A STUDY OF SERUM TOTAL CALCIUM AND SERUM CALCIUM / PHOSPHORUS RATIO IN ESSENTIAL HYPERTENSION AND ITS CORRELATION WITH SEVERITY OF THE DISEASE

DISSERTATION SUBMITTED FOR

MD GENERAL MEDICINE

BRANCH - I

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THE TAMIL NADU

DR. M. G. R. MEDICAL UNIVERSITY

CHENNAI, TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled **"A STUDY OF SERUM TOTAL CALCIUM AND SERUM CALCIUM / PHOSPHORUS RATIO IN ESSENTIAL HYPERTENSION AND ITS CORRELATION WITH SEVERITY OF THE DISEASE"** is the bonafide work of **DR. KUNUNGOYI DOMEH** in partial fulfilment of the university regulations of The Tamil Nadu Dr. M.G.R. Medical University, Chennai, for **M.D General Medicine BRANCH - I** examination to be held in **April 2015**

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I, **DR. KUNUNGOYI DOMEH**, hereby solemnly declare that, this dissertation entitled **"A study of serum total calcium and serum calcium / Phosphorus ratio in essential hypertension and its correlation with severity of the disease"** is a bonafide record of work done by me at the Department of General Medicine, Government Rajaji Hospital, Madurai under the guidance of **Dr. V. T. PREMKUMAR, M.D**, Professor, Department of General Medicine, Madurai Medical college, Madurai.

This dissertation is submitted to the Tamil Nadu Dr. M. G. R. Medical University, Chennai in partial fulfilment of the rules and regulations of the award of **M.D General Medicine Branch - I** examination to be held in **April 2015**.

Place: Madurai

Date: **DR. KUNUNGOYI DOMEH**

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LIST OF ABBREVIATIONS

SBP – systolic blood pressure

DBP – diastolic blood pressure

PIH – pregnancy induced hypertension

DM – diabetes mellitus

LVH – left ventricular hypertrophy

BMI – body mass index

HDL – high density lipoprotein

LDL – low density lipoprotein

Na – sodium

Ca – calcium

P – phosphorus

ATP – adenosine triphosphate

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Abstract:

Introduction: Hypertension significantly increases the risk of mortality and morbidity of cerebrovascular accidents (both ischaemic and haemorrhagic), coronary artery disease, congestive heart failure, chronic kidney failure, and peripheral vascular diseases. Hypertension related complications have become one of the most important contributor to preventable deaths and diseases in our country too, with an alarmingly increasing rate in the rural areas. More than 140 million people are said to be suffering from hypertension in India and it has been estimated to overhaul the 214 million mark by 2025.

Settings and Design: A case-control study involving 100 subjects. Subjects were grouped into 50 healthy controls and 50 cases of essential hypertension. Concentrations of serum total calcium and serum calcium phosphorus ratio were measured in the participants and compared in hypertensive patients and normotensive individuals.

Results: The study showed that serum total calcium were significantly decreased (p Value <0.001) and serum calcium phosphorus ratio is increased (p Value <0.001) in hypertensive patients as compared with normotensive individuals.

Conclusions: Essential hypertension has an inverse association with serum total calcium and positive correlation with calcium phosphorus ratio which suggests a major role in the pathogenesis of essential hypertension.

Keywords: essential hypertension, serum total calcium, serum calcium phosphorus ratio

1. Introduction

Hypertension is an increasingly important medical and public health burden globally. Worldwide, hypertension is estimated to cause 7.7 million deaths,which is about 12 – 15 % of all total deaths.

Hypertension significantly increases the risk of mortality and morbidity of cerebrovascular accidents (both ischaemic and haemorrhagic), coronary artery disease, congestive heart failure, chronic kidney failure, and peripheral vascular diseases. Hypertension related complications have become one of the most important contributor to preventable deaths and diseases in our country too, with an alarmingly increasing rate in the rural areas. More than 140 million people are said to be suffering from hypertension in India and it has been estimated to overhaul the 214 million mark by 2025. The World Health Organization estimated in 2008 that 33 % men and 32 % women older than 25 years in India suffer from hypertension.

Cardiovascular diseases resulted in over 2.3 million deaths in India in 1990. This is expected to double by the next decade in the country. It has been clearly established that Hypertension alone is directly responsible for 57% of all cerebrovascular accident deaths and 20-24 % of all cardiovascular deaths in India.

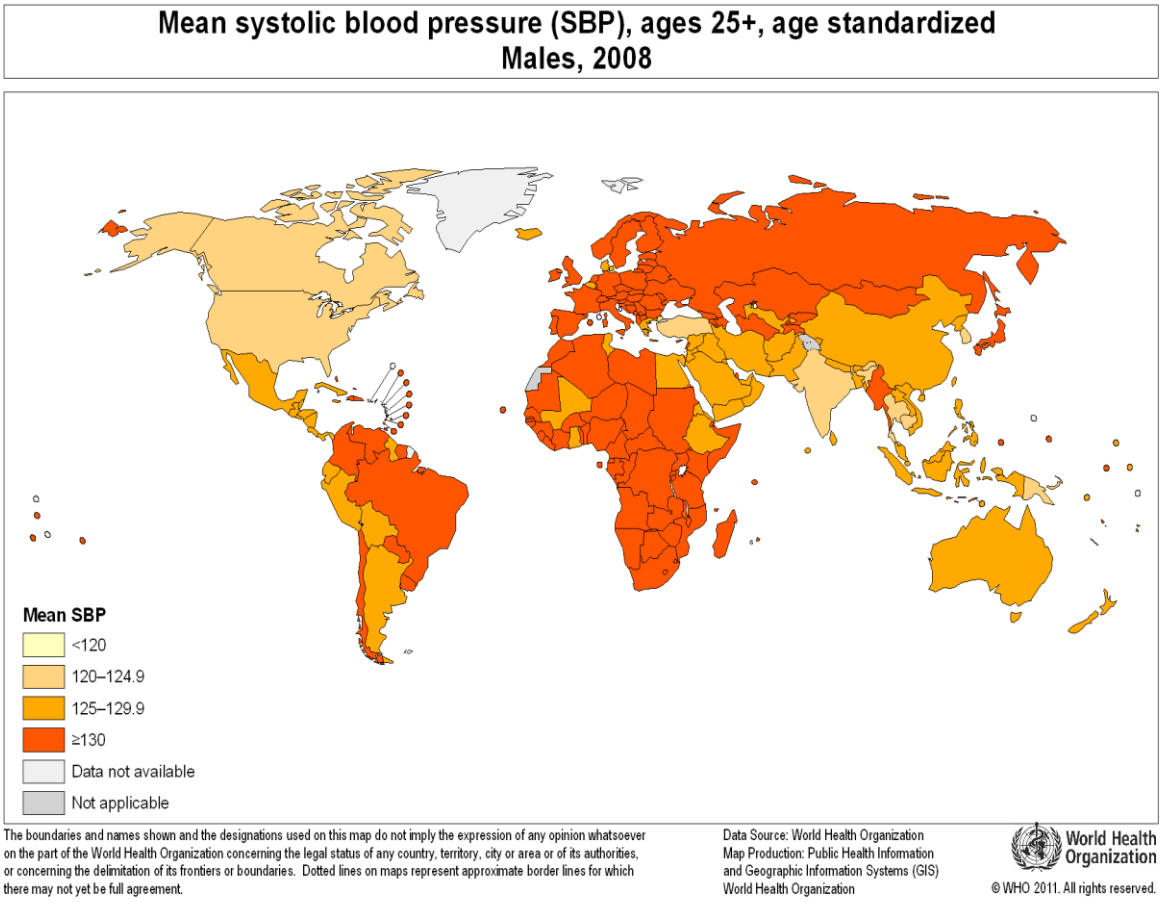


FIG 1: GLOBAL DISTRIBUTION OF HYPERTENSION (WHO 2008)

An Integrated Disease Surveillance Prevalence Survey in 2007-08 indicated that a large number of populations in India are in pre-hypertension category. Mizoram showed 58.5 % people in pre-hypertension stage. However, the actual percentage of population suffering from hypertension was modest comprising of only 19 %. Other states in descending order of prevalence of pre-hypertension were Uttarakhand (48.8 %), Kerala (48.10 %) and Maharashtra (46.2 %) respectively. MP, Tamil Nadu and AP are the other States that have over 40 % of the population in the pre-hypertension stage. The list is given below in the chart.

