subject to so many personal, atmospheric and geographical variations, the normative data varies between laboratories.

Hypertension

Stephen Hales (1733), an eighth century clergyman and pioneer of experimental physiology measured BP for the first time in horses. The height of column of blood in a vertical tube inserted into an artery denoted the BP. In 1886 Riva Rocci invented the pneumatic compression cuff to measure the BP.

Richard Bright (1836) described cardiac hypertrophy in glomerulonephritis and postulated that this was secondary to an increase in renal vascular resistance produced by distorted blood vessels.

Sir Clifford Allbutt in 1896 made a fundamental observation when he recognized the distinction between hypertension due to renal disease and hypertension in which no evidence of renal disease could be discovered (essential hypertension). Tigerstedt and Bergman (1898) isolated a pressor substance from the renal cortex and named it renin.

In 1905, NS Korotkoff, a Russian Physician described Korotkoff sounds and later Erlanger (1921) put forward the concept that muffling of sounds denotes Diastolic Blood Pressure.

In 1934, Goldblatt established a method to produce hypertension in animals. He achieved this by constriction of renal artery in dogs.Essential hypertension was only known little before Australian physiologist Paul Korner in 1940.[82]

Laragh (1963) emphasized the importance of classifying hypertension according to peripheral renin activity into low renin, normal renin, and high renin group.In 1968 Sir George Pickering advocated the concept that hypertension was only a quantitative deviation from the normal so that people were arbitrarily called hypertensives if they were on the higher position of the unimodal distribution curve.

De Wardener and Mc. Gregor in the 1980's postulated a causal role for the sodium present in the vascular tissue and blood cells of most hypertensives. Thus hypertension may not be a distinct disease caused by specific abnormalities. Even today no single or specific cause is known for hypertension.

With the development of potent drug treatment, beginning with ganglion blocking agents, which came into clinical usage in 1950s another revolution in understanding of hypertension, was achieved.

The recent concept of Nephron scarcity by Brenner (1988) and associates states that a major renal abnormality that initiates essential hypertension is a decreased filtration surface area due to reduced number of nephrons and or a decrease in filtration surface area per glomerulus. This may contribute to renal sodium retention and thus to low renin hypertension.

With the advent of newer investigational modalities like CT scan in 1979 and radionuclide studies of late, the diagnosis of various causes of secondary hypertension has been made easy. Zeitler (1971) and Gruwentzig (1978) performed balloon angioplasty of the renal artery successfully and gave an impetus to this new therapeutic technique to treat renovascular hypertension.

At the present time, a number of classes of pharmacological agents are available for clinical use. Nevertheless selection of drug treatment for hypertension remains quite empirical even today.

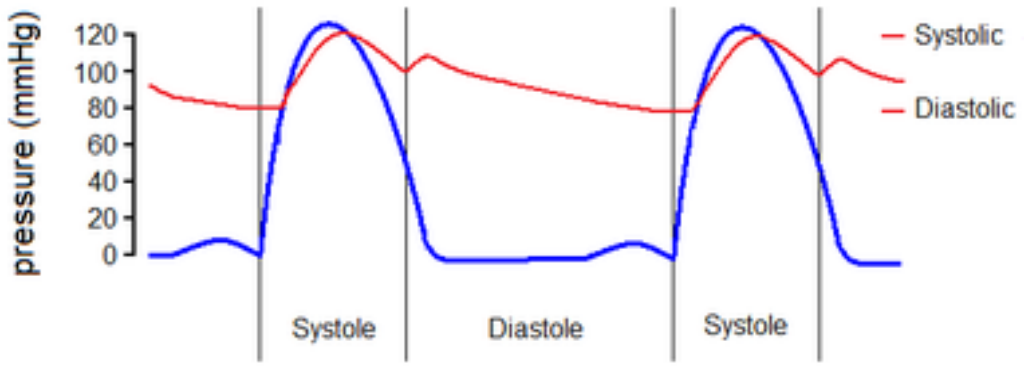
The list of important contributing investigators has grown long as the effort to search for specific etiologic factors in hypertension progresses. A major goal of this research will be reached when the term essential hypertension need no longer be applied to our patients.

DEFINITION

Essential hypertension is high blood pressure for which there is no clearly defined etiology. From a practical perspective, it is best defined as that level of blood pressure at which treatment to lower blood pressure results in significant clinical benefit—a level which will vary from patient to patient depending on their absolute cardiovascular risk.

Essential hypertension (also called primary hypertension or idiopathic hypertension) is the form of hypertension that by definition has no identifiable cause. It is the most common type of hypertension, affecting 90-95% of hypertensive patients,[83] it tends to be familial and is likely to be the consequence of an interaction between environmental and genetic factors. Prevalence of essential hypertension increases with age, and individuals with relatively high blood pressure at younger ages are at increased risk for the subsequent development of hypertension. Hypertension can increase the risk of cerebral, cardiac, and renal events.[7]

Classification



The variation in pressure in the left ventricle (blue line) and the aorta (red line) over two cardiac cycles ("heart beats"), showing the definitions of systolic and diastolic pressure.

Classification	Systolic pressure		Diastolic pressure	
	mmHg	kPa (kN/m^2)	mmHg	kPa (kN/m^2)
Normal	90–119	12–15.9	60–79	8.0–10.5
Prehypertension	120–139	16.1–18.5	81–89	10.8–11.9
Stage 1	140–159	18.7–21.2	90–99	12.0–13.2
Stage 2	≥160	≥21.3	≥100	≥13.3
Isolated systolic hypertension	≥140	≥18.7	<90	<12.0
Source: American Heart Association (2003).[84]				

Failure to reduce blood pressure after taking 3 drug therapy is called as resistant hypertension.[6] In US and UK treating resistant htn guidelines is published.[85]

Hypertension risk factors

Compex disorder is hypertension. Different population have different aetiology and there is no cause for essentional hypertension.[86]

Genetic variation

Family history of hypertension is more prone to develop hypertension in individuals.when compared to whites blacks have 4 times high risk of developing essential hypertension.Study by Kim et al, showed angiotensinogen gene is most common to develop hypertension.[86] On mendelian basis mutations in single gene can cause hypertension.

Age

Age can cause hypertension and the mechanism is vascular compliance reduction and causing arteries stiff. This can build up isolated systolic hypertension with a widened pulse pressure. One more mechanism is glomerular filtration rate decrease due to age which causes decrease in sodium excretion. Salt sensitive hypertension is caused by renal microvascular disease.[87]

Obesity

Risk of hypertension is 5 fold in obesity people when compared to normal weight persons. BMI more than 25 have 85% of cases with hypertension. Animal and clinical studies have shown a link between hypertension and obesity.Renin-angiotensin-aldosterone system and sympathetic nervous system activation are the mechanisms involved.[88]

Salt

It is an environmental factor which causes hypertension. one third of essential hypertension patients are due to high sodium intake.[89] when intake of sodium is exceeded than the body to excrete it through the kidneys, expansion of vascular volume occurs, and cardiac output is increased due to raised arterial pressure.

Intake of alcohol

Due to high calories in alcohol which causes obesity,and eventually leads to hypertension with excessive alcohol consumption.[90] Smoking is risk factor of hypertension, by causing other cardiovascular disease.

Renin

Increase in renin is risk factor for hypertension. The mechanism involved is increased renin causes increased angiotensin 2 and than increased vasoconstriction ,

thrist and aldosterone and causes increased sodium reabsorption in kidneys which leads to hypertension.

Diabetes

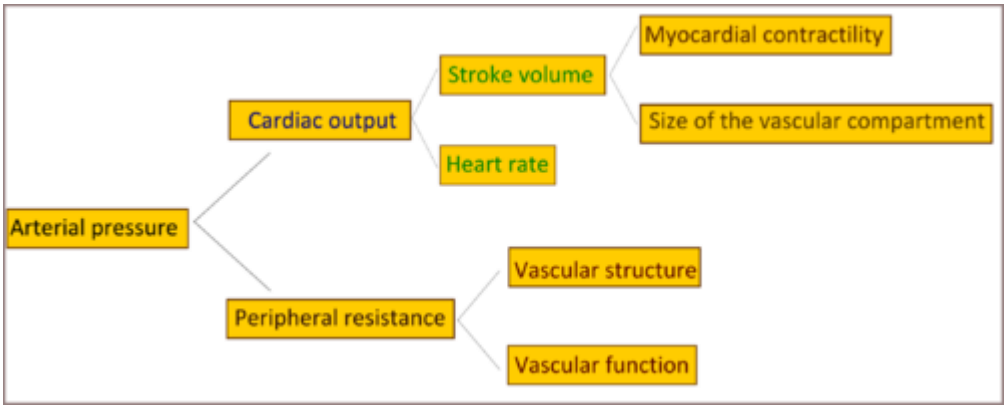
Insulin resistance or hyperinsulinemia called syndrome X or metabolic syndrome causes hypertension. Insulin has vasodilatory action by stimulating sympathetic activity which reduces the mean arterial pressure.

Hypertension is caused by obesity by renin angiotensin system activation in adipose tissue and also insulin resistance.

Deficiency of vitamin

Cardiovascular risk factors are seen in vitamin D deficiency.[63] people with deficiency of vitamin D have low systolic and diastolic blood pressures,due to inhibition of renin and its activation.

Pathophysiology



Factors Affecting Arterial Pressure

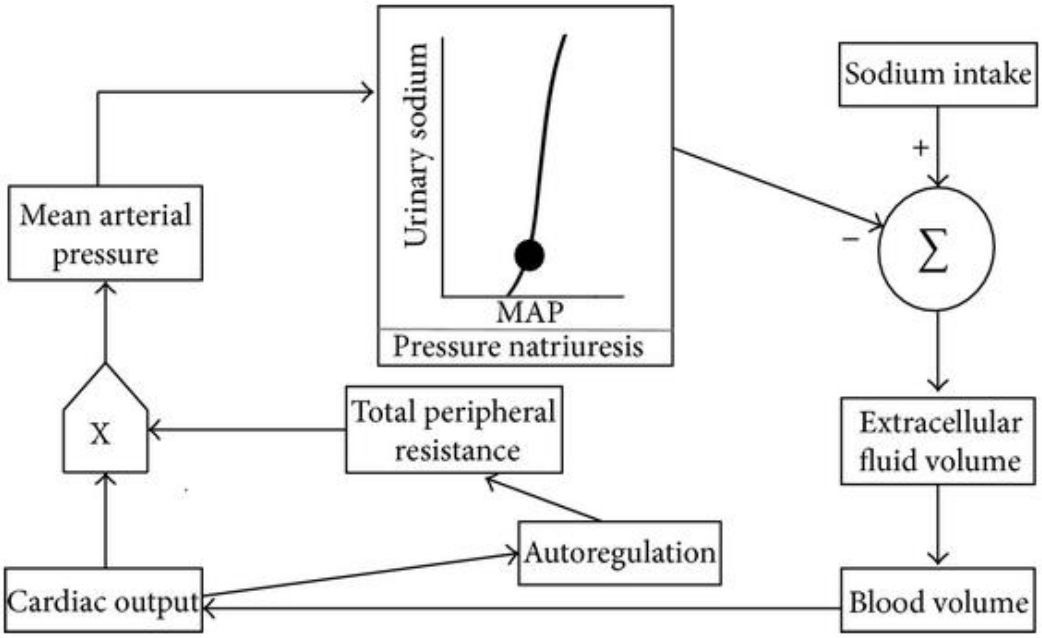
An overactive Renin-angiotensin system leads to vasoconstriction and retention of sodium and water. The increase in blood volume leads to hypertension.

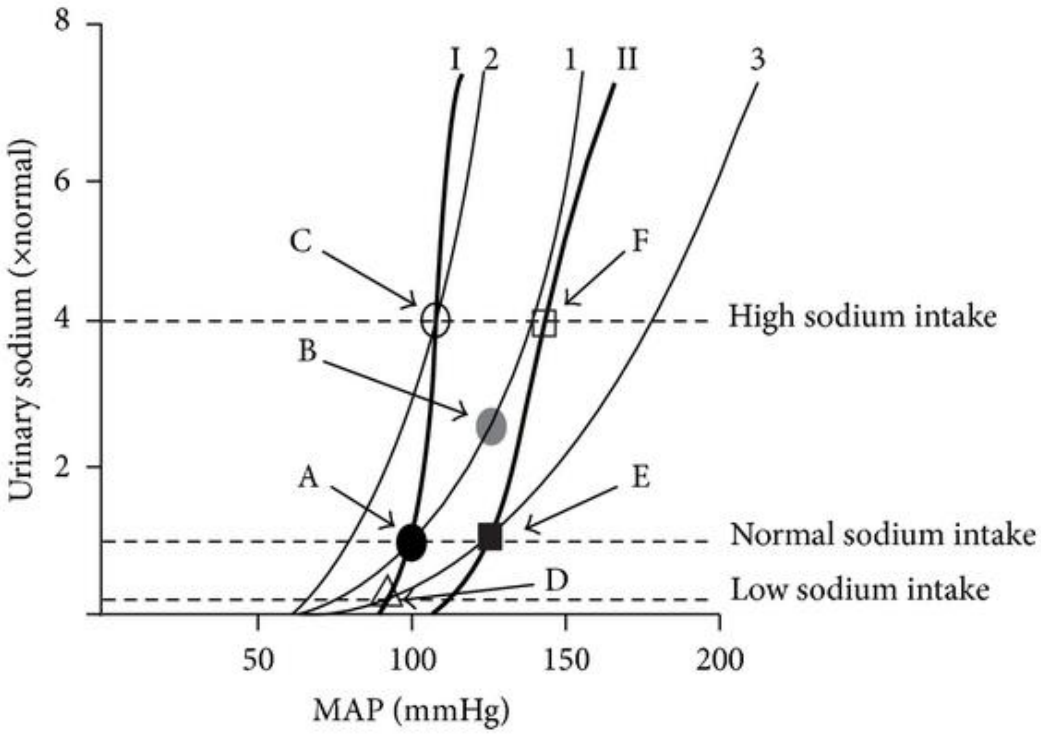
An overactive sympathetic nervous system, leading to increased stress responses.