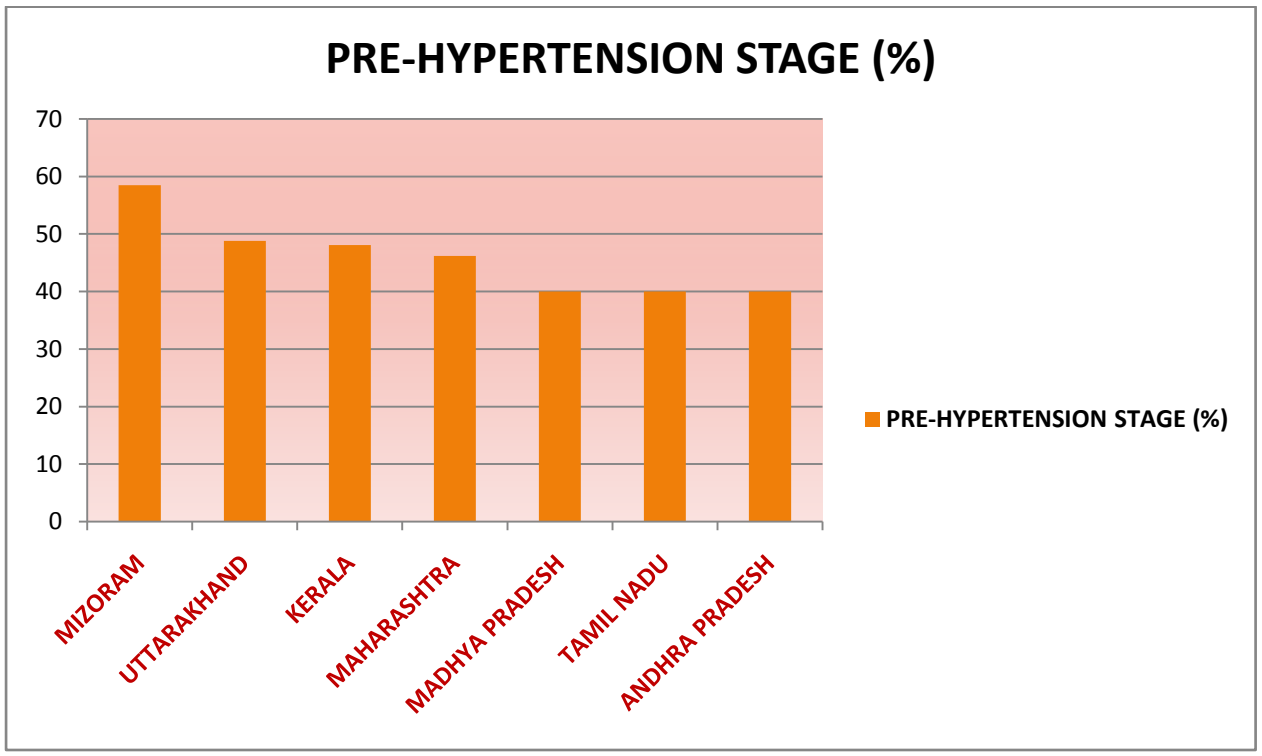


FIG 2: STATES IN INDIA WITH HIGH PREVALENCE OF PREHYPERTENSION STAGE

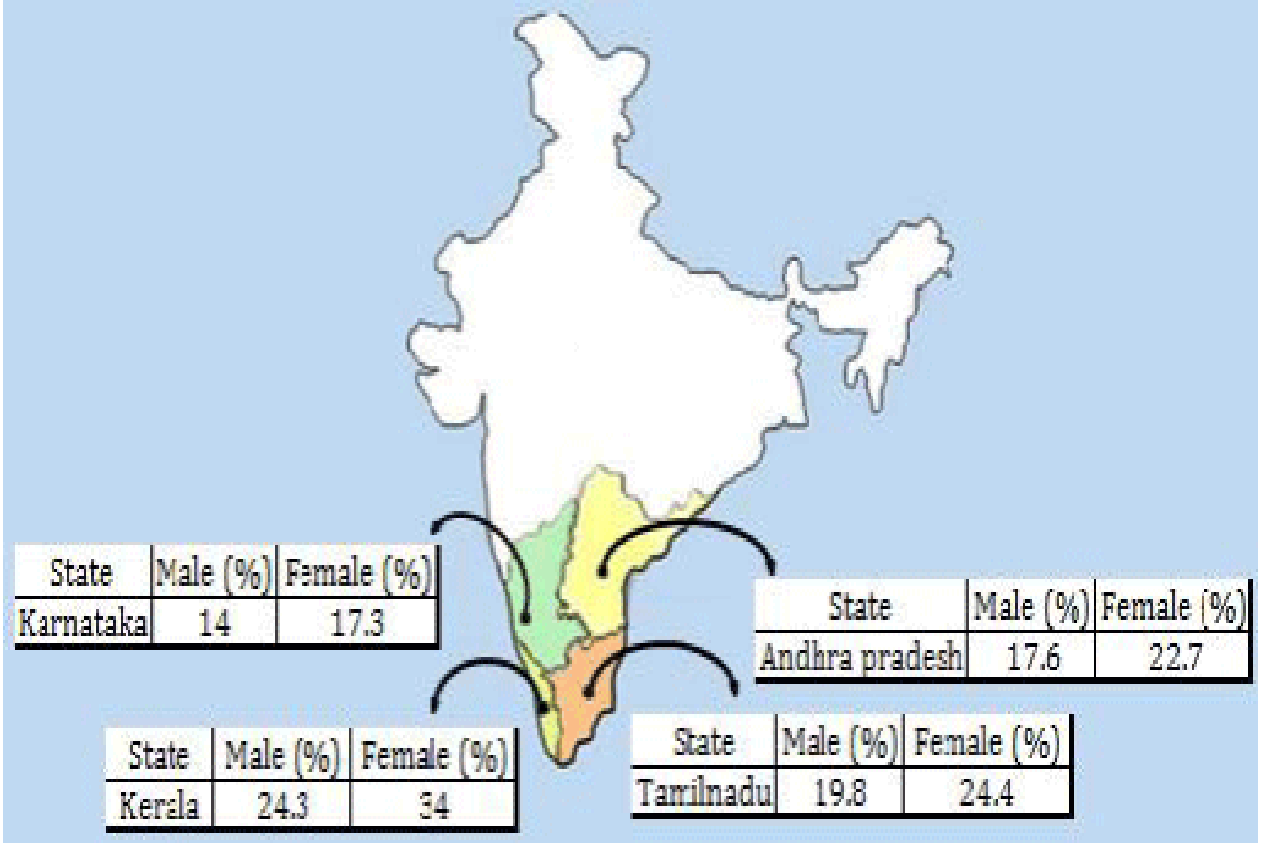


FIG 3: PREVALENCE OF PRE-HYPERTENSION POPULATION IN SOUTH INDIA

Primary hypertension has remained a major modifiable risk factor for cardiovascular disease even though tremendous advances have been made in understanding its pathogenesis and effective treatment guidelines have been laid down by the medical fraternity. Hypertension increases the risk of Cardiovascular disease for millions of people globally, a contradiction in the face of modern medicine and worse, it is increasing at an alarming rate.

Essential hypertension is that type of hypertension where all the secondary causes have been ruled out. It comprises of 85-95% of adults hypertension.

Essential hypertension is that type of hypertension, for which, no cause can be identified, affecting around 95% of all hypertensive individuals.

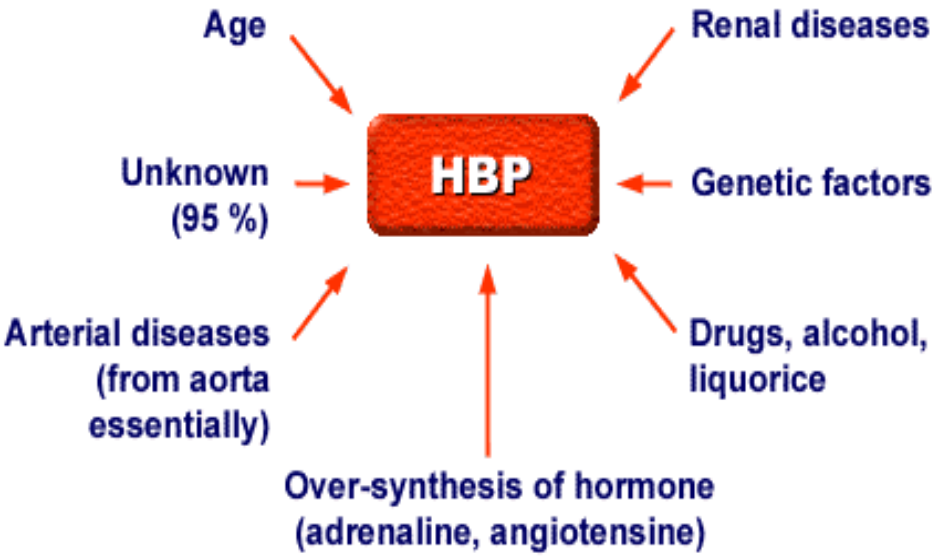


FIG 4: CAUSES OF HYPERTENION IN ADULTS

The primary characteristic of essential hypertension is an increased tone in vascular smooth muscle which results in an increase in total peripheral resistance.

It has been postulated in many studies is that changes in the serum calcium is the key regulator in the contraction of vascular smooth muscle cells. Therefore, Any situation which is associated with an increased strength of smooth muscle contraction must either alter the Calcium level itself, change the behaviour of the contractile apparatus to Calcium, or exert both effects.

Apart from its role in the contraction of vascular and cardiac smooth muscle, Calcium also plays a major role as an intracellular messenger in the response to noradrenaline, adrenaline, and angiotensin, the release of noradrenaline, the rate of secretion of aldosteron by adrenal glomerulosa cells, and probably the release of renin from juxtaglomerular cells. Furthermore, many vasodilatory and other antihypertensive agents exert calcium dependent mechanisms, their effects by interference with or by reduction of intracellular calcium concentration.

Whatever mechanisms are involved in the pathogenesis of primary hypertension the final consequence must be, in all likelihood, a disorder of cellular calcium mechanism or, more specifically, in the functioning of the cell membrane. In

recent years a various models of calcium metabolism involving cellular calcium level, membrane binding transport, altered premeability and transport kinetics, have been described in individuals with hypertension and in animal models as well. Also, many studies have suggested that general calcium handling may be altered in essential hypertension. These findings indicate a likely role for the calcium ion in the pathophysiology of essential hypertension. In this study, we attempt to review the evidence that altered calcium metabolism, thereby change in serum calcium levels are implicated in primary hypertension.

Epidemiology:

The incidence and prevalence of hypertension varies among different countries and geographical regions or even communities within a country. In India urban areas have higher prevalence rate as compared with rural areas. However, recent data suggests that the trend is changing during the last three decades. In adults, diastolic BP increases with age till 50 – 60 years of age, after which, it follows a decreasing pattern.

Recent studies had indicated that over 1 billion adults had high blood pressure in 2000, which is expectedted to increase to 1.56 billion by 2030. The prevalence of high blood pressure in the l9th and 20th centuries was variable in different studies in India, which ranged from 3-16% in Urban population and 3-7% in Rural population. Review of recent data indicate that prevalence of high blood pressure is increasing exponentially in both urban and rural population and currently is 20-25% in small towns and cities and 10-16% in villages. The global status on Non Communicable Diseases report in 2011 showed that over 2.6 million deaths occurred from Cardiovascular disease alone in our country in 2008, mainly from coronary artery disease and cerebrovascular accident.

Several surveys on the prevalence of high blood pressure in India has been done

since post – independence era. In fact, one of the earliest well known study was done in 1963 by Mathur and associates at Agra in the urban population. Since then, many regional and national studies have been done in both rural and urban population. Some of the earliest as well as most recent studies done in India are mentioned below in the table.