It is also known that hypertension is highly heritable and polygenic (caused by more than one gene) and a few candidate genes have been postulated in the etiology of this condition.[91]

Etiology and Neurogenic Pathophysiology,Control of Blood Pressure by the Kidneys

The relative stability of arterial blood pressure leads to the conclusion that it is a highly controlled variable. Arterial pressure is maintained at the level satisfactory to ensure an adequate tissue perfusion. Baroreflexes and vasoactive hormones produce tight regulation over relatively short time spans. Long-term regulation is, most generally, thought to be achieved through the renal fluid volume regulation mechanism. Regulation of mean arterial pressure (MAP) requires integrated actions of the physiological systems affecting its major determinants. In the simplest formulation, determinants of MAP are approximated by Ohm's law modified for fluid dynamics (pressure = flow × resistance). Blood flow depends on cardiac output and blood volume, whereas resistance is primarily determined (as total peripheral resistance) by the contractile state of small arteries and arterioles throughout the body, which is itself determined by the tissues blood flow autoregulation mechanism. Blood volume depends on extracellular fluid volume (ECFV), which itself is determined by the total body sodium content. The latter depends on the balance (sodium equilibrium) between sodium intake and urinary sodium excretion (natriuresis; the main route of body sodium loss). Natriuresis is itself determined by the kidney's perfusion pressure, therefore the application of the pressure-natriuresis concept.[3],[5]


Urinary sodium
MAP
Pressure natriuresis
Sodium intake
+
−
Σ
Mean arterial pressure
X
Total peripheral resistance
Extracellular fluid volume
Autoregulation
Cardiac output
Blood volume


Urinary sodium (×normal)
8
6
4
2
I
2
1
II
3
C
F
B
A
E
D
High sodium intake
Normal sodium intake
Low sodium intake
50
100
150
200
MAP (mmHg)

[The renal-mean arterial pressure (MAP) set-point model as proposed by Guyton et al.[3],[4],[5] (a) Basic renal-body fluid feedback mechanism for long-term regulation of blood pressure and body fluid volumes. (b) Normalized urinary sodium excretion is plotted as a function of the MAP to show the pressure natriuresis relationships, at different sodium intake levels, corresponding to the normal condition (acute renal function curves 1, 2, and 3 and chronic renal function curve (I)) and to a mild hypertension condition (chronic renal function curve (II)]

Long-term regulation of MAP is intimately associated with extracellular fluid volume homeostasis. Sodium equilibrium is critical to extracellular fluid volume homeostasis, and the kidneys, as the principal route through which sodium is eliminated from the body, are therefore central to the long-term stability of MAP. This concept was expressed quantitatively in a systems analysis approach that predicts that the kidney acts as an overriding regulator of arterial pressure through a "renal-body fluid feedback" mechanism. A key component of this feedback is the pressure natriuresis or the effect of arterial pressure on renal sodium and water excretion, exemplified in acute and chronic renal function curves; thin and thick curves, resp.). Arterial pressure is set at the level required by the kidney to allow sodium and water excretion to match the intake. Basal-acute and normal-chronic renal function curves (curves 1 and I, resp.) coincide at this pressure level. Kidney perfusion studies show that, in the absence of a change in sodium intake, a rise in MAP (or renal perfusion pressure) is matched by increased renal sodium excretion (point B; sodium excretion exceeds intake), or pressure natriuresis, which reduces extracellular fluid volume, cardiac output and returns MAP to normal. is operative, even when sodium intake is normal. In the presence of hypertension, if sodium intake is increased, a higher than normal MAP increase is necessary to obtain sodium balance (point F).[3],[5] With the

intrinsic kidney function being normal, in the early stage of essential hypertension, an abnormal pressure natriuresis relationship can only result from an abnormal regulation of the kidney function.

Salt Intake

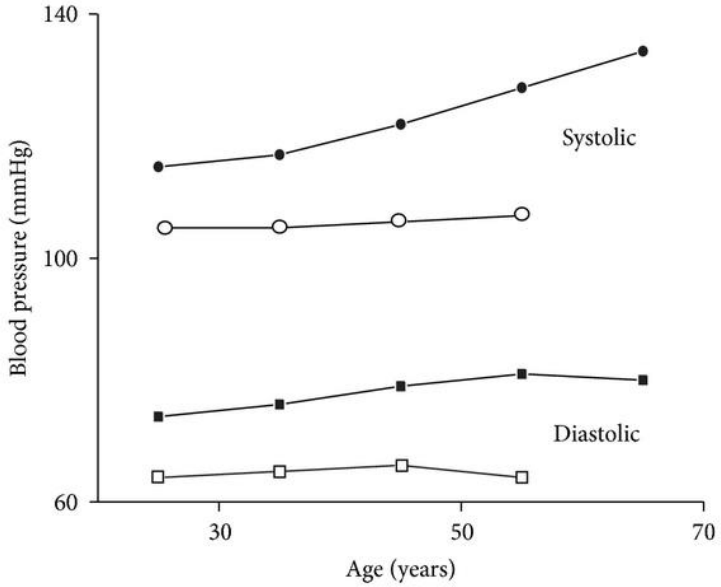
If the start of human evolution is arbitrarily set at the beginning of the Paleolithic, during 3 million years, the ancestors of humans, like all other mammals, ate a diet containing little sodium and much potassium: some 0.6 g and 7 g per day, respectively, a Na+/K+ relationship close to 0.09. The ability to easily increase blood pressure is a characteristic that might have conferred an evolutionary advantage until modern times. Blood pressure is directly proportional to total body sodium content. To promote sodium ingestion, sodium appetite is a motivated behavioral state, arising in response to sodium deficiency that drives humans to seek and ingest food and fluids containing sodium.[14] To prevent sodium loss, the most powerful mechanism is the renin-angiotensin-aldosterone system (RAAS), which controls kidney's tubular sodium reabsorption. Depletion of sodium or emotional stress activates the sympathetic nervous system, which, acts mainly via stimulation of the RAAS and further prevents urinary sodium loss. Besides sodium appetite, evolution has provided humans with a pleasant liking of salt taste, which motivates man to ingest sodium in excess of need, when it is available.[14]

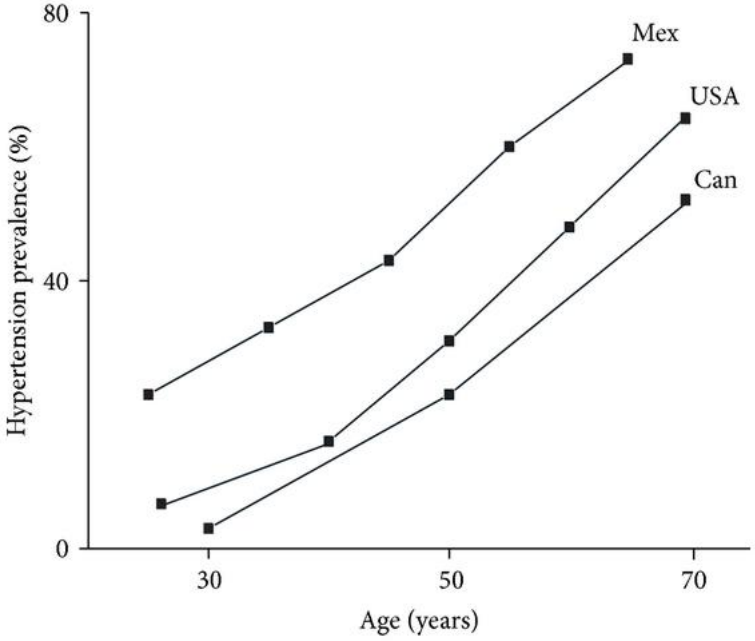
Many large observational epidemiological investigations conducted worldwide link high salt intake and hypertension.[92] In one of the first global studies on sodium intake,[93] 24 h urine sodium and urinary sodium/potassium relationship were positively associated with blood pressure as well as the increase in blood pressure with age. Furthermore, populations with low average daily sodium intake (some tribal societies which do not add salt to the food) had relatively low blood

pressure and very little or no increase in blood pressure with age. Hypertension was fairly uncommon in these societies, but individual's blood pressure rose after migration to an urban environment. However, migration involves more change than just a change in salt intake, because other factors, such as mental stress and changes in physical activity and diet, may contribute to the rise in blood pressure. Blood pressure increase with age is higher in urban than in rural environments, reflecting the environmental influence on blood pressure. On the other hand, in two clinical studies performed on some 200 individuals in which, within their usual diet, dietary sodium intake was randomly and sequentially adjusted at low (1.15 g/day), intermediate (2.30 g/day), and high (3.45 g/day) levels, during three 30-day periods, a positive relationship was found between blood pressure and sodium ingestion, supporting the conclusions of the population studies described above.[94],[95]

Diet with low potassium induces sodium retention and increases blood pressure. On the contrary, potassium supplementation promotes natriuresis and blood pressure is reduced.[96]

5-6g[97] and some 3.5g of potassium, is the suggested intake of salt in a day. roughly half and twice of the current average intake of sodium and potassium, respectively. Humans have sodium deficiency for a long time and so developed hedonistic taste for salt.


140
100
60
Blood pressure (mmHg)
Systolic
Diastolic
30
50
70
Age (years)


80
40
0
Hypertension prevalence (%)
Mex
USA
Can
30
50
70
Age (years)

These are the graphs showing difference between blood pressure and age in years and hypertension prevalence and age.[7],[8]

Due to this it is very difficult to reduce sodium intake. Nonetheless, we must realize that the human body is not equipped to handle the unnatural amount of sodium present in our current diet; hence, hypertension, to some extent, may be classified as a disease of "affluence".[98]

Abnormal Regulation of the Kidney Function

At the end of the 19th century, the renal sympathetic nerves were known to contain fibers which upon stimulation decreased renal blood flow and urinary flow rate. It was also known that renal blood flow and urinary flow rate increased after renal sympathetic nerves transection.[99] In the early decades of the 20th century, faced with the high mortality of severe hypertension and the absence of effective pharmacological therapy, a number of operations on the sympathetic nervous system, such as radical splanchnicectomy, were devised in an attempt to lower blood pressure. By the late 1960s, most of the available antihypertensives, which by then had been developed, antagonized the sympathetic nervous system. The potency and clinical usefulness of these drugs helped to sustain the argument that the sympathetic nervous system was important in the pathogenesis of essential hypertension.

The sympathetic nervous system exerts a basal excitatory activity over the kidney. Increases in this activity result in (1) increase in renin secretion, (2) increase in renal tubular sodium reabsorption, and (3) renal vasoconstriction. Experimental studies established the concept that subvasoconstrictor levels of renal sympathetic nerve activity can produce increased renin secretion and increased tubular sodium reabsorption (without changes in renal blood flow and glomerular filtration rate), which result in a shift in the renal-pressure natriuresis function to the right, so that a

higher than normal arterial pressure is required to attain sodium balance. Within this evidence is the finding that normotensive young men with a family history of hypertension have a higher sympathetic activity than those without a family history. During mental stress, sympathetic activity and blood pressure increase in normotensive offspring of parents with essential hypertension, but do not increase in those with normotensive parents. One-third of patients with borderline hypertension display so-called hyperkinetic circulation, characterized by an elevation in resting heart rate combined with a high cardiac output and an increase in the circulating plasma level of the adrenergic neurotransmitter norepinephrine.[100]

In the same way, renal sympathetic activity is augmented two- to threefold (on average) in young patients (<45 years) with essential hypertension. These patients also show an increased renin release and plasma renin activity. On the other hand, in patients with resistant hypertension, responding inadequately to concurrent treatment with multiple antihypertensive drug classes, radiofrequency ablation of the renal sympathetic nerves lowers blood pressure remarkably. Nowadays, it is deemed that a neurogenic origin (sympathetic activation) of essential hypertension could account for up to 50% of all cases of high blood pressure.

However, increased sympathetic nerves activity is most clearly expressed in the early stages of hypertension development and is less consistent as the time passes. Once chronic hypertension is installed, and after the early stages of essential hypertension, hypertensive blood pressure levels may be maintained, even in the absence of an increased renal sympathetic nerve activity, mainly by a secondary kidney disease, characterized by glomerulosclerosis, interstitial fibrosis and proteinuria.[101]

Origins of Sympathetic Nervous System Activation in Essential Hypertension

The specific causes of the increased sympathetic activity in essential hypertension are only partially known. Genetic influences (a family history) are evident, and behavioral (as salty food preference), psychosocial (as mental stress) and lifestyle (as physical inactivity) factors appear to be involved. Of prime importance, no doubt, is obesity. The prevalence of hypertension in middle-age obese subjects is 40–50%. Obesity increases the sympathetic (including the renal sympathetic) nervous system activity through the high sodium intake-related mechanisms that will be discussed below and through other mechanisms, such as hyperleptinemia, that will not be reviewed in this paper. Likewise, although the prevalence of hypertension increases with aging and 60% of all adults aged 60–69 years are hypertensive, owing to the pathogenic factors associated with an increased sympathetic nervous system activity in the elderly, such as high dietary sodium intake and increasing obesity, and only the former will be discussed here.

On the other hand, clinical and epidemiological studies indicate the importance of chronic mental stress in the pathogenesis of essential hypertension.[102] Hypertensive subjects may decrease their blood pressure with a meditation program. Psychosocial stress can increase the activity of the sympathetic nervous system by potentiating the neural mechanisms activated by a high salt intake. Race and ethnicity may also influence the predisposition to the sensitivity of blood pressure to salt. Black Africans have a higher prevalence of hypertension and more frequent severe hypertension; they also have a greater blood pressure sensitivity to salt intake than do people of other ethnic origins. Physical inactivity also appears to be important. Aerobic fitness and physical activity are each inversely related to the development of

hypertension. Aerobic exercise training in sedentary normotensive and hypertensive people reduces blood pressure and renal and muscle sympathetic nerves activity.[103]

In hypertensive individuals with their usual salt intake (9–18g/day) the concentrations of plasma sodium and cerebrospinal fluid (CSF) sodium are slightly increased (by 0.5–3mM) as compared with values observed in the same individual on a low (3-4g/day) salt intake. The same variation is probably also observed in normotensive individuals in the same circumstance. Similar changes are observed in animal models of hypertension, on a high sodium diet.[104]

Studies performed in animal models allow proposing that these increase and/or activate brain's sodium/osmoreceptors, located mainly at the hypothalamic lamina terminalis, to trigger sympathoexcitation.[105] These osmoreceptors do not appear to reset significantly with prolonged change in osmolality and therefore can provide a sustained signal to chronically increased sympathetic tone. Similarly, water deprivation-induced increase in osmolality acts, at least in part, in the brain to promote sympathoexcitation and support blood pressure.

The lamina terminalis comprises three structures aligned in the anteroventral region of the third ventricle. The third structure, the median preoptic nucleus (MnPO), located within the blood-brain barrier, has reciprocal connections with the two other structures and integrates the humoral sensory information raised in the SFO and OVLT.[106–108] SFO and OVLT increase their activity in response to an increase in plasma and/or CSF Ang II concentration, resp.). Diverse studies have shown that SFO and OVLT express Na+ conducting nonselective cationic channels (which serve as extracellular Na+-levels sensors) and the Angiotensin II type 1 receptor (AT1 receptor), whose expression is enhanced by a high sodium diet and by blood-borne Angiotensinogen II. The latter may explain, at least partially, the antihypertensive and

sympathoinhibitory actions of systemic AT1 receptor blockers, such as losartan or valsartan. From the SFO and OVLT, perhaps after integration in the MnPO, signals are conveyed from the lamina terminalis to the presympathetic hypothalamic parvocellular neurons of the paraventricular nucleus (pPVN), mainly through Angiotensin II type 1 receptor mediated synapses, though some participation of glutamatergic synapses at the pPVN level has been described.

The angiotensinergic nature of most synapses involved in this conduction may explain, at least partially, the antihypertensive and sympathoinhibitory actions of systemic angiotensin-converting enzyme type 1 (ACE1) inhibitors, such as enalapril or captopril.[109] At this point, it is necessary to mention that all known components of the RAAS, including the precursor and enzymes required for the production and metabolism of angiotensin peptides and specific AT1 and AT2 receptors, as well as aldosterone (Aldo, which may even cross the blood-brain barrier) and mineralocorticoid receptor (MR), have been identified in the brain. It is thought that the direct SFO-OVLT-pPVN pathway described above, participates mainly in rapid sympathetic responses to changes in cardiovascular reflexes or acute psychogenic stress response; however, in generating the chronic sympathoexcitation observed in essential hypertension, another indirect or neuromodulatory SFO-OVLT-pPVN pathway is involved.

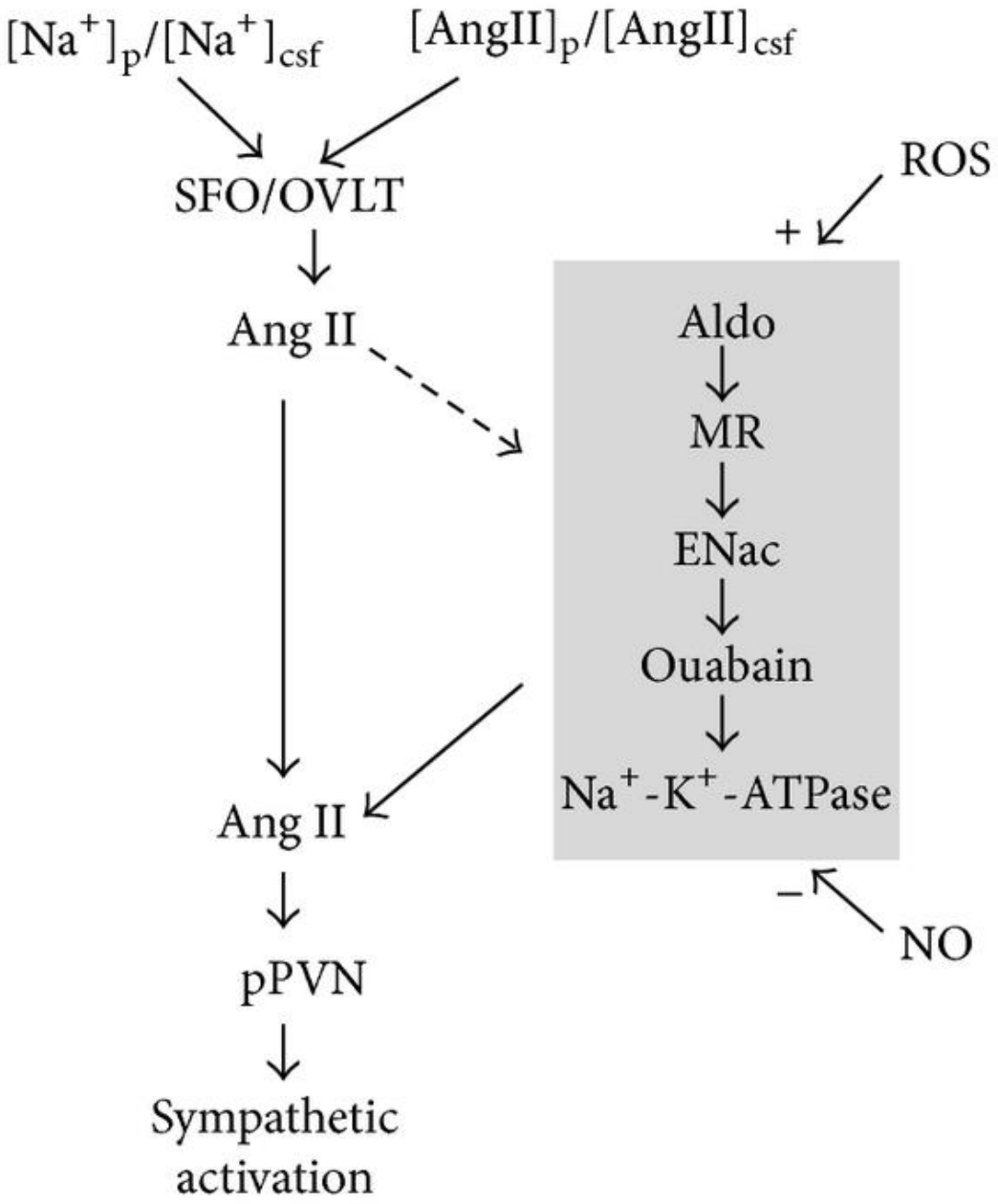
This neuromodulatory pathway is characteristically dependent on protein phosphorylation and changes in protein expression, and its activation promotes increases in renin, ACE1, AT1 receptors, Aldo synthase, and NADPH oxidase and decrease in neuronal nitric oxide (NO) synthase in the hypothalamus.[110] This polysynaptic pathway is slowly activated (days or weeks) by a chronic increase in and/or or in and/or and may be inhibited by the systemic ACE1 inhibitors and AT1

receptor blockers, mentioned above, as well as by systemic spironolactone, an Aldosterone antagonist.

This neuromodulatory pathway appears to rise from SFO and OVLT projections directed to the magnocellular neurons of the paraventricular and supraoptic nucleus of the hypothalamus, but its exact anatomical location is uncertain. Angiotensinergic conduction, involving Ang II and AT1 receptors, is present at least at the beginning (SFO and OVLT) and end (pPVN) of this pathway, but it involves the sequential participation of diverse neuromodulatory agents, receptors, and ion transport mechanisms, such as Aldosterone, mineralocorticoid receptor, benzamil-blockable epithelial Na+ channels (ENaC), endogenous ouabain-like compounds (ouabain), and ouabain-sensitive Na+-K+-ATPase. The activity of this pathway is regulated by the balance between the inhibitory influence of NO and the stimulatory influence of reactive oxygen species (ROS), such as superoxide and peroxynitrite; however, as a consequence of the activation of this pathway by the factors mentioned above, production of Angiotensin II and Aldosterone increases, and this increase, by promoting reactive oxygen species generation and inhibiting nitric oxide synthesis, shifts the NO/reactive oxygen species balance to an enhanced excitation.

This abnormal balance may be corrected, at least to some extent by chronic systemic administration of the long-acting dihydropyridine calcium channel blockers azelnidipine, cilnidipine, and amlodipine; and the antidyslipidemic agents simvastatin and pravastatin (chronic peripheral administration of both kind of drugs appears to result in gradual access of drug to the central nervous system) and by regular exercise training; each of them increases NO and decreases reactive oxygen species in the brain, thereby partially explaining the antisympathetic and antihypertensive actions of these therapeutics and life style agents.[106]

The activity of the neuromodulatory pathway maintains an enhanced activity of the pPVN neurons, which increases the sympathetic nervous system activity through a direct pathway, mainly vasopressinergic, to the sympathetic preganglionic neurons located at the intermediolateral (IML) cell column of the spinal cord, and through an indirect pathway (vasopressinergic, angiotensinergic, and glutamatergic) to the presympathetic neurons in the rostral ventrolateral medulla (RVLM). In turn, the presympathetic neurons in the RVLM activate the intermediolateral sympathetic preganglionic cells through a glutamatergic pathway.[107] Hence, activation of the neuromodulatory pathway maintains an increased activity of the pPVN, the RVLM, and the IML presympathetic and sympathetic neurons, leading to sympathoexcitation and hypertension.



To summarise Control of blood pressure requires complex integration of regulatory mechanisms across multiple physiological systems. A sustained increase in arterial pressure therefore reflects a failure of one or more of these controls. The hallmark of essential hypertension, and of all types of chronic hypertension from whatever origin, is an abnormal renal-pressure natriuresis relationship, which is shifted to the right, so that sodium equilibrium is obtained at a higher than normal pressure level. A high salt intake (9–12 g/day) is a pleasant component of our current

normal diet, but it is abnormal from an evolutionary point of view. This high salt intake induces a slight increase in plasma and CSF sodium, which, when sensed by the lamina terminalis sodium/osmoreceptors, triggers in susceptible individuals, a hypothalamic neuromodulatory signaling chain, activating the sympathetic nervous system.

An increased sympathetic nervous system excitatory activity toward the kidney results in increased renin secretion and renal tubular sodium reabsorption and, consequently a shift to the right of the pressure natriuresis relationship. Hence, in essential hypertension, the abnormal pressure natriuresis relationship is due to an increased activity of the renal sympathetic nerves. However, this increased renal sympathetic activity is most clearly expressed in the early stages of essential hypertension development and is less consistent as time passes. This review has focused on two of the main etiological and pathophysiological mechanisms responsible for the onset and maintenance of uncomplicated essential hypertension. Once chronic hypertension is installed, it may be maintained, even in the absence of an increased renal sympathetic nerves activity, mainly by a secondary kidney disease.

Deficiency of vitamin D Leads to Hypertension?

Hypertension or increase in blood pressure is considered the leading factors in causing worldwide disability of life years.[108] Age, race, family history, obesity, sedentary lifestyle, using tobacco,intake of high salt, stress, and consuming alcohol in a larger quantity are the etiological factors of hypertension.

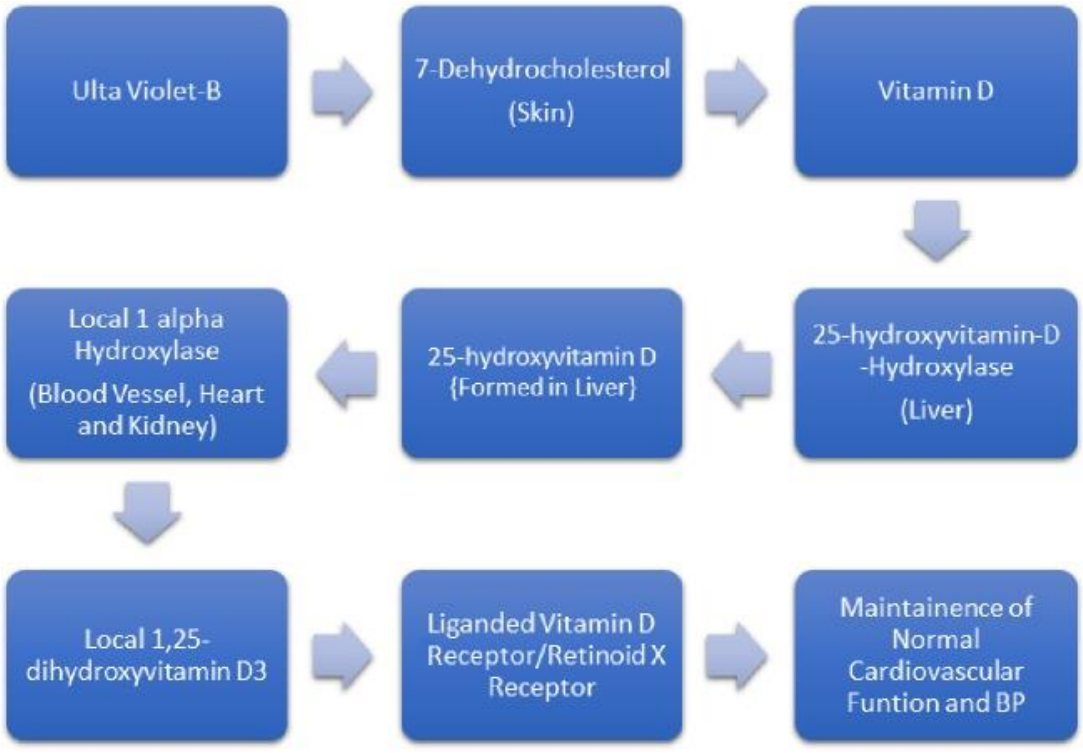
cerebrovascular accident (CVA), myocardial infarction (MI), congestive heart failure (CHF), peripheral artery disease (PAD), atrial fibrillation (AF), and end-stage renal disease (ESRD) risk factor for hypertension and fundamental reason for mortality.