Table 1: Post-Independence era studies from 1963 – 1999 on prevalence of hypertension in Urban adults of India

Authors	Area	Year	Age	Prevalence		Prevalence	
				Men		Women	
				%age	sample	%age	sample
Mathur	Agra	1963	>20	3.98	1408	6.64	227
Malhotra	Railways	1970	20-58	6.2	2638	----	----
Gupta	Rohtak	1978	>20	6	1151	7	872
Chaddha	New Delhi	1990	25-64	11.66	637	13.68	17351
Gupta R	Jaipur	1995	>20	30	1415	34	797

Table 2 : Recent studies from 2000 – 2012 on prevalence of hypertension in Rural adults of India

Authors	Area	Year	Age	Prevalence		Prevalence	
				Men		Women	
				%age	sample	%age	sample
Gupta SP	Haryana	1977	20-69	3.5	1154	3.69	891
Baldwa	Rajasthan	1984	>20	6.93	447	8.81	465
Jajoo	Maharashtra	1993	>20	2.89	2247	4.06	1789
Agarwal	UP	1994	>20	1.57	3760	----	----
Malhotra	Haryana	1999	16-70	3.0	2259	5.80	----

Table 3 : Recent studies from 2000 – 2012 on prevalence of hypertension in Urban adults of India

Authors	Year	Area	Age (yr)	Sample Size	Prevalence(%)
Anand MP	2000	Mumbai	30-60	1662	34.0
Gupta PC	2004	Mumbai	≥ 35	88653	47.9
Prabhakaran	2005	Delhi	20-59	2935	30.0
Reddy KS	2006	National	20-69	19973	27.2
Mohan V	2007	Chennai	≥ 20	2350	20.0
Kaur P	2007	Chennai	18-69	2262	27.2
Yadav S	2008	Lucknow	≥ 30	1746	32.2

Table 4 : Recent studies from 2000 – 2012 on prevalence of hypertension in Rural adults of India

Author	Year	Area	Age (yr)	Sample	Prevalence(%)
Hazarika	2004	Assam	>30	3180	33.3
Thankappan	2006	Kerala	>30	2159	36
Krishnan	2008	Harayana	15-64	2828	9.3
Todkar SS	2009	Maharashtra	≥ 20	1297	7.2
Vijaykumar	2009	Kerala	≥18	1990	36.1
Bhardwaj	2010	Himachal	≥ 18	1092	35.9
Kinra	2010	National	20-69	1983	20.0

Factors responsible for the increasing trend of hypertension has been attributed to increased longevity, lifestyle modifications, example sedentary life, obesity, less physical work, etc., dietary changes like smoking, high fat content, increased salt intake, environmental and genetic factors.

Despite the high prevalence rate of hypertension in India, awareness of hypertension and its implications is still low among the general population and the patients seeking treatment for hypertension, still lower. Preventive measures and mass education at grass root level is in need today to deal with hypertension which has become a national health burden.

DEFINITION AND CLASSIFICATION:

In clinical terms, hypertension is defined as that level of blood pressure at which the institution of therapy reduces blood pressure related morbity and mortality.

Currently, the criteria for defining hypertension is based on average of 2 or more blood pressure readings in sitting position in at least two outpatient visits. The current recommended criteria for diagnosis of hypertension at home is the average awake blood pressure of more than 135/85 mmHg and asleep blood pressure of more than 120/75 mmHg which are roughly equal to a clinic blood pressure of 140/90 mmHg.

Classification of Hypertension:

JNC – VII Classification: American Medical Association

Blood Pressure Classification in Adults (JNC 7)

BP Classification	SBP (mm Hg)	DBP (mm Hg)
Normal	< 120	and < 80
Prehypertension	120 – 139	or 80 – 89
Stage 1 Hypertension	140 – 159	or 90 – 99
Stage 2 Hypertension	≥ 160	or ≥ 100

Prehypertension per se is not a disease category. Rather it is a term used to recognize individuals who are at high risk of developing hypertension so as to facilitate the awareness to both the patient and clinician to the risk and proper strategies implemented to prevent or delay the disease from progressing further. Such persons should ideally be advised to change lifestyle and diet to minimize the risk of high blood pressure in their later lives.

On the other hand, individuals with prehypertension who also have other associated comorbitities should be started with appropriate drug therapy if their blood pressure remains elevated more than 130/80 mmHg .

Several terms have been coined by various authors for describing hypertension associated with specific conditions or settings.

White coat Hypertension - Hypertension in the hospital/clinic

Systolic Hypertension - Systolic BP more than 140 mmHg but less than 80 mmHg diastolic (probably age related)

Malignant Hypertension - Very high Blood Pressure with signs of end organ damage

Labile Hypertension - Blood Pressure which shows variable readings

Accelerated Hypertension - very high Blood Pressure detected recently

Pseudo Hypertension - High blood pressure due to atherosclerosis

Pulmonary Hypertension - High Blood Pressure due to congestion in the lungs

Renovascular Hypertension - High blood pressure owing to narrowed blood vessel in the kidney

Pre-eclampsia - High blood pressure induced by pregnancy

Secondary Hypertension - Hypertension due to a known causating factor

Borderline Hypertension - Blood Pressure in the grey zone

RISK FACTORS:

Age: The risk of hypertension increases through middle age (>50 years) and is slightly more common in men. Women are more likely to develop high blood pressure after age 65 i.e., post menopausal age.

Smoking: It induces arterial stiffness, lipid modification and endothelial dysfunction. Smoking leads to transient elevation of systolic BP by 10-20 mmHg with a single cigarette which in turn elevates the average daytime BP.

Alcohol: The risk of hypertension is slightly lower in moderate drinkers (< 2 drinks/day) than teetotalers. However it is increased in heavy drinkers (> 3 drinks/day). 1 drink being equal to about 13 gm of alcohol. The mechanism of alcohol leading to hypertension is not exactly known. Some possible mechanisms are increased sympathetic activity, impaired baroreceptors, increased cortisol levels and activation of rennin-angiotensin-aldosterone pathway.

Dietary habits: Increased salt intake leads to retention of fluid and rennin-angiotensin system stimulation, thereby increasing the incidence of

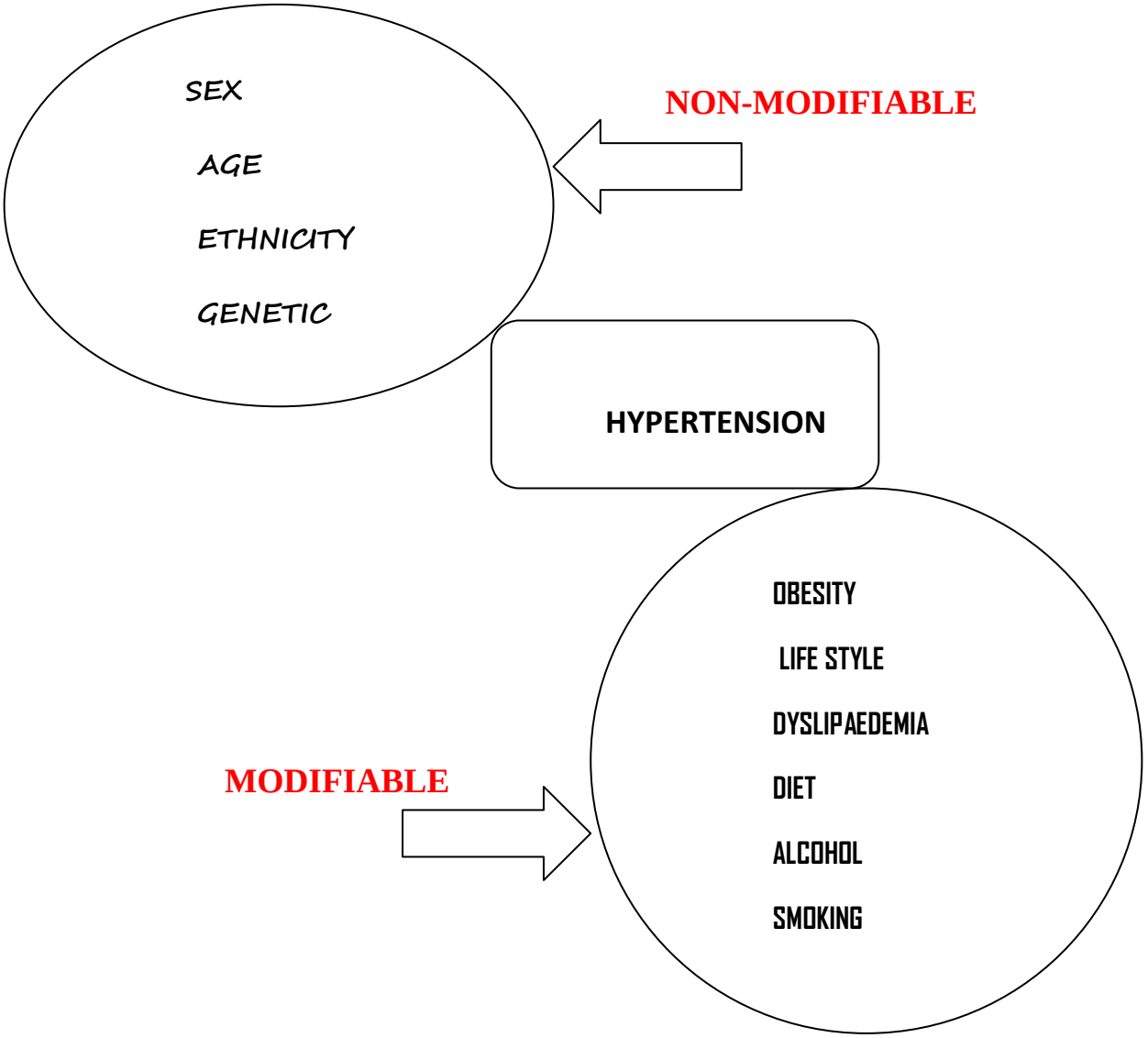
hypertension. Recent studies have shown that hypertension is associated with low vitamin D. Also, High dietary content in fat and carbohydrate has been linked with increased α1 and β adrenergic receptors causing increased sympathetic activity.