Hypertension is more prevalent in the zones where sun radiation is reduced.Hypertension will rise to 2.5 mmHg and 2.5% for every 10 degrees of deviation from the equator.(111)

Mehta stated that low levels of vit d , sugar,and fats are new risk factors for causing hypertension.(112)

Hypertension and Vitamin D levels association

Ultraviolet-B from sun develops pre vit d3 from 7-hydrocholesterol and causes thermal isomerization to form vit D3. Hydroxylation occurs to form 25-hydroxyvit D 25(OH)D in liver and converts to 1,25-dihydroxyvit D3 1,25(OH2)D3 in kidneys blood vessels and heart.(113)



Vitamin D role in Maintaining Blood Pressure(113)

Clinical studies

Kota, et al. stated SBP, DBP, mean arterial pressure Mean arterial pressure is increased in people with vit D inadequacy and proposed that vit D deficiency is related to RAAS regulation.[114]

one group of scientists studied vit D supplementation on hypertension risk people with 1000 IU every day , 2000 IU every day, 4000IU every day, had declined in SBP for every increase in vit D where, DBP remained the same.[115]

Experiment in Denmark 2012 had caused lowering of blood pressure in hypertensive patients by vit D supplements. people with low levels of vit D had more effect in lowering blood pressure with vitamin D.[116]

Carrara,et al.2013 a Italian study reviewed vit D supplementation on blood pressure. In this study 25000IU of vit D is given per week for 8 weeks and blood pressures were reduced, and concluded supplementation of vit D can reduce risk of hypertension.[117]

Vimaleswaran, et al. did a study to know 25(OH)D is associated with blood pressure and risk of HTN. In this study genes of different variants which effect synthesis of 25(OH)D are used. By this study they concluded increased 25(OH)D may decrease htn risk.[118]

Caro,et al. conducted a study and concluded that 25(OH)D has no statistical association with blood pressure.[119]

A Chinese study by Lee, et al. stated that parathyroid hormone levels and vit D have no relation with hypertension.[120] Kashi, et al, cross sectional study concluded that serum 25(OH)D, calcium and PTH has no relation with hypertension.[121] 251 people with age 40 years are included.

Hypertension management.

Pharmological treatment is advised along with lifestyle changes. In 1958 Thiazide diuretics were first introduced and are effective antihypertensive.[122] Goals of treatment are to reduce cardiovascular diseases and to improve mortality. Various classes of treatment for hypertension are:

1. Diuretics thiazides.Bendroflumethiazide, hydrochlorothiazide, indapamide are the commonly used drugs;
2. calcium channel blockers; compensatory activation of baroreceptors is induced by amlodipine and nifedipine.
3. Adrenoceptor antagonists; drugs included in this class are atenolol and metoprolol.
4. Blockers of the renin-angiotensin-aldosterone system (RAAS): The group of angiotensin-converting-enzyme inhibitors (ACEi) includes drugs such as enalapril and captopril, angiotensin II receptor blockers (ARBs) include losartan, azilsartan and valsartan and
5. Direct renin inhibitors comprise drugs like aliskiren.

Effects of Vitamin D on the Local Renin-Angiotensin System (RAS)

Tissues of heart,vessels,kidneys,lung,adrenal gland,and nervous system has RAS.[123] Blood pressure is controlled by RAS by acting on cardiovascular, renal, and adrenal functions.

Renal damage is caused by intrarenal RAS. Renal injury is caused by local activation of RAS in the kidney due to vit D deficiency.[124] There is suppression of renin as RAS is negative regulator by vitamin D. Lung fibrosis can also occur due to chronic RAS activation due to vit D deficiency. Bone metabolism is also effected by 1,25(OH)2D3 due to RAS which was demonstrated on mice which causes

osteoporosis.[125] Vitamin D deficiency also effects pancreatic islet RAS by which secretory function of islet beta cells is improved.

Vitamin D and Essential Hypertension

In U.S. population a survey is done which provides health statistics known as National Health and Nutrition Examination survey (NHANES)[126] Martins et al. in a study showed the prevalence of hypertension and association between 25(OH)D level by NHANES 3 data(period 1988-1994).[127] It is a crosssectional study. It includes individuals of age above 20 years with n=15,088. Based on 25(OH)D levels the individuals are divided into quartile.

25(OH)D <21 ng/ml comes under 1st quartile with prevalence of 20.46% of hypertension.

25(OH)D> 37 ng/ml comes under 4th quartile with prevalence of 15.10% of hypertension. Based on age,sex,race and comparsion between 1st and 4th quartile gives odds ratio (OR) which is 1.30.

Imbalance between vasodilation and vasoconstriction causes essential hypertension. Essential hypertension is developed due to epigenetic and environmental factors. Many epideomological studies have been done to prove this. Supplementation of Vitamin D to protect cardio vascular diseases, studies have been done in mice furthermore. So renin synthesis is done by vitamin D injections. Antihypertensive drug which is cost effective can be used as Cholecalciferol.

Ke L et al in 2015[27] in a systematic review and meta-analysis of all observational studies published "map trends in the evidence on the association between blood vitamin D levels and the risk of hypertension. The authors findings suggested that the better the assessed quality of the respective study design", "the stronger the relationship between higher 25OHD levels and hypertension risk (RR

=0.67 (0.51–0.88); OR =0.77 (0.72–0.89))". "There was significant heterogeneity among the findings for both prospective and cross-sectional studies" but no evidence of publication bias was shown. "There was no increased risk of hypertension when the participants were of older age or when they were vitamin D deficient. Younger females showed strong associations between high 25OHD levels and hypertension risk", "especially in prospective studies (RR =0.36 (0.18–0.72); OR =0.62 (0.44–0.87))".

"The authors concluded that despite the accumulating evidence of a consistent link between vitamin D and blood pressure, these data are observational", "so questions still remain in relation to the causality of this relationship". "Further studies either combining existing raw data from available cohort studies or conducting further Mendelian analyses are needed to determine whether this represents a causal association". "Large randomized controlled trials are also needed to determine whether vitamin supplementation may be beneficial in the prevention or the treatment of hypertension".

Qi D et al in 2017[29] in a Prospective study and meta-analysis sought to determine the link between vitamin D concentrations and incident hypertension. The authors found during a median follow-up of 2 years, 42.6% of the cohort (n = 1047) developed hypertension. Compared with the 25-hydroxyvitamin D >30ng/ml, 25-hydroxyvitamin D <20 ng/ml was associated with a greater hypertension risk (OR: 1.225 [95% CI: 1.010 to 1.485] p = 0.04), although the association was attenuated and not statistically significant after adjusting for potential confounders (OR: 1.092 [95% CI: 0.866 to 1.377] p = 0.456). This meta-analysis included seven prospective studies for 53,375 participants using adjusted HR founded a significant association between vitamin D deficiencies and incident hypertension (HRs = 1.235 (95% CI: 1.083 to

1.409, p = 0.002)). The authors concluded that Lower serum 25-hydroxyvitamin D concentrations were not associated with a greater risk of incident hypertension. More research is needed to further determine the role of 25-hydroxyvitamin D in hypertension prevention and therapy.

"O'Callaghan KM et al in 2018[128]" in a interventional, observational "narrative systematic review evaluated growing evidence of an association between low maternal vitamin D status and increased risk of hypertensive disorders".

"The authors found Conflicting data for an association of vitamin D with gestational hypertensive disorders in observational studies arises from a number of sources" "including large heterogeneity between study designs, lack of adherence to standardized perinatal outcome definitions, variable quality of analytical data for 25-hydroxyvitamin D (25(OH)D)" "and inconsistent data reporting of vitamin D status. While evidence does appear to lean towards an increased risk of gestational hypertensive disorders at 25(OH)D concentrations <50 nmol/L, caution should be exercised with dosing in trials, given the lack of data on long-term safety". "The possibility that a fairly narrow target range for circulating 25(OH)D for achievement of clinically-relevant improvements requires further exploration". "As hypertension alone, and not preeclampsia specifically, limits intrauterine growth" "evaluation of the relationship between vitamin D status and all terms of hypertension in pregnancy is a clinically relevant area for research and should be prioritised in future randomised trials".

Sample Size Calculation

With prevalence of hypertension 7.1[(129)] at 95% confidence level and at 5% absolute error, sample size was calculated by using following formula:

$$n= [z^2p(1-p)]/d^2$$

Where: Z = table value of alpha error from Standard Normal Distribution table (0.95)

p= prevalence rate = 7%

d= margin of error =±5%

$$n= [0.95 \times 0.95 \times 0.07 (0.93)] / 0.05 \times 0.05 = 47.7$$

50 patients of primary hypertension and 50 controls of age and sex matched individuals were included in the study.

STATISTICAL ANALYSIS

Quantitative data is presented with the help of Mean and Standard deviation. Comparison among the study groups is done with the help of unpaired t test as per results of normality test. Qualitative data is presented with the help of frequency and percentage table. Association among the study groups is assessed with the help of Fisher test, student 't' test and Chi-Square test. 'p' value less than 0.05 is taken as significant**.**

Pearson's chi-squared test

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where X2 = Pearson's cumulative test statistic.

Oi = an observed frequency;

Ei = an expected frequency, asserted by the null hypothesis;

n = the number of cells in the table.

Results were graphically represented where deemed necessary. MS Excel, SPSS ver. 20 were used for statistical analysis and graphical representation.

OBSERVATIONS AND RESULTS

A hospital based observational case control study was conducted with 100 patients to analyze whether low plasma vitamin D level is risk factor for primary hypertension or not. The patients are divided into two groups of 50 patients each as follows:

Cases: Patients of primary hypertension

Control: Patients that were age and sex matched individuals

Distribution of patients according to Age

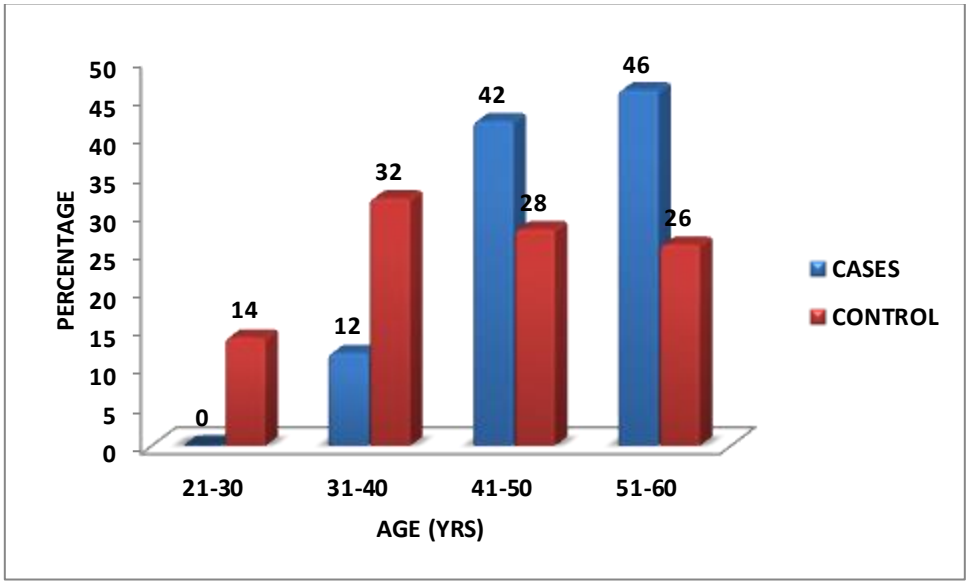
Majority of the patients (32%) in controls were from the age group of 31-40 years followed by 28% from the age group of 41-50 years, 26% from the age group of 51-60 years, 14% from the age group of 21-30 years. The mean age in controls was 21.2 ± 11.5 years.

Majority of the patients (46%) in cases were from the age group of 51-60 years followed by 42% from the age group of 41-50 and 12% from the age group of 31-40 years. The mean age in cases was 18.02 ± 6.3 years. The difference in mean age of the patients in the two groups was statistically significant as per Student t-test ($p<0.05$).

Table 1: Distribution of patients according to Age

AGE	CASES		CONTROL		p value
	N	%	N	%	
21-30	0	0	7	14	0.001*
31-40	6	12	16	32	
41-50	21	42	14	28	
51-60	23	46	13	26	
Total	50	100	50	100	
Mean±SD	18.02±6.3		21.2±11.5		

Note: * significant at 5% level of significance ($p<0.05$)



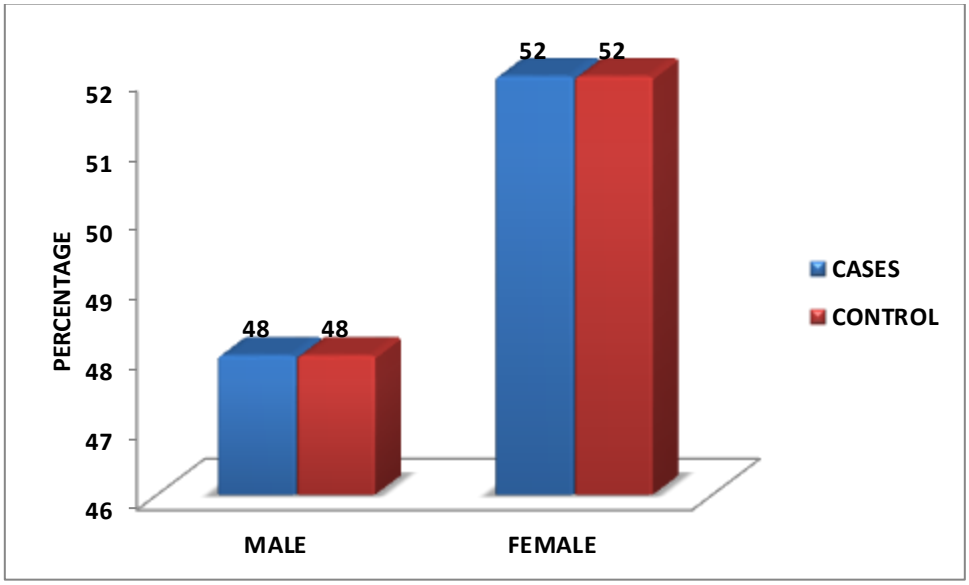
Graph 1: Distribution of patients according to Age

Distribution of patients according to Gender

The number of patients in both the groups was predominantly female (52%) respectively while male patients constituted 48% of the study population. The distribution of patients between two groups on basis of gender were comparable and statistically not significant as per Chi-Square test (p>0.05).

Table 2: Distribution of patients according to Gender

SEX	CASES		CONTROL		p value
	N	%	N	%	
MALE	24	48	24	48	>0.05
FEMALE	26	52	26	52	
Total	50	100	50	100	
M/F RATIO	0.92:1		0.92:1		



Graph 2: Distribution of patients according to Gender

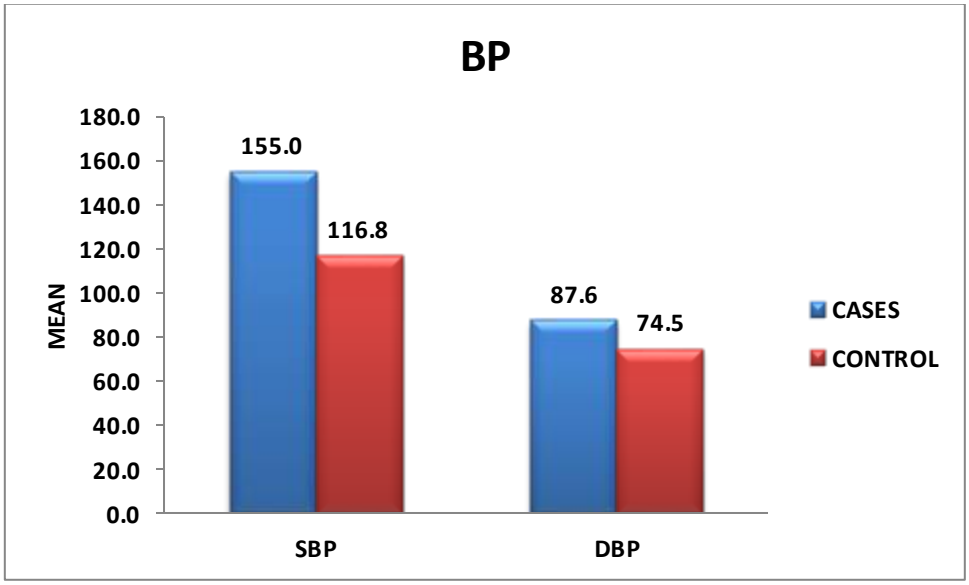
Comparison of Blood Pressure parameters between groups

The mean systolic blood pressure (SBP) value in controls was significantly lower as compared to cases (116.8 ± 6.7 vs. 155.0 ± 8.4 mmHg). The mean diastolic blood pressure (DBP) values in controls was significantly lower as compared to cases (74.5 ± 4.8 vs. 87.6 ± 5.2 mmHg).There was significant difference between the groups as per Student t-test (**p<0.05**).

Table 3: Comparison of Blood Pressure parameters between groups

PARAMETERS	CASES		CONTROL		p value
	Mean	SD	Mean	SD	
SBP	155.0	8.4	116.8	6.7	<0.001*
DBP	87.6	5.2	74.5	4.8	<0.001*

Note: * significant at 5% level of significance (p<0.05)



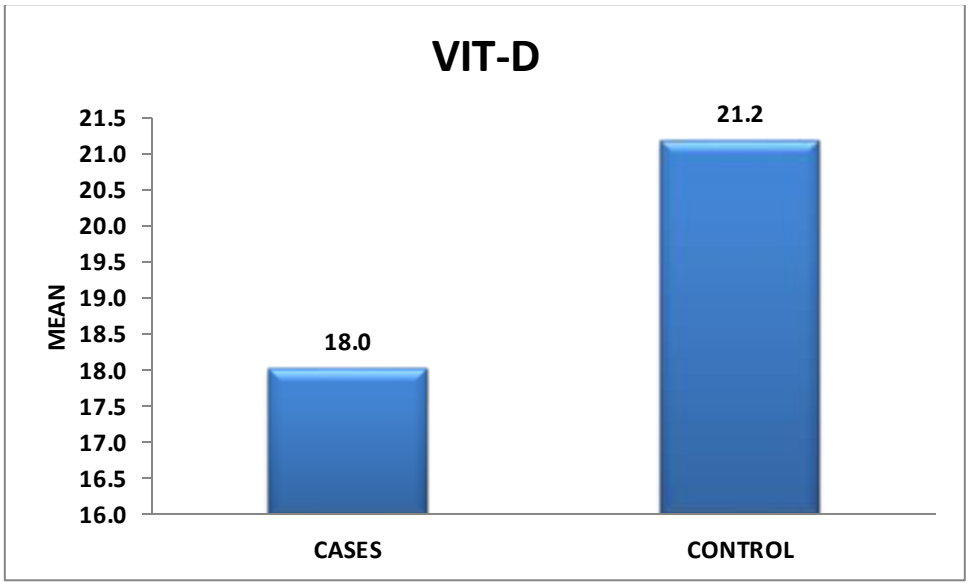
Graph 3: Comparison of Blood Pressure parameters between groups

Comparison of Vitamin D levels between groups

The mean Vitamin D level in controls was higher as compared to cases (21.2± 11.5 vs. 18.0 ± 6.3ng/ml). However there was no significant difference between the groups as per Student t-test (p>0.05).

Table 4: Comparison of Vitamin D levels between groups

PARAMETERS	CASES		CONTROL		p value
	Mean	SD	Mean	SD	
VIT-D (ng/dl)	18.0	6.3	21.2	11.5	0.093



Graph 4: Comparison of Vitamin D levels between groups

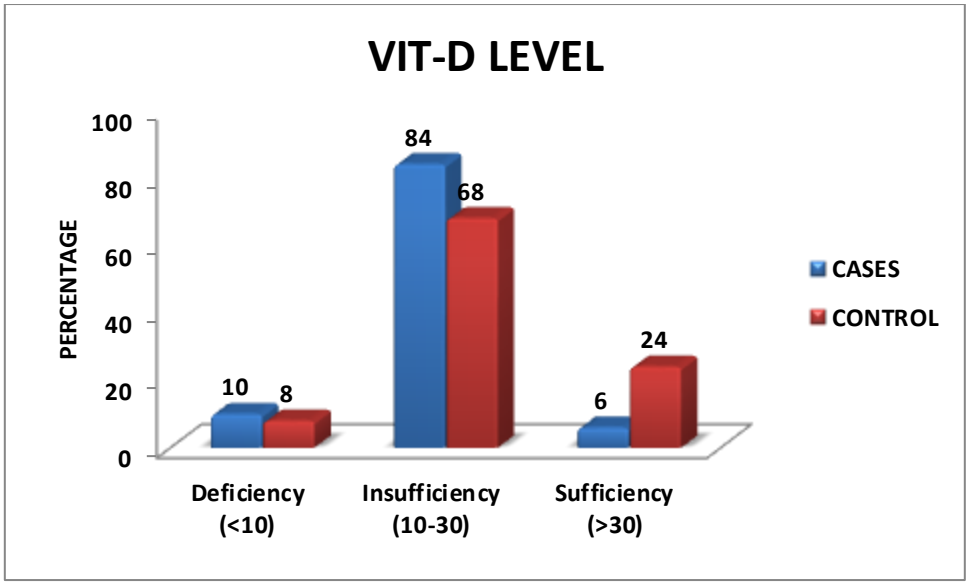
Comparison of Deficiency in Vitamin D levels between groups

In controls, 8% patients had Vitamin D deficiency while 24% patients had Vitamin D sufficiency. In cases, 10% patients had Vitamin D deficiency while 84% patients had Vitamin D insufficiency. There was significant difference between the groups as per Chi-Square test (**p<0.05**).

Table 5: Comparison of Deficiency in Vitamin D levels between groups

VIT-D LEVEL	CASES		CONTROL		p value
	N	%	N	%	
Deficiency (<10)	5	10%	4	8%	0.042*
Insufficiency (10-30)	42	84%	34	68%	
Sufficiency (>30)	3	6%	12	24%	
Total	50	100	50	100	

Note: * significant at 5% level of significance (p<0.05)



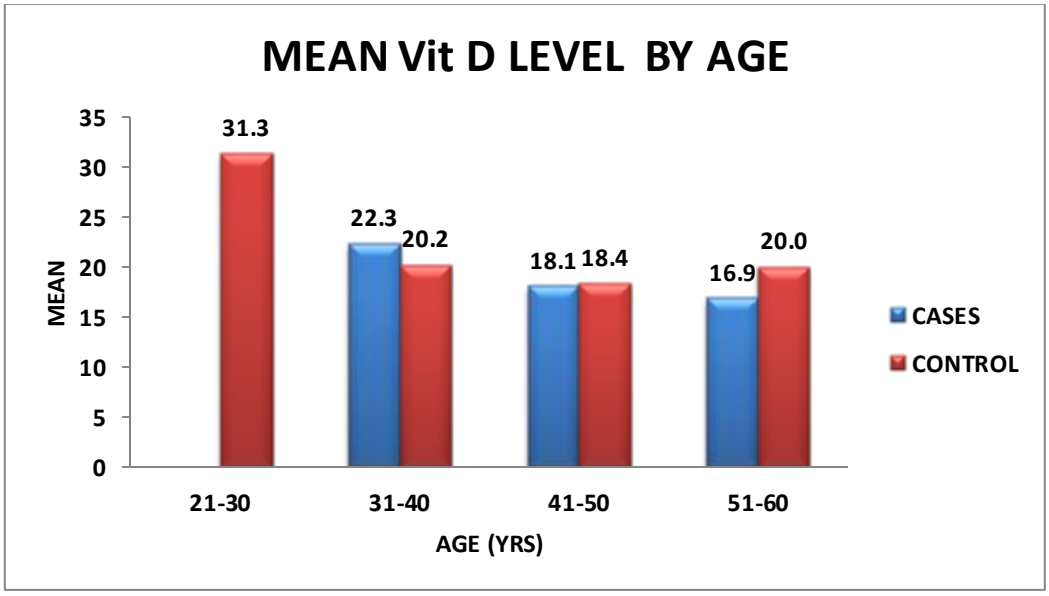
Graph 5: Comparison of Deficiency in Vitamin D levels between groups

Association of Vitamin D levels and Age between groups

In controls, the mean vitamin D levels was comparable across all age groups as per ANOVA test ($p>0.05$). In cases the mean vitamin D levels was lower in patients >40 years of age.

Table 6: Association of Vitamin D levels and Age between groups

AGE	CASES		CONTROL	
	Mean	SD	Mean	SD
21-30	-	-	31.3	25.1
31-40	22.3	11.1	20.2	7.3
41-50	18.1	4.7	18.4	7.3
51-60	16.9	5.9	20.0	5.5
Total	18.0	6.3	21.2	11.5
ANOVA p value	0.174		0.085	



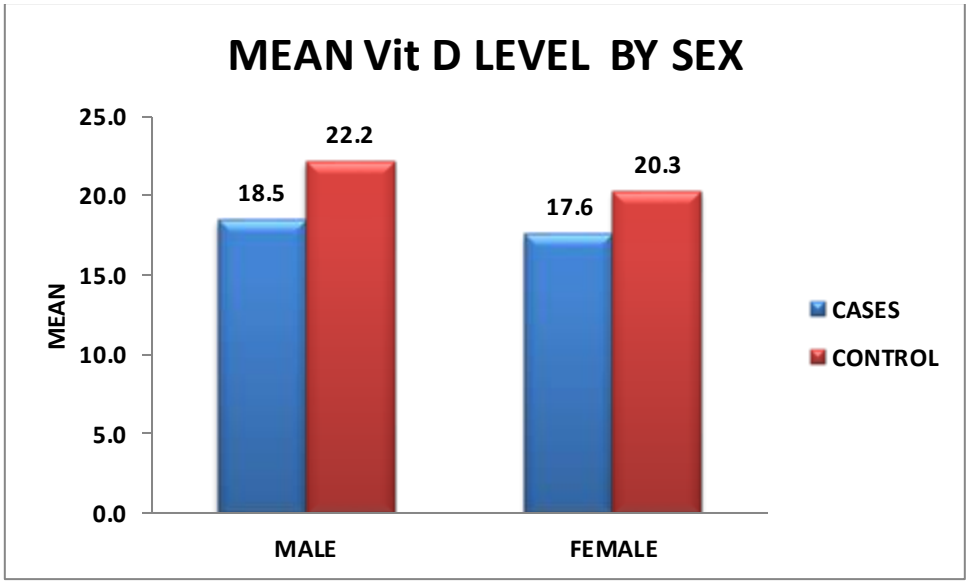
Graph 6: Association of Vitamin D levels and Age between groups

Association of Vitamin D levels and Sex between groups

In both groups, the mean Vitamin D levels were comparable between male and female patients. There was no significant association of vitamin D levels and sex as per Student t-test ($p>0.05$).