Body mass index: Hypertension prevalence increases linearly with increase BMI. Obese individuals have increased norepinephrine turnover in peripheral tissues, impairment of pressure natriuresis, structural changes in the kidneys, endothelial dysfunction and changes in vascular tone.

Genetics: Concordance of blood pressure is significantly higher in families than in individuals not related by family, higher between monozygotic than dizygotic twins and higher between biological than adoptive siblings living in the same household. Several mutations in the gene have been implicated in the development of hypertension in susceptible individuals. For example, single gene mutations like Angiotensinogen gene, Glucorticoid receptor gene, Congenital adrenal hyperplasia, Lipoprotein lipase gene, and Multiple gene mutations include genes encoding for Renin–angiotensin–aldosterone system, Cellular ion-transport systems (Na–Li; Na–H; Na–K–Cl), Insulin resistance, Metabolic syndrome, etc have been associated with hypertension.

In 90-94% of the individuals, a single reversible cause of hypertension cannot be identified, hence the term idiopathic hypertension. Among the remaining 5-10%, a more discreet cause can be pointed at, which is then called secondary hypertension.

HYPERTENSION AND THE RISK FACTORS



MECHANISM OF ESSENTIAL HYPERTENSION

Some mechanisms implicated in causing hypertension are:

Genetic predisposition: It has been postulated that hypertension is a representation of polygenic disorder where in, a combination of genes acts in association with environmental and demographic factors. Several methods including animal models have been studied to identify the genetic loci and genes associated with hypertension. Current studies suggest the likelihood of various gene mutations which may be responsible for the development of hypertension. Some of the genes which have been exclusively studied in this regard are genes encoding for rennin-angiotensin-aldosterone system, alpha adducin, β adrenoreceptor, angiotensinogen 1 receptor, aldosterone synthase, lipoprotein lipase , and epithelial sodium channel in Liddle syndrome .

Foetal environment: Low birth weight as a result of foetal malnourishment has been repeatedly found to be followed by an increased incidence of hypertension in later life with an overall estimate that 1 kg lower birth weight is associated with 2-4 mmHg higher systolic blood pressure in adulthood.

Renin-angiotensin system: Approximately, 20% of the patients who have hypertension have suppressed plasma rennin activity.

They display increased retention of sodium with suppression of rennin owing to increased production of an unknown mineralocorticoid. They have higher salt sensitivity to blood pressure and respond to diuretics. Additionally, in some studies, when patients were categorically placed based on renin-Na, serum calcium was distinguishably different among different subgroups. Lower serum calcium was seen in Low renin subgroup and vice-versa. Of importance, angiotensin II, which activates angiotensin II type 1 receptor and stimulates aldosterone production, induces cardiac as well as vascular hypertrophy and hyperplasia.

Vascular reactivity and remodelling: Hypertensive patients show greater response to norepinephrine than normotensive individuals. The downregulation of noradrenergic receptors is impaired in hypertension. As expected, alpha and beta adrenergic antagonists reduce blood pressure effectively thus indicating the role of sympathetic system in hypertension. There is evidence of alterations in structure, function and mechanical properties of small vessels.

Endothelial dysfunction: Nitric oxide, a potent vasodilator, which is released by endothelial cells in response to changes in blood pressure, is decreased in hypertensive patients.

Sympathetic nervous system: Hypertensive patients have increased sympathetic activity with decreased parasympathetic tone. Alteration in baroreceptor and chemoreceptor pathways occur both at central and peripheral levels resetting to a higher pressure. Conversely, this resetting is reversed when blood pressure is brought down to a lower or normal level with antihypertensive drugs.

Intravascular volume: Sodium, primarily an extracellular ion, is responsible for extracellular fluid volume which influences blood pressure over long term. Excess sodium due to decreased excretion via kidneys leads to hypertension as higher blood pressure is required to achieve sodium balance.

Inherited cardiovascular factors and hyperinsulinemia: Approximately, 40% of the hypertensive patients have hypercholesterolemia. There is a strong association between hypertension and type 2 diabetes mellitus. Hyperinsulinemia in hypertension arises as a consequence of insulin resistance to the peripheral utilisation of glucose. In obese as well as 20% of non obese hypertensive individuals, insulin resistance is noted and decreased hepatic uptake of insulin which contributes to hyperinsulinemia.

Altered calcium metabolism: Essential hypertension is associated with altered calcium metabolism. Changes in the regulation of intracellular free Calcium and disturbed extracellular calcium homeostasis are noted in patients diagnosed with primary hypertension. Extracellular calcium provides calcium ions for the maintenance of intracellular calcium levels, blood coagulation, bone mineralization and plasma membrane potential. It is widely accepted that the increase in peripheral vascular tone that characterises the established phase of hypertension is due to increased active tension in the smooth muscle cells. Calcium influx through receptor and voltage-gated calcium channels initiates vascular contraction and the fall in the intracellular free calcium concentration results in relaxation or vasodilatation.

SECONDARY HYPERTENSION

It is the arterial hypertension of known etiology. It constitutes 5-10% of all hypertension. Some common causes of secondary hypertension are given below:

Kidney: Parenchymal kidney disease, Renal vascular pathology, malignancy, Liddle's syndrome, polycystic kidney disease, nephritic syndrome

Endocrine: Acromegaly, Hyperthyroidism, Hypothyroidism, Cushing syndrome,

Hyperparathyroidism, Primary aldosteronism, Pheochromocytoma, Carcinoid syndrome

Drugs and Substance abuse like NSAIDs, oral contraceptive pills, alcohol, cocaine, anabolic steroids

Neurologic: Increase intracranial pressure, familial dysautonomia, Obstructive sleep apnea

Coarctation of the aorta and Diseases of the aorta

Pregnancy induced hypertension

NATURAL HISTORY OF UNTREATED HYPERTENSION:

In untreated hypertensive individuals, as the age progresses, Systolic blood pressure increases while diastolic blood pressure falls. The risks associated with high blood pressure are more firmly associated with systolic blood pressure than to diastolic blood pressure in the individuals who are over the age of 60 years.

SYMPTOMS AND SIGNS

Patients with uncomplicated hypertension are mostly asymptomatic and many are unaware of the disease. Some patients may complain of headache, dizziness, tinnitus, palpitations, feeling of faint, all of which, may be encountered even in

normotensive patients.

PATIENT'S RELEVANT HISTORY

Clinical history should include the following assessments:

- Previous history and duration of hypertension
- Personality and social life
- Family history of hypertension as well as cardiovascular disease
- Risk factors: Diet, drugs, addiction habits (smoking, alcoholism, etc), Sedentary lifestyle, Obesity, other co-morbidities (diabetes, dyslipidaemia, etc)
- Features of secondary hypertension:
  - Kidney disease: urogenital infections, haematuria, Chronic analgesic users, autosomal polycystic kidney;
  - Pheochromocytoma: episodic occurence of diaphoresis, headaches, anxiety, Flushing, palpitations.
  - Hyperaldosteronism: episodic weakness of muscles, tetany
  - Medication or substance abuse: oral contraceptive pills, liquorice, carbenoxolone, cocaine abuse, amphetamines, nasal drop, corticosteroids, NSAIDS
- Features of target end-organ damage:
  - Central Nervous System: headaches, Giddiness, Decreased

visual acuity, transient ischemic attack, Focal neurological deficits;

− Cardiovascular system: Breathlessness, palpitations, chest pain, pedal oedema

− Kidney: excessive thirst, polyuria, nocturia, dark/brown coloured urine

− Peripheral arteries: cold extremities, intermittent claudication.

PATHOLOGIC CONSEQUENCES OF HYPERTENSION:

Hypertension is a well known risk factor for all the clinical manifestations associated with atherosclerosis. It is an independent predisposing factor of congestive heart failure, coronary heart disease, cerebrovascular accident, kidney disease and peripheral vascular diseases.

Effects on Cardiovascular system:

Cardiovascular complication is the most common cause of death in patients with high blood pressure. Increased systemic pressure imposes excessive workload on heart. At first heart compensates by increase in wall thickness of left ventricle leading to concentric left ventricle hypertrophy.

This is followed by left ventricular dysfunction, left ventricular cavity dilatation and congestive cardiac failure ensues. Anginal chest pain usually appear because of the following combinations:

1. Accelerated atherosclerosis
2. Increased myocardial oxygen demand as a result of increased left ventricular mass.

Hypertension is a major risk factor for myocardial infarction and ischemia. Prevalence of silent MI is significantly increased in hypertensive subjects and they have a greater risk for mortality after an initial MI.

Effects on Nervous system:

Neurological effects of long standing high blood pressure can be divided into retina related changes in the central nervous system. Retina is the only tissue in the body which the blood vessels can be examined directly. Funduscopy examination gives the opportunity to study the vascular effects of hypertension. A useful guide is the Keith Wagener Barker classification of fundoscopic changes.

High blood pressure is directly associated with increased risk of cerebrovascular accidents (both ischaemic and haemorrhagic). The incidence of cerebrovascular accidents increases progressively with increased blood

pressure level, especially systolic BP in patients who are more than 65 years. Hypertension may accelerate brain function decline with age. Blood flow in the brain usually remains unchanged within a wide range of arterial pressure through a process termed auto-regulation of blood flow. In patients with malignant hypertension there is failure of auto-regulation system of cerebral blood flow resulting in vasodilation and excessive perfusion.

With severe hypertension, hypertensive encephalopathy may occur which manifests as severe headache, nausea with or without projectile vomiting, focal neurological deficits, and altered sensorium. It is a medical emergency and if immediate treatment is not provided, patients may progress to stupor, coma, seizure, or even death.

Effects on kidney:

High blood pressure is a well known risk factor for kidney injury and chronic renal failure. The risk of kidney injury appears to be more closely related to systolic rather than diastolic blood pressure. Whether the injury is due to primary or secondary hypertension, it causes damage to the renal arterioles which is also known as hyaline arteriolosclerosis that ultimately progresses and cause loss of function. Sclerotic lesion of both the afferent arteriole and efferent arteriole along with glomerular capillary tufts is the most common finding among the renal vascular lesions in patients with high blood pressure which leads to reduction of glomerular filtration rate and loss of tubular function.