Table 7: Association of Vitamin D levels and Sex between groups

GROUP	CASES		CONTROL	
	Mean	SD	Mean	SD
MALE	18.5	5.7	22.2	7.7
FEMALE	17.6	7.0	20.3	14.3
p value	0.63		0.57	



Graph 7: Association of Vitamin D levels and Sex between groups

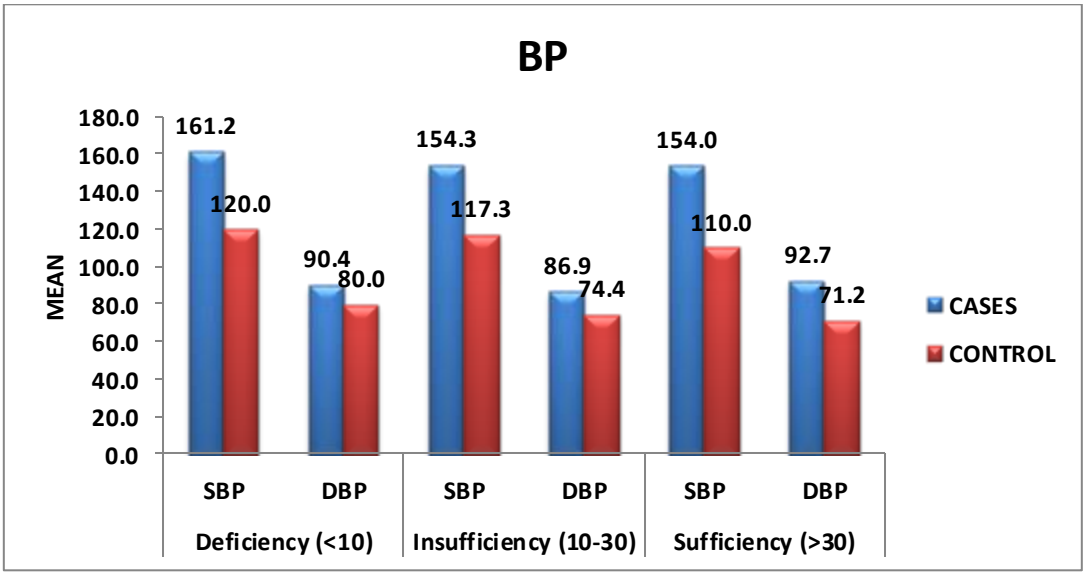
Association of Deficiency in Vitamin D levels and Blood Pressure parameters

The systolic and diastolic blood pressure values were significantly higher in cases as compared to controls in Vitamin D deficient, insufficient and sufficient patients. The association of Vitamin D levels was found to be significant with SBP and DBP values (**p<0.05**).

Table 8: Association of Deficiency in Vitamin D levels and Blood Pressure parameters

VIT-D (ng/dl)	BP	CASES		CONTROL		p value
		Mean	SD	Mean	SD	
Deficiency (<10)	SBP	161.2	5.2	120.0	0.0	<0.001*
	DBP	90.4	0.9	80.0	0.0	<0.001*
Insufficiency (10-30)	SBP	154.3	8.8	117.3	6.9	<0.001*
	DBP	86.9	5.4	74.4	4.8	<0.001*
Sufficiency (>30)	SBP	154.0	2.0	110.0	0.0	<0.001*
	DBP	92.7	3.1	71.2	1.8	<0.001*

Note: * significant at 5% level of significance (p<0.05)



Graph 8: Association of Deficiency in Vitamin D levels and Blood Pressure parameters

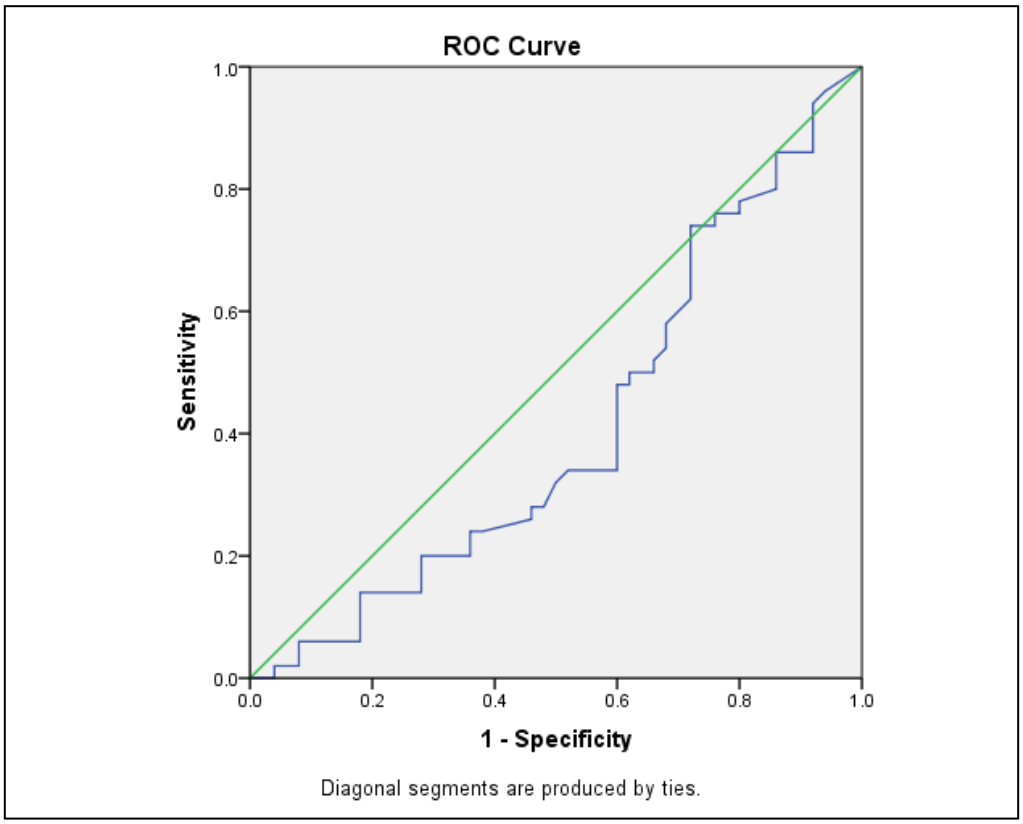
Receiver operating characteristic (ROC) analysis for Vitamin D to analyze Hypertension

For Vitamin D, a cut off 17.75 had highest sensitivity 42.0% and specificity 40.0%. The area under the ROC curve was low and cannot be a predictor of hypertension.

Table 9: ROC analysis for Vitamin D to analyze Hypertension

Area Under the Curve	SE	P VALUE	95% Confidence Interval	
			Lower Bound	Upper Bound
0.408	0.057	0.113	0.295	0.521

Positive if Greater Than or Equal To	Sensitivity	1 - Specificity
7.1	100%	100%
8.5	96.0%	94.0%
12.3	84.0%	86.0%
14.2	74.0%	76.0%
15.9	62.0%	72.0%
16.55	54.0%	68.0%
17.45	46.0%	60.0%
17.55	44.0%	60.0%
17.75	**42.0%**	**60.0%**
17.95	38.0%	60.0%
18.2	36.0%	60.0%
18.5	34.0%	60.0%



Graph 9: ROC analysis for Vitamin D to analyze Hypertension

DISCUSSION

A hospital based observational case control study was conducted with 100 patients to analyze whether low plasma vitamin D level is risk factor for primary hypertension or not. The patients are divided into two groups of 50 patients each as follows:

Cases: Patients of primary hypertension

Control: Patients that were age and sex matched individuals

Vitamin D deficiency is an emerging risk factor for multiple comorbidities worldwide, despite abundant sunshine. So far, various studies have been done to prove an association between Vitamin D deficiency and HTN. This relationship has already been established in the Western population but needs further validation in the Indian scenario.[29][118]

The persistence of elevated childhood and adolescent blood pressure and its progression into adult hypertension has been demonstrated in the past. Repeated high BP measurements in adolescence are a predictor of adult hypertension. Blood pressure monitoring in young adults is therefore useful for the early detection and management of hypertension.

In the present study, majority of the patients (32%) in controls were from the age group of 31-40 years followed by 28% from the age group of 41-50 years, 26% from the age group of 51-60 years, 14% from the age group of 21-30 years. The mean age in controls was 21.2 ± 11.5 years. Majority of the patients (46%) in cases were from the age group of 51-60 years followed by 42% from the age group of 41-50 and 12% from the age group of 31-40 years. The mean age in cases was 18.02 ± 6.3 years. The difference in mean age of the patients in the two groups was statistically significant as per Student t-test ($p<0.05$).

The number of patients in both the groups was predominantly female (52%) respectively while male patients constituted 48% of the study population. The distribution of patients between two groups on basis of gender were comparable and statistically not significant as per Chi-Square test ($p>0.05$). This is similar to the studies of Priya S et al[130], Reddy VS et al[129] and Akbari R et al.[131]

Priya S et al[130] cross- sectional case–control study establishing a causal association between Vitamin D deficiency and HTN found mean age of study population was 40.69 ± 8.28 years, with maximum patients being in the age group of >40 years. Among cases, 52.9% were male and 47% were female. There was no statistically significant between the case and control group in relation to age and gender distribution. ($P = 0.06$ and $P = 0.07$).

Reddy VS et al[129] cross-sectional survey estimating the prevalence of Hypertension among young adults and associated with hypertension including age, sex, socio-economic status, Body Mass Index, dietary habits, tobacco use and alcohol consumption found nearly 70% of the students were between 18 and 19 years and over 95% were under 21 years. The proportion of males (47.7%) and females (52.3%) was comparable, as was the proportion of rural (49.2%) and urban (50.8%) participants.

Akbari R et al[131] case-control study investigating hypertension and serum vitamin D levels in hypertensive subjects found gender distribution was similar in both the comparison group by 41% males and 59% females (mean age of patients and controls was comparable (53.7±6.4 and 52.3±7.4 years) respectively. There was no significant difference in the mean age of men and women between the two groups.

In our study, the mean systolic blood pressure (SBP) value in controls was significantly lower as compared to cases (116.8 ± 6.7 vs. 155.0 ± 8.4 mmHg). The

mean diastolic blood pressure (DBP) values in controls was significantly lower as compared to cases (74.5 ± 4.8 vs. 87.6 ± 5.2 mmHg).There was significant difference between the groups as per Student t-test (**p<0.05**).

The study of Reddy VS et al[129] reported Hypertension was detected in 29 of the 407 subjects (prevalence=7.1%), of which the majority were newly diagnosed (76%). The prevalence among men (15.0%) was higher as compared to women (5.0%). In addition, 46.7% of the subjects were found to have blood pressures in the pre-hypertensive range.

It was observed in our study that the mean Vitamin D level in controls was higher as compared to cases (21.2± 11.5 vs. 18.0 ± 6.3ng/ml). However there was no significant difference between the groups as per Student t-test (p>0.05).

Priya S et al[130] cross‐sectional case–control study establishing a "causal association between Vitamin D deficiency" and HTN reported mean level of 25(OH) D among cases was 15.15 ± 12.51 ng/ml, while among controls, the corresponding value was 33.59 ± 16.69 ng/ ml. The difference was statistically significant (P = 0.0001). Among cases, 80.4% were Vitamin D deficient and 9.8% had insufficient levels of Vitamin D. Among controls, 16.2% each had Vitamin D deficiency and insufficiency.

It was observed in the present study that in controls, 8% patients had Vitamin D deficiency while 24% patients had Vitamin D sufficiency. In cases, 10% patients had Vitamin D deficiency while 84% patients had Vitamin D insufficiency. There was significant difference between the groups as per Chi-Square test (**p<0.05**). In controls, the mean vitamin D levels was comparable across all age groups as per ANOVA test (p>0.05). In cases the mean vitamin D levels was lower in patients >40 years of age. In both groups, the mean Vitamin D levels were comparable between male and female

patients. There was no significant association of vitamin D levels and sex as per Student t-test ($p>0.05$).

The systolic and diastolic blood pressure values in our study were significantly higher in cases as compared to controls in Vitamin D deficient, insufficient and sufficient patients. The association of Vitamin D levels was found to be significant with SBP and DBP values (**$p<0.05$**). This is comparable to the studies of Priya S et al[130], Akbari R et al[131] and Martins D et al[132].

Priya S et al[130] cross‐sectional case–control study establishing a "causal association between Vitamin D deficiency" and HTN reported association of Vitamin D levels was found to be significant with systolic BP ($P = 0.02$) but same was not true for diastolic BP.

Akbari R et al[131] case-control study investigating the "relationship between serum vitamin D level and hypertension (HTN)" in hypertensive subjects reported mean of PTH serum level in two groups showed no significant difference between the men of the two groups; however, this difference was significant between the females of both groups. Serum vitamin D level was significantly higher in patients with HTN than healthy group ($P=0.001$). Proportion of serum 25-OHD deficiency, insufficiency and sufficiency in patients were 27%, 40% and 33% and in the control group 40%, 53% and 7% respectively. Lower concentration of serum vitamin D and PTH in hypertensive patients was observed only in age ranging from 45 to 60 years.

Martins D et al[132] study found significantly lower mean 25- OHD in patients with HTN as compared with healthy controls.

In another study, the prevalence of HTN in subjects with sufficient vit D was 20% lower as compared with those with vit D deficiency. A significant inverse correlation was observed between serum vit D level and high blood pressure of white

people based on the data of National Health and Nutrition Examination Survey (NHANESIII).[133]

Margolis KL et al[134] found increased systolic blood pressure by taking supplemental vit D and calcium in postmenopausal women. Nevertheless in another study Mateus-Hamdan L et al[135] "found a significant correlation between the serum level of PTH and hypertension but not" with vitamin D. Discrepancies in relationship between serum vit D across various studies may be attributed to several factors including age, sex, ethnic characteristics, and prevalence of coexisted comorbidities particularly HTN and vit D deficiency in the general population and the study groups. Additionally, several common chronic diseases such as vitamin D deficiency, diabetes, obesity, metabolic syndrome, and hypertension are prevalent in this geographic region[136][137]. These factors affect HTN and vitamin D status differently.

For Vitamin D, a cut off 17.75 had highest sensitivity 42.0% and specificity 40.0%. The area under the ROC curve was low and cannot be a predictor of hypertension. Similar observations were noted in the studies of Priya S et al[130], Ke L et al[27], Qi D et al[29] and O'Callaghan KM et al[128].

Priya S et al[130] cross‐sectional case–control study establishing a causal association between Vitamin D deficiency and HTN reported receiver operating characteristic curve revealed a low predictive value of 25(OH) D levels for predicting HTN with low sensitivity and specificity.

"Qi D et al[29] prospective study and meta-analysis determining the link between vitamin D concentrations and incident hypertension reported during a median follow-up of 2 years, 42.6% of the cohort (n = 1047) developed hypertension". "Compared with the 25-hydroxyvitamin D >30ng/ml, 25-hydroxyvitamin D <20 ng/ml was associated with a greater hypertension risk (OR: 1.225 [95% CI: 1.010 to

1.485] p = 0.04)", "although the association was attenuated and not statistically significant after adjusting for potential confounders (OR: 1.092 [95% CI: 0.866 to 1.377] p = 0.456)".

"O'Callaghan KM et al[128] interventional, observational narrative systematic review evaluating growing evidence of an association between low maternal vitamin D status" "and increased risk of hypertensive disorders reported conflicting data for an association of vitamin D with gestational hypertensive disorders in observational studies arises from a number of sources" "including large heterogeneity between study designs, lack of adherence to standardized perinatal outcome definitions, variable quality of analytical data for 25-hydroxyvitamin D (25(OH)D), and inconsistent data reporting of vitamin D status".

SUMMARY

A hospital based observational case control study was conducted with 100 patients to analyze whether low plasma vitamin D level is risk factor for primary hypertension or not. The patients are divided into two groups of 50 patients each as follows:

Cases: Patients of primary hypertension

Control: Healthy Patients that were age and sex matched individuals

The following observations were noted:

1. Majority of the patients (32%) in controls were from the age group of 31-40 years followed by 28% from the age group of 41-50 years, 26% from the age group of 51-60 years, 14% from the age group of 21-30 years. The mean age in controls was 21.2 ± 11.5 years. Majority of the patients (46%) in cases were from the age group of 51-60 years followed by 42% from the age group of 41-50 and 12% from the age group of 31-40 years. The mean age in cases was 18.02 ± 6.3 years. The difference in mean age of the patients in the two groups was statistically significant as per Student t-test ($p<0.05$).
2. The number of patients in both the groups was predominantly female (52%) respectively while male patients constituted 48% of the study population. The distribution of patients between two groups on basis of gender were comparable and statistically not significant as per Chi Square test ($p>0.05$).

3 The mean systolic blood pressure (SBP) value in controls was significantly lower as compared to cases (116.8 ± 6.7 vs. 155.0 ± 8.4 mmHg). The mean diastolic blood pressure (DBP) values in controls was significantly lower as compared to cases (74.5 ± 4.8 vs. 87.6 ± 5.2 mmHg).There was significant difference between the groups as per Student t-test (**$p<0.05$**).

4 The mean Vitamin D level in controls was higher as compared to cases (21.2± 11.5 vs. 18.0 ± 6.3ng/ml). However there was no significant difference between the groups as per Student t-test ($p>0.05$).

5 In controls, 8% patients had Vitamin D deficiency while 24% patients had Vitamin D sufficiency. In cases, 10% patients had Vitamin D deficiency while 84% patients had Vitamin D insufficiency. There was significant difference between the groups as per Chi-Square test (**$p<0.05$**).

6 In controls, the mean vitamin D levels was comparable across all age groups as per ANOVA test ($p>0.05$). In cases the mean vitamin D levels was lower in patients >40 years of age.

7 In both groups, the mean Vitamin D levels were comparable between male and female patients. There was no significant association of vitamin D levels and sex as per Student t-test ($p>0.05$).

8 The systolic and diastolic blood pressure values were significantly higher in cases as compared to controls in Vitamin D deficient, insufficient and sufficient patients. The association of Vitamin D levels was found to be significant with SBP and DBP values (**$p<0.05$**).

9 For Vitamin D, a cut off 17.75 had highest sensitivity 42.0% and specificity 40.0%. The area under the ROC curve was low and cannot be a predictor of hypertension.

CONCLUSION

Low Vitamin D levels are risk factors for primary hypertension. “Despite the accumulating evidence of a consistent link between vitamin D and blood pressure, the questions still remain in relation to the causality of this relationship”. “Further studies are needed to determine whether this represents a causal association. Large randomized controlled trials are also needed to determine whether vitamin supplementation may be beneficial in the prevention or the treatment of hypertension”. “More research is needed to further determine the role of 25-hydroxyvitamin D in hypertension prevention and therapy”.

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ANNEXURE-I

ETHICAL CLERANCE CERTIFICATE

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04/10/2016 *at* 3-00pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title A Study of plasma vitamin D levels as risk factor in primary Hypertension: a case control study in BLDE University

Name of P.G. student Shivaraj reddy kandala
General Medicine

Name of Guide/Co-investigator Dr M.S. Biradar
Professor of Medicine

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE II

CONSENT FORM

INFORMED CONSENT FORM	**:**	**TITLE OF RESEARCH: "A STUDY OF PLASMA VITAMIN D LEVELS AS A RISK FACTOR IN PRIMARY HYPERTENSION: A CASE CONTROL STUDY.**
GUIDE	**:**	**DR.M.S.BRADAR MD**
P.G.STUDENT	**:**	**DR.SHIVARAJ REDDY.K**

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to access plasma vitamin D levels as a risk factor in primary hypertension.

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved in this study and I may experience mild pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to study the plasma vitamin D levels is a risk factor in primary hypertension in this part of state.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulation of hospital. If the data is used for publication the identity will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further medical compensation.

_____ _____

(Signature of Guardian) (Signature of patient)

STUDY SUBJECT CONSENT FORM:

I confirm that **Dr.Shivaraj reddy.k** has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all above in detail in my own language and I understand the same. I agree to give my consent to participate as a subject in this research project.

SIGNATURE OF PARTICIPANT DATE

SIGNATURE OF WITNESS DATE

ANNEXURE III

PROFORMA

BLDEDU's SHRI B.M.PATIL MEDICAL COLLEGE

HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA

Name: **CASE NO:**

Age: **IP NO:**

Sex: **DOA:**

Religion: **DOD:**

Past Occupation:

Present Occupation:

Residence:

Chief complaints:

History of present illness:

Past History:

History of IHD

History of tuberculosis

History of diabetes mellitus

History of Hepatic or Renal diseases

Personal History:

Diet/appetite:

Sleep:

Bladder and bowel habits:

Smoking/Tobacco chewing/Snuff Inhalation/alcohol:

Family History:

TB: Asthma: Malignancy: DM: HTN:

Treatment History:

General Physical Examination

Height :

Weight:

Body Mass Index :

Vitals

Pulse rate:

Blood prssure:

Respiratory rate:

Temp:

Head to toe examination:

SYSTEMIC EXAMINATION.

- **Respiratory System**

- **Cardiovascular System**

- **Central Nervous System**

- **Per abdomen**

INVESTIGATIONS

HAEMATOLOGY –

Haemoglobin	gm %
Total WBC counts	Cells/mm^3
Differential counts -	
Neutrophils	%
Lymphocytes	%
Eosinophils	%
Monocytes	%
Basophils	%
ESR	mm after 1 hour

SR. CREATININE:

SR. SODIUM:

SR. POTASSIUM:

ECG: **FUNDOSCOPY:**

CHEST X RAY:

PLASMA VITAMIN D:

URINE EXAMINATION -

Albumin	
Sugar	
Microscopy	

FINAL DIAGNOSIS

TREATMENT GIVEN

OUTCOME:

Improved:

ANNEXURE III

KEY TO MASTER CHART

%	-	PERCENTAGE
S.NO	-	SERIAL NUMBER
IP.NO	-	IN PATIENT NUMBER
OP.NO	-	OUT PATIENT NUMBER
M	-	MALE
F	-	FEMALE
yr	-	YEAR
mts	-	MONTHS
H/O	-	HISTORY OF
HTN	-	HYPERTENSION
IHD	-	ISCHEMIC HEART DISEASE
DM	-	DIABETES MELLITUS
PR	-	PULSE RATE
SBP	-	SYSTOLIC BLOOD PRESSURE
DBP	-	DIASTOLIC BLOOD PRESSURE
bpm	-	BEATS PER MINUTE
FBS	-	FASTING BLOOD SUGAR
ECG	-	ELECTROCARDIOGRAM
BMI	-	BODY MASS INDEX
HB	-	HAEMOGLOBIN
TC	-	TOTAL COUNT
CX RAY	-	CHEST X RAY
Na	-	SODIUM
K	-	POTASSIUM
Kg/m^2	-	KILOGRAM PER METER SQUARE
Mg/dl	-	MILLI GRAM PER DESI LITER
Ng/ml	-	NANO GRAM PER MILLILITER
Mmol/l	-	MILLIMOLES PER LITER
N	-	NORMAL
wnl	-	WITHIN NORMAL LIMIT

MASTER CHART

CASES

										BP									
S.NO	**PATIENT NAME**	**IP/OP NO.**	**SEX**	**AGE yrs**	**H/o HTN**	**H/O DM**	**H/O IHD**	**VIT.D ng/ml**	**PR bpm**	**SBP mmHg**	**DBP mmHg**	**FBS mg/dl**	**ECG**	**SR.CREATININE mg/dl**	**BMI kg/m2**	**FUNDOSCOPY**	**HB%**	**TC**	**CX RAY**	**Na mmol/l**	**K mmol/l**
1	LESAPPA	1786	M	42	1 yr	NO	NO	17.9	76	154	90	75	wnl	1	20	N	16	9060	N	131	4.1
2	PARVATHI.T	19086	F	55	2yr	NO	NO	15.8	84	146	80	93	wnl	0.6	22.4	N	12.4	6850	N	131	4.2
3	MAHADEVI BIRADAR	446680	F	45	2yr	NO	NO	24.2	88	150	90	88	wnl	0.6	22.5	N	11.6	9880	N	136	3.9
4	SHIVAGANGAWWA	42731	F	50	1yr	NO	NO	16	68	180	90	110	wnl	1	19.7	N	12.7	11200	N	133	3.5
5	ANNAPURNA	448	F	60	1yr	NO	NO	15.3	68	160	90	98	wnl	0.7	20.7	N	13.6	10620	N	133	4.4
6	BABUDAREPPA	430	M	48	4yr	NO	NO	23	74	160	90	104	wnl	1.4	23	N	10.5	6480	N	136	5.6
7	SUMITRA	953	F	60	3yr	NO	NO	16.6	74	160	90	97	wnl	0.8	23.6	N	10	11740	N	131	4
8	VITTAL NINGAPPA	177	M	53	2yr	NO	NO	27.4	72	150	80	97	wnl	0.9	25.7	N	15.3	4570	N	138	3.5
9	VITTAL	39450	M	35	6mt	NO	NO	17.9	76	150	80	130	wnl	0.8	22	N	13.6	7000	N	136	4.5
10	FATIMA	40014	F	60	1yr	NO	NO	16.5	76	160	100	92	wnl	0.8	21	N	10.6	10680	N	140	4.5
11	YALLAWWA LAXMAN	29474	F	60	6mt	NO	NO	10.8	88	148	90	100	wnl	0.7	21.7	N	13.8	10080	N	138	4.4
12	HANUMAWWA	23450	F	60	1yr	NO	NO	27.8	84	160	94	103	wnl	0.8	23	N	12	8990	N	142	3.8
13	BOURAMMA	637	F	60	2yr	NO	NO	12	82	140	80	95	wnl	0.8	22.7	N	14.3	10150	N	140	4.5
14	BASAVARAJ.N	588	M	45	2yr	NO	NO	15.8	76	146	90	109	wnl	1.2	23.3	N	13.7	5830	N	124	5.1
15	SHANTAMMA	2100	F	45	6mt	NO	NO	12.6	74	130	90	110	wnl	0.6	20.7	N	12.5	9000	N	138	4
16	SAROJINI	3979	F	50	2mt	NO	NO	9	72	170	90	107	wnl	0.8	21.4	N	11.6	8920	N	142	4.9
17	NARESH.K	54371	M	46	4mt	NO	NO	17.5	72	150	90	88	wnl	1	23.5	N	14.9	4560	N	139	4.7
18	MAHADEV	5176	M	55	5yr	NO	NO	15.5	72	150	90	102	wnl	0.8	21.8	N	15.4	5790	N	137	4.6
19	APPU CHADAKAVATE	287660	M	55	3yr	NO	NO	16.4	68	152	88	98	wnl	1	24	N	18.9	8990	N	138	4
20	HANUMANTHARAYYA	5816	M	32	2mt	NO	NO	8.1	76	160	90	126	wnl	0.7	21.4	N	15.6	4460	N	132	4.6
21	SIDDARAMAPPA	6055	M	55	1week	NO	NO	13.7	84	160	90	91	wnl	0.8	21.4	N	11.1	10020	N	134	4.5
22	SHANTABAI	7040	F	55	2mt	NO	NO	18.4	94	152	80	64	wnl	0.8	22.2	N	14	10000	N	132	3.6
23	S.P SHIVAGADDAGI	60788	M	32	2week	NO	NO	12.6	78	150	90	117	wnl	0.9	23.7	N	16.2	7610	N	143	4
24	SIDHLINGAWWA	6460	F	60	6mt	NO	NO	15.2	70	156	90	92	wnl	1	22.7	N	10.2	7690	N	137	5.2