Renal disease may manifest as a mild to moderate elevation of serum creatinine concentration, microalbuminuria, or proteinuria. Microalbuminuria in hypertensive patients has been correlated with LVH and carotid artery thickness. Any agent or group of agents that adequately lowers BP to levels less than 130/85 mm Hg will delay the progression of nephropathy.

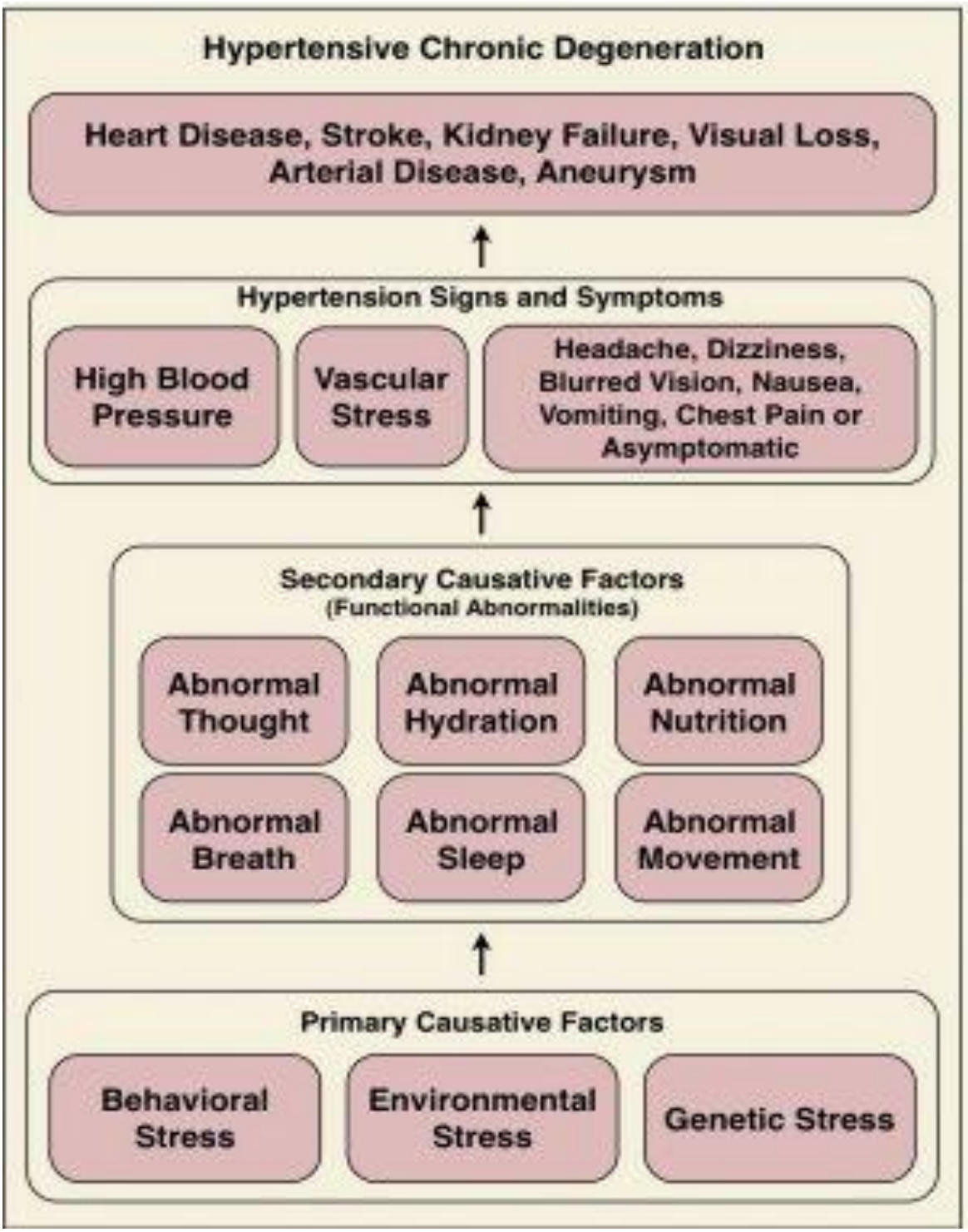


FIG 5: FLOW CHART OF STRESSOR AND EFFECT IN HYPERTENSION

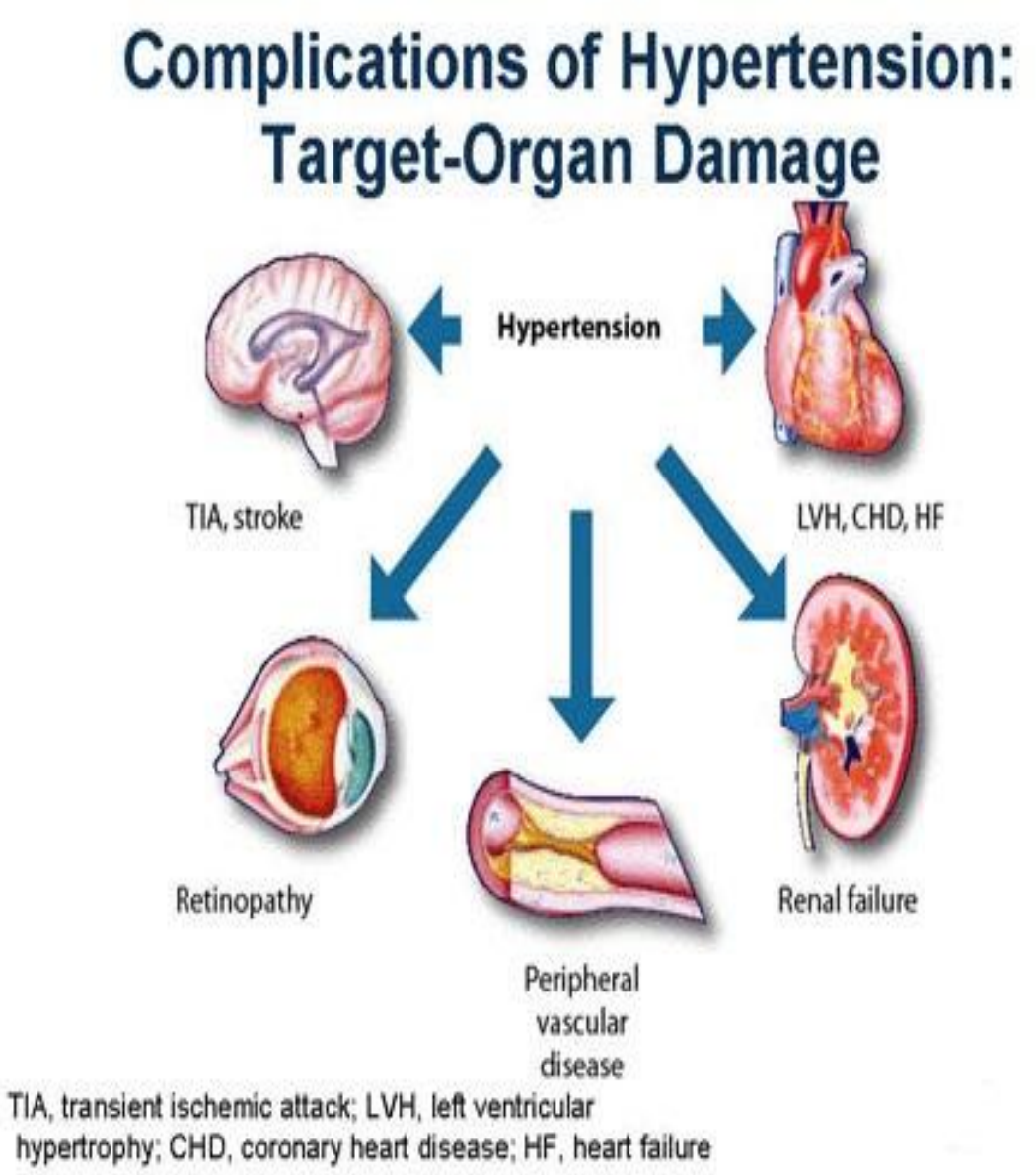


FIG 6: COMPLICATIONS OF HYPERTENSION

EVALUATION OF A HYPERTENSIVE PATIENT:

Evaluation of patients with high blood pressure has the following objectives:

1.To study the lifestyle and identify other cardiovascular risk factors or associated disorders that may later influence the prognosis and treatment.

2. To look for the cause or causes of high blood pressure and if identified, formulate a strategy of treatment.

3. To evaluate the presence or absence of end organ damage and Cardiovascular diseases.

Physical examination:

Some of the examination which should be included routinely are:

- Measurement of blood pressure using correct technique and apparatus, measurement and comparison of the contralateral arm,
- Examination of fundus,
- Body mass index (to be calculated as weight in kilograms divided by square of height in metres.
- Waist – hip ratio and circumference
- Auscultation for abnormal sounds in the arteries such as carotid bruit, abdominal bruit and femoral bruit,
- Examination of Thyroid gland (to rule out thyrotoxicosis and tumours)
- Examination of cardiovascular system
- Examination of respiratory system,
- Examination of abdomen for palpable kidney, tumours and abdominal aortic pulsations.
- Palpation of the lower limbs for pedal/ankle oedema and lower limb pulses along with complete neurological assessment.

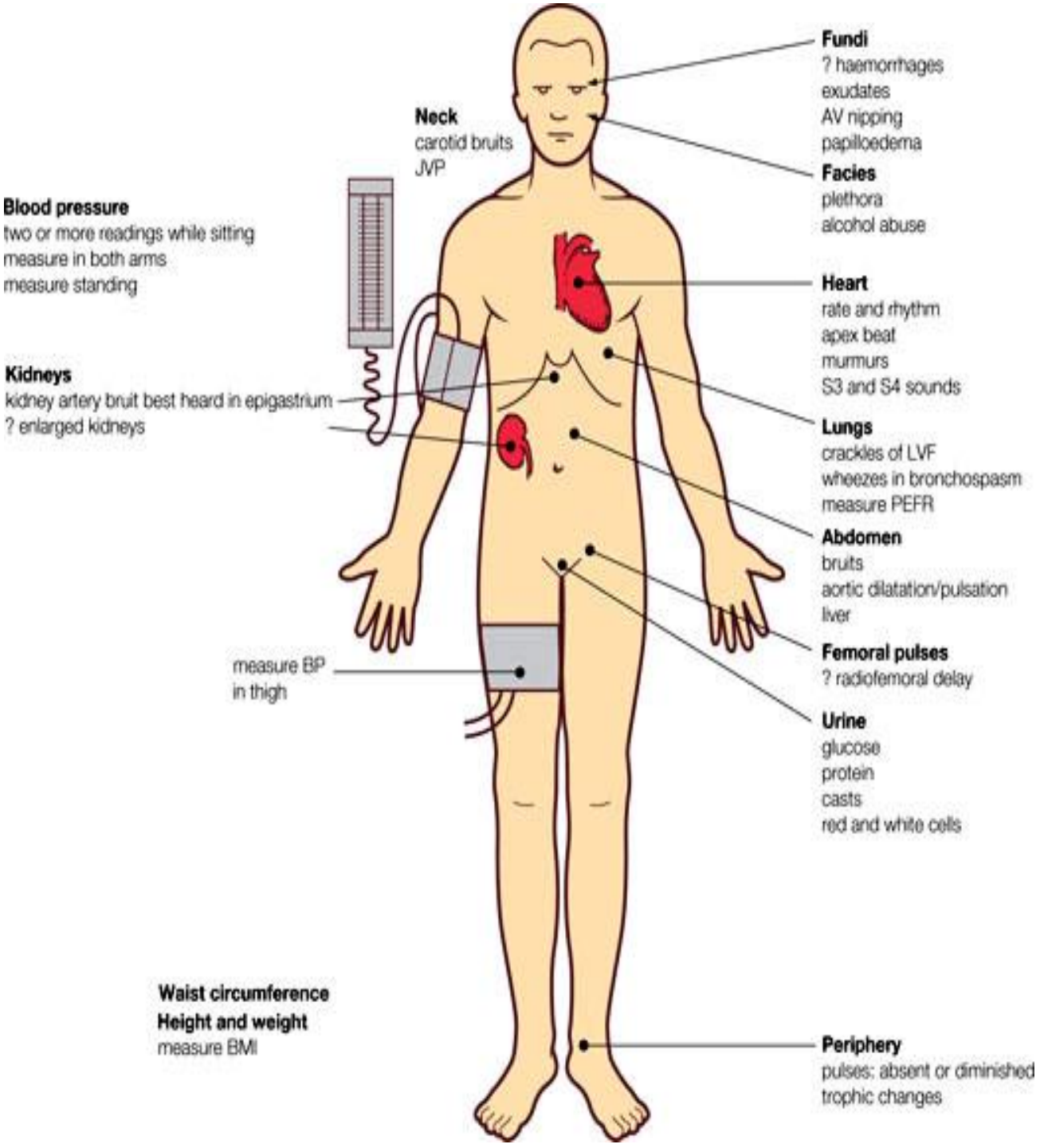


FIG 7: SYSTEMIC EVALUATION OF A HYPERTENSIVE PATIENT

Biochemical Investigations:

1. Urine analysis: Protein, Blood, Glucose, Microscopy
2. Haematocrit
3. Fasting glucose
4. Serum Creatinine and Blood Urea Nitrogen
5. Serum $^{+}$ calcium, phosphorus and other electrolytes
6. Lipid profile that including Total cholesterol, HDL Cholesterol, LDL Cholesterol and Triglycerides.
7. Electrocardiogram
8. Echocardiogram

Hence it is evident that urine albumin excretion is one important parameter which should ideally be included in the Evaluation of hypertensive patient.

CALCIUM :

Calcium is the most abundant mineral in the human body. A normal healthy adult body contains a total calcium of roughly 1 kg, 99% of which, are present in the bones in the form of calcium phosphate salts. The extracellular fluid contains around 22.6 mmol, out of which about 9 mmol is found in the serum. Almost 500 mmol of calcium is exchanged between bone and the Extracellular fluid daily. Serum level of calcium is tightly regulated with normal total calcium of 8.07-10.2 mg/dl and a normal ionised calcium of 4.4-5.5 mg/dl. The amount of total calcium varies accordingly with the level of serum albumin to which calcium is bound. The biological effects of calcium in the body are influenced by the content of ionised calcium, rather than the total calcium. Ionised calcium does not vary with the albumin level, and therefore it is an advantage to measure the level of ionised calcium whenever serum albumin is expected to vary or any pathological condition that changes serum albumin concentration or a pre-existing calcium disorder is suspected in the patient.

Dietary sources of Calcium:

Some products containing high calcium in India are mentioned below.

- Milk (skimmed > whole)

- Cheese
- Soya beans, broccoli, spinach,
- Dried fruits and nuts
- Yoghurt
- Figs, Oranges

Calcium metabolism:

The mechanism of calcium homeostasis is complex and diverse, involving calcium itself and other related minerals such as magnesium and phosphorous, and three calcitropic hormones-parathyroid hormone, calcitonin, and active form of vitamin D_3 which is 1,25 dihydroxy cholecalciferol.

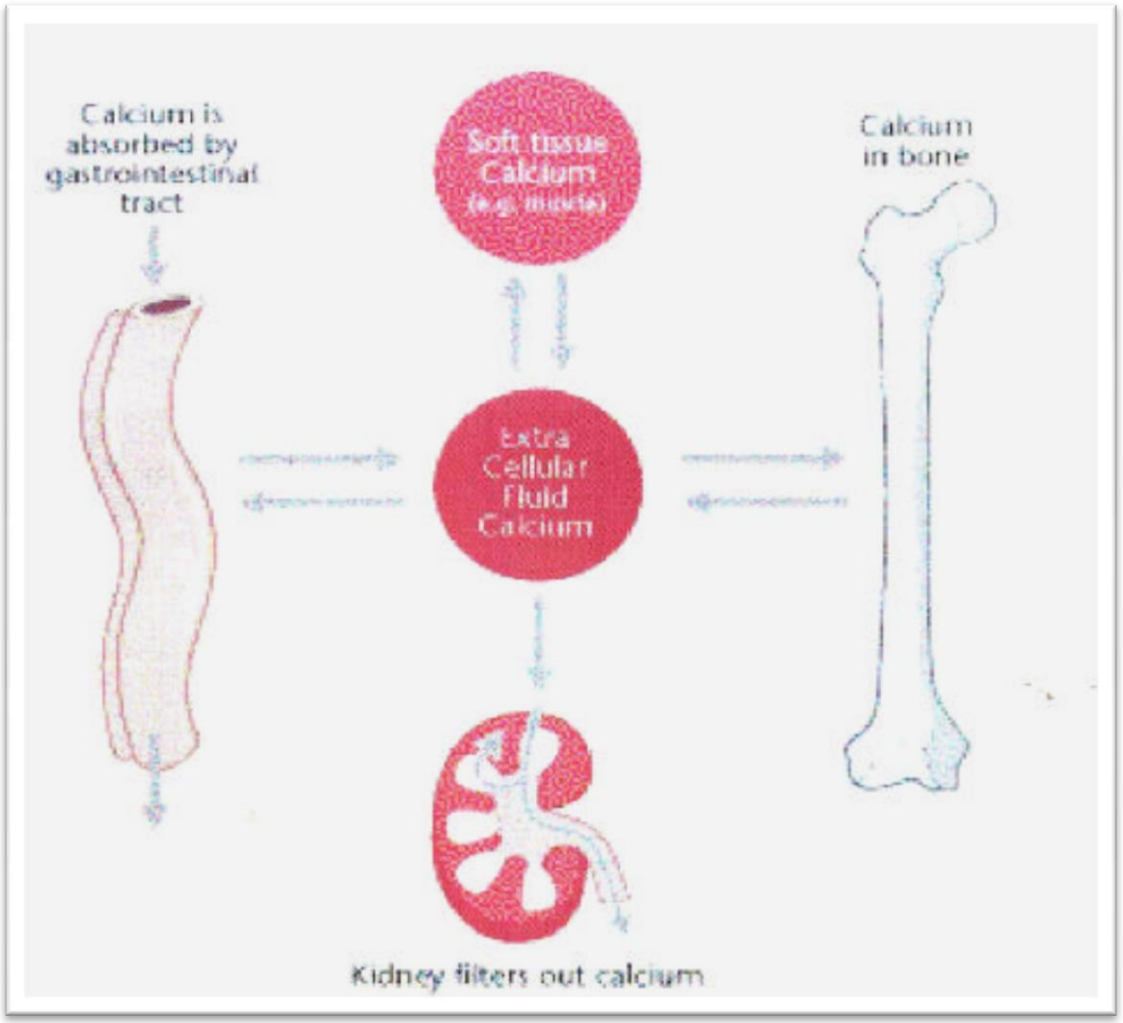


FIG 8: CALCIUM HOMEOSTASIS IN THE BODY

There are three principal organs involved in calcium homeostasis:

- Gastrointestinal tract
- Bone
- Kidney

Gastrointestinal tract:

Intestinal absorption of calcium is mediated by two mechanisms:

- An active process, regulated by calcitriol (1,25- dihydroxycholecalciferol)
- A passive process

Active process involves a calcium binding protein whose synthesis is increased by calcitrol. It occurs mainly in duodenum and upper jejunum. Calcium enters the luminal surface of the cells through specific apical calcium channels, whose number is regulated. It then moves across the cell membrane in association with a specific calcium binding protein (calbindin). Ca^{2+}-ATPases and Na/Ca exchangers actively extrude calcium across the basolateral surface and thereby maintain the transcellular calcium gradient. At high levels of calcium intake, synthesis of calcitriol is reduced, in turn decreases calbindin synthesis and decreases rate of active intestinal calcium absorption. The opposite occurs with decreased dietary calcium intake.