25	BORAMMA	75035	F	54	3yr	NO	NO	15	84	146	80	90	wnl	0.6	24.6	N	10.2	6670	N	140	4.2
26	MANGALA.B	44140	F	45	1yr	NO	NO	13	88	150	80	110	wnl	0.8	23.6	N	14.2	9000	N	136	3.8
27	MEHABOOB	6903	M	55	2yr	NO	NO	20	96	154	80	97	wnl	1.1	23.2	N	15.7	5670	N	138	4.5
28	TIPPANNA BELAGAL	202192	M	56	2yr	NO	NO	21.9	74	160	86	96	wnl	1.3	20.8	N	18.4	7860	N	136	4.2
29	RAJU.M	9867	M	50	8mt	NO	NO	19.6	78	156	90	92	wnl	0.9	22.1	N	16.7	6100	N	136	4
30	PARVATHI.B	10406	F	45	8mt	NO	NO	28.9	72	150	90	108	wnl	0.6	22.4	N	13.8	7960	N	134	4.7
31	SAROJINI	10448	F	40	2weeks	NO	NO	35.3	74	154	92	77	wnl	1.6	20	N	11.7	9840	N	138	3.6
32	BASUGOND.V	10874	M	40	1yr	NO	NO	32.4	74	156	96	75	wnl	1.2	21.4	N	11.8	5700	N	126	3.8
33	AYUB.K	121310	M	53	6mt	NO	NO	21.9	76	150	96	107	wnl	0.9	22.8	N	14.1	7990	N	124	4.3
34	BOURAMMA.B	11302	F	45	1week	NO	NO	17.4	80	160	90	67	wnl	0.7	24.8	N	12.5	4560	N	142	3.6
35	RAJSHEKAR	11724	M	55	2yr	NO	NO	8.1	74	156	92	82	wnl	1.1	22.8	N	11.5	5670	N	144	3.5
36	BASAGONDA	11289	M	60	1yr	NO	NO	18	84	146	80	85	wnl	1	24	N	15.3	9990	N	143	4.6
37	REVAPPA BIRADAR	288313	M	44	3mt	NO	NO	14	86	160	80	100	wnl	0.8	23.3	N	16.3	3400	N	134	3.6
38	SHREESHAIL.S	129346	M	55	1mt	NO	NO	21	84	150	80	78	wnl	0.9	23.2	N	14.5	6400	N	138	4.1
39	IRAMMA.S	12888	F	57	6mt	NO	NO	30	84	152	90	102	wnl	0.6	20.7	N	13.7	8640	N	132	4.4
40	MANGALA BIRADAR	44140	F	45	2yr	NO	NO	13	74	160	90	100	wnl	0.6	19.5	N	12.4	9700	N	132	4.2
41	BOURAMMA	12612	F	49	1yr	NO	NO	17.6	96	148	90	97	wnl	0.7	20	N	12.1	7150	N	140	3.6
42	SHANTABAI	12413	F	55	2weeks	NO	NO	10.1	66	180	90	85	wnl	0.6	22.4	N	11.4	7800	N	132	3.8
43	CHANDRABAGA	143217	F	60	1yr	NO	NO	16.8	72	156	80	99	wnl	1	20.8	N	12.6	6578	N	141	3.8
44	MAMATAZBI.D	12766	F	44	1yr	NO	NO	23.7	90	160	86	103	wnl	0.8	21.4	N	12	8880	N	141	4.1
45	ANANDA.S	15945	M	41	1mt	NO	NO	27.4	84	160	86	100	wnl	1.2	20.7	N	12.4	8220	N	139	2.8
46	NIRMALA	137399	F	45	1mt	NO	NO	20.1	82	152	80	84	wnl	0.6	18.7	N	14	9010	N	138	3.9
47	KASHINABEE.B	12190	F	55	1yr	NO	NO	9.3	76	160	90	95	wnl	0.7	21.4	N	12.3	9670	N	144	5
48	RAJESHWARI.V	8092	F	53	8mt	NO	NO	8.1	78	164	90	108	wnl	0.8	23.2	N	14.9	8140	N	133	3.4
49	SIDDARVD.N	54872	M	40	1week	NO	NO	27.6	78	156	90	104	wnl	0.8	23	N	14.8	7710	N	140	4.3
50	RIYAZ.H	23055	M	45	8mt	NO	NO	17.2	80	164	88	98	wnl	1	20	N	14.6	7020	N	136	3.8

CONTROLS

										BP									
S.NO	**PATIENT NAME**	**IP/OP NO.**	**SEX**	**AGE yrs**	**H/O HTN**	**H/O DM**	**H/0 IHD**	**VIT.D ng/ml**	**PR bpm**	**SBP mmHg**	**DBP mmHg**	**FBS mg/dl**	**ECG**	**SR. CREATININE**	**BMI kg/m2**	**HB%**	**TC**	**CX RAY**	**Na mmol/l**	**K mmol/l**
1	BABUGOUDA	1674	M	40	NO	NO	NO	22.3	76	110	70	71	Wnl	0.6	20.2	12.1	9780	N	132	3.5
2	VIJAYALAXMI	19223	F	33	NO	NO	NO	20.7	78	116	70	96	Wnl	0.5	19.2	12.8	5150	N	130	4.5
3	IRAMMA	42475	F	45	NO	NO	NO	30	84	110	70	100	Wnl	0.8	22	12.3	11600	N	135	4.1
4	YANKAVVA	18411	F	40	NO	NO	NO	19.5	86	120	70	90	Wnl	0.7	17.8	12	10530	N	130	4.1
5	HASINA	18085	F	45	NO	NO	NO	13	86	116	70	100	Wnl	0.7	21.4	10.7	11710	N	132	4.6
6	MALLANAGOUDA	20826	M	55	NO	NO	NO	25	84	110	70	70	Wnl	1.1	20.7	13.6	8150	N	141	4.3
7	SUNDRABAI	22315	F	45	NO	NO	NO	25	68	126	80	110	Wnl	0.9	21.4	10.1	14490	N	131	5.4
8	PARASURAM	22662	M	37	NO	NO	NO	19.5	78	120	80	105	Wnl	0.6	21.4	15.5	7650	N	136	3.8
9	MAIBOOB	39387	M	33	NO	NO	NO	34.1	76	110	70	70	Wnl	0.8	24.2	15.1	7620	N	138	4.2
10	SUSHILABAI	427	F	55	NO	NO	NO	16	74	116	80	109	Wnl	0.6	20	11.2	8600	N	135	4.4
11	RATNABAI .B	18035	F	56	NO	NO	NO	21	74	108	74	96	Wnl	0.6	23.7	11.8	8630	N	137	4
12	BHAGAWWA	22507	F	60	NO	NO	NO	13.9	74	126	70	105	Wnl	0.6	19.1	12.5	10220	N	147	3.5
13	SUNITHA.G	28473	F	35	NO	NO	NO	30.1	96	110	70	89	Wnl	0.8	21.7	12.2	6100	N	139	4.7
14	BASAVARAJ.G	26885	M	47	NO	NO	NO	8.1	88	120	80	103	Wnl	0.7	22	11	8600	N	135	4.3
15	LAXMIBAI	24251	F	45	NO	NO	NO	13	78	110	76	99	Wnl	0.6	21.1	11.5	8250	N	141	5.1
16	REKHA.G	1281	F	42	NO	NO	NO	13	86	126	70	90	Wnl	0.6	23.6	13.5	7460	N	140	4.3
17	AVINASH	4404	M	28	NO	NO	NO	17.3	76	120	80	90	Wnl	0.8	21.4	15.1	7280	N	140	5.4
18	SANJAY.G	24329	M	40	NO	NO	NO	11.3	76	126	70	103	Wnl	1	23.2	10.9	5400	N	136	3.9
19	BABU BHIMARAYA	29502	M	60	NO	NO	NO	16	86	112	74	83	Wnl	0.8	22.8	12.6	7510	N	137	4.3
20	MANJUNATH.S	1498	M	23	NO	NO	NO	21	82	120	80	84	Wnl	0.6	23	14	8270	N	126	4.9
21	BASHA.A	6037	M	59	NO	NO	NO	20	84	120	80	87	Wnl	1.3	22.1	14.5	9610	N	138	3.9
22	MANJULA.A	23479	F	39	NO	NO	NO	25.5	72	116	70	87	Wnl	0.6	20.8	10.7	9700	N	132	3.8
23	SANTOSH.B	4434	M	22	NO	NO	NO	8.1	72	120	80	100	Wnl	1.4	24.2	13.6	5400	N	144	4.6
24	GURULINGAWWA	7285	F	60	NO	NO	NO	13.8	74	126	80	84	Wnl	1.8	21.7	12.6	9870	N	138	4.3
25	S.L HEBALLATI	43885	F	35	NO	NO	NO	8.1	76	120	80	91	Wnl	0.7	24.5	12.3	8790	N	134	3.7
26	SUVARNA.M	4042	F	37	NO	NO	NO	17	78	110	70	97	Wnl	0.6	21.8	10.3	3530	N	140	3.4
27	T.M SHEKAR	62743	M	52	NO	NO	NO	14.8	96	120	70	100	Wnl	0.9	24.5	14.3	8150	N	134	3.7
28	GARIBSAB.SAIFANSAB	29496	M	24	NO	NO	NO	18.6	68	122	80	91	Wnl	1	22.1	13	4200	N	133	3.6
29	AYYANNA	10524	M	51	NO	NO	NO	22.9	64	126	80	90	Wnl	0.7	20.7	15.2	10520	N	141	4.3
30	BHARATHI.V	8948	F	50	NO	NO	NO	8.9	76	120	80	87	Wnl	0.6	20.4	11.6	4370	N	135	3.8
31	SUMITRA	10529	F	44	NO	NO	NO	14.4	78	116	80	105	Wnl	0.6	20.9	11.4	8360	N	140	4

32	PARASURAM.M	10066	M	40	NO	NO	NO	18.6	72	110	70	96	Wnl	1.1	21.4	15.6	9450	N	147	3.5
33	M.V DAYABERI	136643	M	33	NO	NO	NO	29.8	76	120	80	109	Wnl	1.1	23.7	16	7760	N	134	3.8
34	KAVITHA.T	2834	F	37	NO	NO	NO	21	88	126	80	84	Wnl	0.8	22	12.4	9820	N	138	4.7
35	MALLAPPA.B	12521	M	43	NO	NO	NO	22	76	110	70	106	Wnl	1	21.4	15.6	9950	N	143	5.3
36	SAHEBGOUDA	28716	M	45	NO	NO	NO	30	74	126	80	106	Wnl	0.9	24.2	12.4	9200	N	130	4.2
37	CHANDRASHEKAR	29050	M	48	NO	NO	NO	26	74	122	80	100	Wnl	1	21.4	14.8	9510	N	136	3.8
38	Y.K BAJANTRI	144464	M	30	NO	NO	NO	29.9	68	114	70	90	Wnl	0.8	20	16.2	9490	N	144	3.7
39	GUNASAGARI	12278	F	54	NO	NO	NO	16.9	92	126	80	83	Wnl	0.7	21.2	11.1	5170	N	146	3.8
40	SUJATHA.B	17023	F	28	NO	NO	NO	36.1	90	128	72	95	Wnl	0.7	20.6	11.7	2100	N	134	3.7
41	SUNANDA.S	23382	F	31	NO	NO	NO	11.6	84	120	80	100	Wnl	0.6	21.6	11.9	2290	N	138	3
42	LAXMIBAI	16864	F	55	NO	NO	NO	19.6	82	100	70	98	Wnl	1.6	17.1	12.1	11000	N	142	3
43	BHAGYASHREE	13124	F	49	NO	NO	NO	16.6	72	118	70	93	Wnl	0.8	20	10.8	7030	N	142	4.3
44	MEENAZ.S	12816	F	25	NO	NO	NO	21.6	78	126	70	97	Wnl	0.6	19.1	10.2	8680	N	138	3.8
45	HANUMATH INGOLI	287955	M	50	NO	NO	NO	22	70	110	70	104	Wnl	0.8	22.4	10.8	4520	N	134	3.4
46	BHARATHI.R	23870	F	29	NO	NO	NO	84.4	86	110	72	94	Wnl	0.6	22.7	12	9840	N	140	3.8
47	SUREKHA	7737	F	32	NO	NO	NO	11.7	84	130	80	102	Wnl	0.8	22.2	11.6	10270	N	137	3.2
48	KASTURIBAI	7658	F	60	NO	NO	NO	21	84	108	70	100	Wnl	1	24	12.4	9800	N	131	4.1
49	UTTAM	25990	M	28	NO	NO	NO	36.9	80	110	74	92	Wnl	0.8	23.2	13.4	10900	N	133	4.5
50	SATAYAPPA.A	23242	M	58	NO	NO	NO	33.4	72	110	78	108	Wnl	0.6	21.8	11.1	7600	N	135	4.1