Passive process occurs in the small intestine and possibly in the colon. It approximates 5% of daily calcium intake.

The absorption of calcium is influenced by other dietary constituents. The presence of anions such as phosphate, oxalates and phytates decrease calcium solubility and so its absorption.

Kidneys:

The ionized and complex fraction of plasma calcium is filtered at the glomeruli, amounting to approximately 250mmol per 24hr. Of this, about 98% is reabsorbed, from the proximal tubules. Calcium reabsorbtion in the proximal tubules is a passive process and is closely linked with sodium and is not regulated by hormone.

The fine tuning of calcium excretion is carried out in the distal parts of nephrons where nearly 15% of the filtered load is reabsorbed in the tubule. This region comprises of the distal convoluted tubule, the connecting tubule and the beginning of the cortical collecting duct. Here, reabsorption is an active process and occurs against an electrochemical gradient. This active reabsorption is subject to hormonal regula tion -principally by parathormone, but also by calcitrol, calcitonin, oestrogens and androgens.

Bone:

The influx of calcium into bone equals its rate of efflux and bone mass remains constant. But it is different in bone growth and senescence. Bone remodelling probably accounts for changes in bone density that takes place with ageing or disease.

In a normal adult, about 5% of entire skeleton is remodelled in one year. In contrast radioisotope studies have indicated that 1-2 % of body calcium can be exchanged between the bone and ECF over a period of several days.

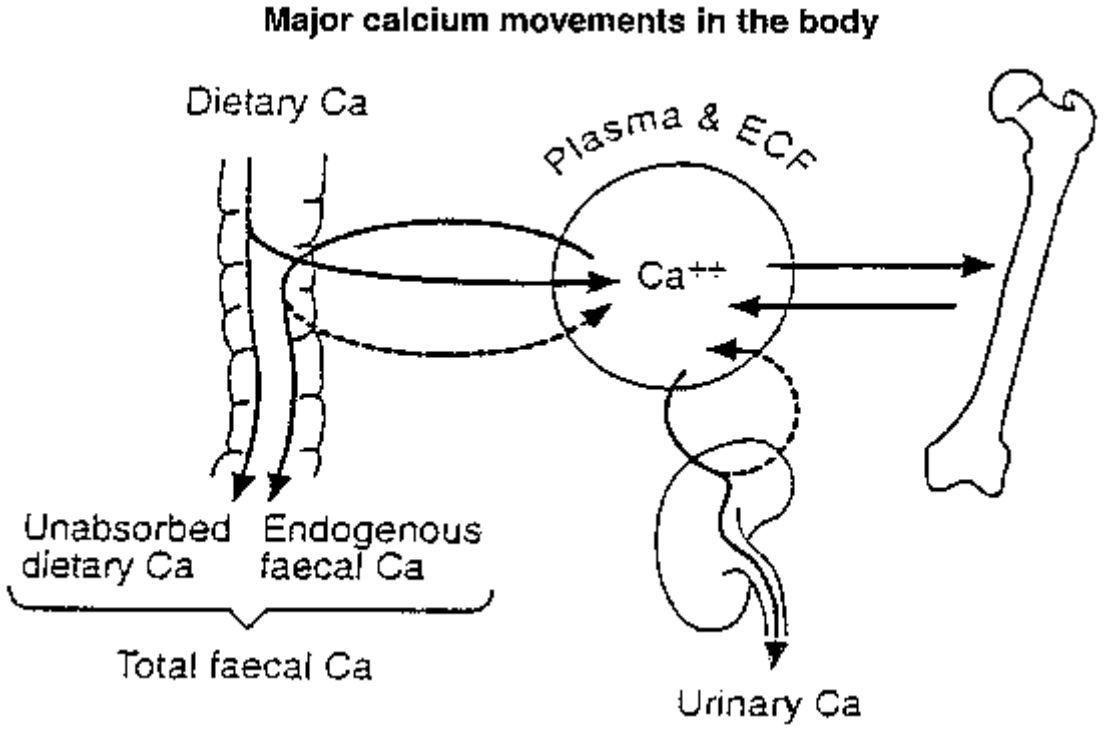


FIG 9: SITES OF CALCIUM EXCRETION FROM THE BODY

HORMONAL REGULATION OF CALCIUM METABOLISM:

Regulation of calcium is given briefly in the diagram given below:

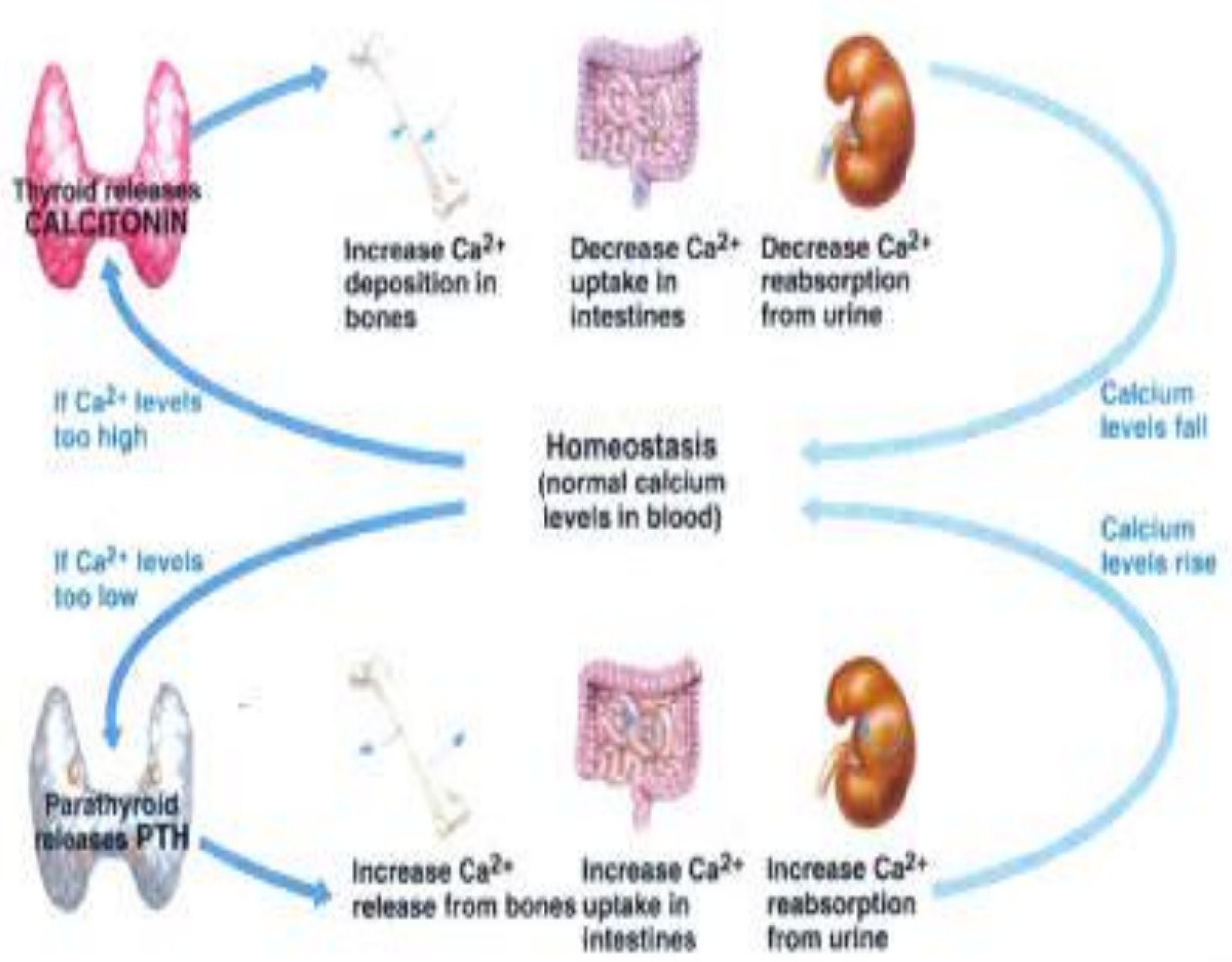


FIG 10 : CALCIUM HOMEOSTASIS

1. PARATHYROID HORMONE: The principal regulator of PTH secretion is the ECF ionized calcium content in the body, low level stimulates secretion and high level inhibits it.

2. CALCITONIN: It is a 32 amino acid peptide chain secreted by the parafollicular C-cells present in the thyroid gland. Secretion of calcitonin is stimulated by an increase in plasma calcium concentration and is also released in response to some gut hormones such as gastrin, glucagon, secretin. It reduces plasma calcium concentration by decreasing calcium reabsorption from kidneys, decreases uptake from intestines and increases deposition in bones.

3. VITAMIN - D: Vitamin D3, either ingested in a preformed state or synthesised in the skin by the action of ultraviolet light, is normally hydroxylated in the 25-position in the liver to form 25-hydroxyvitamin D3, the principal form of vitamin in the circulation. 25-hydroxyvitamin D3 is normally hydroxylated in l-a position in the kidney to form 1, 25 dihydroxycholecalciferol, which is biologically the active form of vitamin.

Calcium metabolism in essential hypertension:

Primary hypertension is strongly linked with altered calcium metabolism. Calcium ion acts as an intracellular second messenger in the contractility of cardiac muscle and smooth muscle cells. A rise in total peripheral vascular resistance is a common finding in all forms of hypertension irrespective of the causes. The free intracellular calcium level determines the tension in vascular smooth muscle cells which leads to in peripheral vascular resistance. Calcium affects on the peripheral vascular tone directly.

Alterations in the level of intracellular calcium level are believed to be involved in the final common pathway which mediates the synthesis, secretion and action of many hormones, such as the pressure actions of catecholamines and angiotensin II. Calcium regulating hormones like 1,25dihydroxy cholecalciferol, renin level contribute to pathogenesis of primary hypertension.

Several disturbances of calcium metabolism have been associated with hypertension. It has been suggested that low dietary calcium intake significantly increases the risk for hypertension. In addition, some hypertensive subjects appear to have lower serum calcium levels, increased urinary excretion of calcium i.e.hypercalciuria, raised intracellular calcium levels, and reduced cellular membrane calcium binding.

PHOSPHORUS

It is the sixth most abundant element in the human body. A normal healthy adult contains almost 700 g of phosphorus in the body, most of which, is located in the bones as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The remaining Phosphorus is present in extracellular fluid and soft tissue organs, mainly as part of proteins,

Lipids, nucleic acids, phospholipids and nucleotides.

FUNCTIONS:

- Phosphorus is an essential nutrient in bone mineralization.
- It is required in energy generation, cell signals via phosphorylation.
- It provides structural integrity of phospholipids, various nucleotides, proteins, lipids and nucleic acids.
- Intracellular phosphate is present in many of the compounds that are phosphorylated compounds, like adenosine triphosphate , guanosine triphosphate ,etc. which are important basic needs in metabolism of energy and activation of enzymes.
- It acts as a buffer for both extracellular and intracellular compartments through the interchaange of HPO42- and H2PO4-, thus helping to maintain normal pH in the body.

Dietary sources of Phosphorus:

Some of the products rich in phosphorus found in India are mentioned below.

1. Vegetable sources: Soya beans, Yellow/white beans, Lentils, Peas

   Seeds of pumpkin, squash, watermelon

   Cashew nuts, almond, whole grains

2. Animal sources: Cheese and yoghurt

   Sea foods like salmon fish, oyster and shellfish

   Pork liver and chicken liver

PHOSPHORUS METABOLISM:

Similar to calcium metabolism, the main Phosphorus regulation sites are:

- The Digestive system (for absorption),
- The Renal system (for excretion)
- The Skeletal system (for storage)

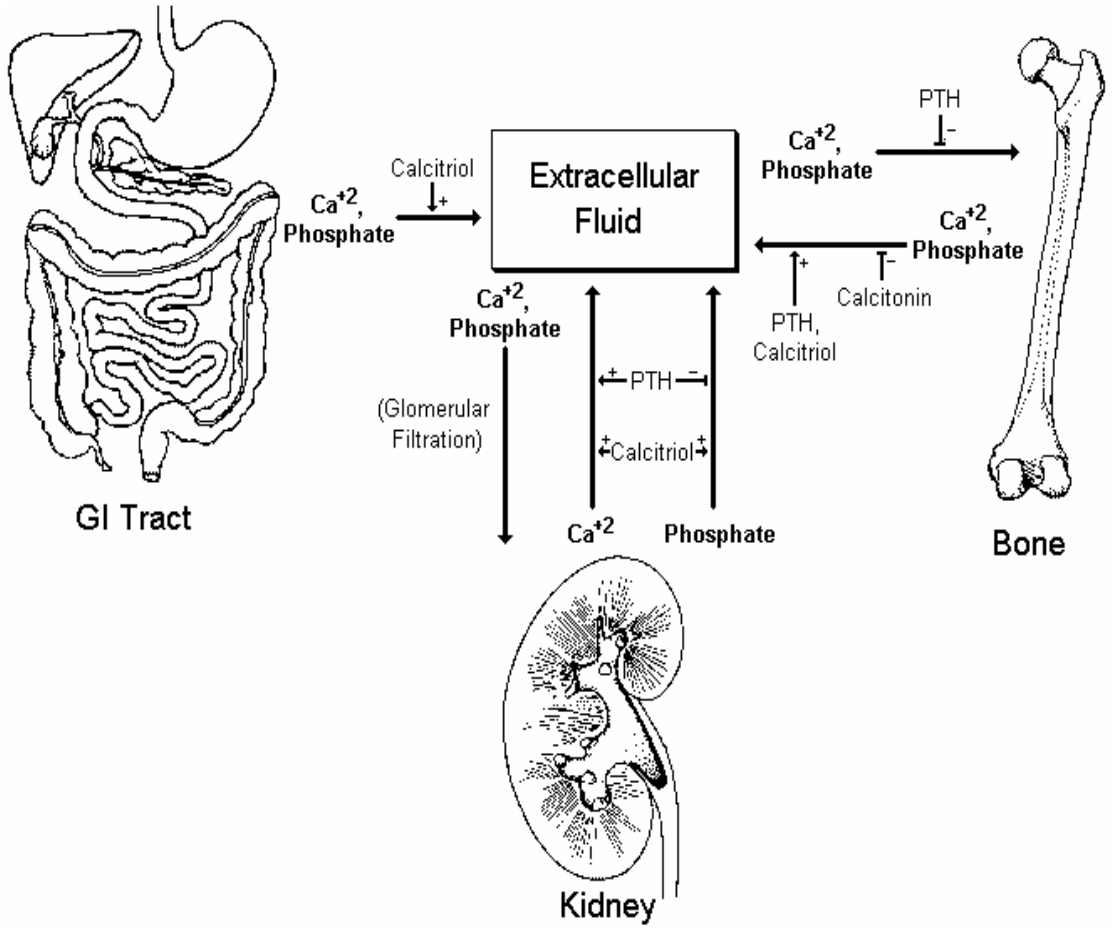


FIG 11 : PHOSPHORUS METABOLISM

The main regulation occurs in the kidneys, and homeostasis is maintained by excreting Phosphorus via urine. In an average adult with normal dietary Phosphorus intake, approximately 6-7 g of Phosphorus is filtered daily by kidney. More than 80% of Phosphorus is reabsorbed in the proximal convoluted tubule and the remaining 10% in the distal convoluted tubule and around 10% is excreted via urine. Phosphorus in a human body is considered to be in balance when the output (loss of Phosphorus via urine, stools and sweat) is equal to that of the amount of Phosphorus absorbed which occurs through intestines.

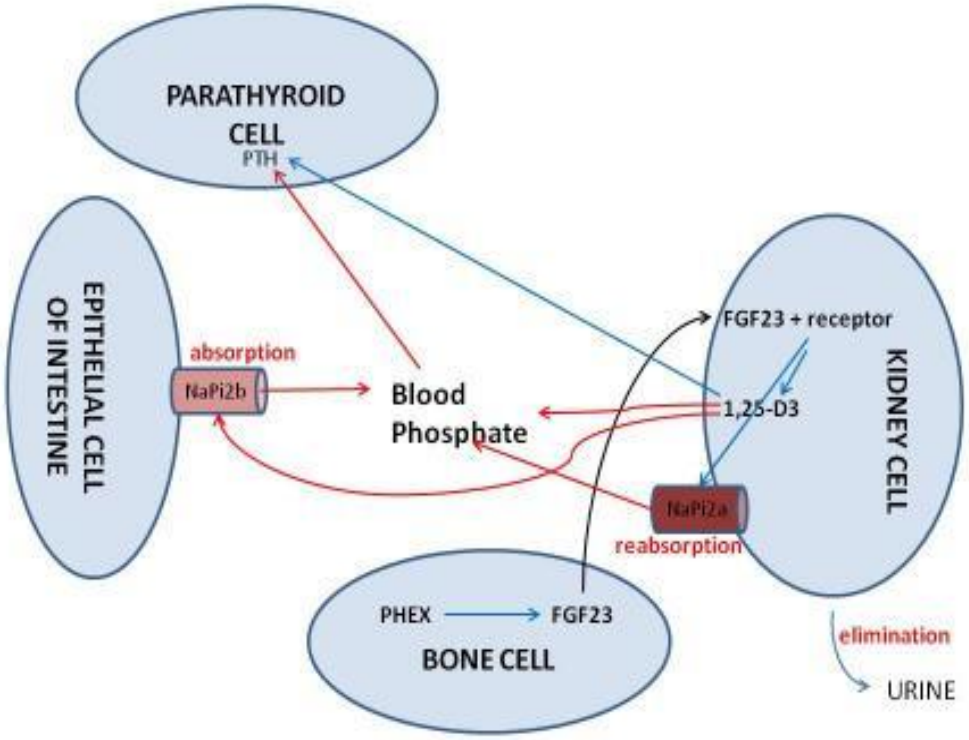


FIG 12: REGULATION OF PHOSPHORUS METABOLISM

2. AIM AND OBJECTIVES

- To study the levels of serum Total Calcium and serum Calcium / Phosphorus ratio in patients with primary hypertension.
- To correlate the serum Total Calcium level and serum Calcium / Phosphorus ratio with blood pressure

STUDY POPULATION

A total of 100 subjects, of which 50 cases of essential hypertension satisfying inclusion and exclusion criteria visiting medicine OPD and admitted in Government Rajaji hospital, Madurai and 50 age and sex matched normotensive controls from 1st June 2014 to 30th November 2014 will be taken up for study.

3. REVIEW OF LITERATURE

HISTORY

Though Hypertension was believed to exist since antiquity it was possible to recognize it only after the discovery of a device to measure it. Blood circulation was discovered as early as the year 1616 by William Harvey. First report of direct blood pressure dates from 1726 by Stephen Hales who cannulated horse crural artery and assessed the height of blood column before and after hemorrhage.

The monoaural stethescope was discovered by Lennac in 1819. Biaural stethoscope came to America in 1852. Vierordt, a German scientist was the first person to device an instrument to measure blood pressure in the year 1853.Though this instrument was cumbersome he is the pioneer in establishing the principles of estimating blood pressure by obliterating the pulse which is followed even today.

In the year 1896 Riva Rocci introduced the sphygmomanometer cuff. In the year 1905 Nikolai Korotkoff devised auscultatory method of measuring arterial pressure and measured diastolic and systolic pressure levels for first time. Evaluation of antihypertensive therapy began in 1920.Progress began

with arrival of thiazide diuretics in late 1960s.

In the year 1986, H Kesteloot and J V Joossens conducted an epidemiological study on the association between food habits and cardiovascular risk factors. Serum sodium and Serum potassium, Serum calcium and serum phosphorus, and total protein were measured in a total of 4167 men and 3891 women with a mean age of 49 years, and observed a very significant relationship between serum calcium, serum phosphorus with hypertension along with other cations. A random sample of the population was obtained in all Belgian counties by using the voting lists. A detailed dietary history was obtained from the participants by trained dietitians, and blood pressure was measured in the sitting position. Heart rate was measured at the time of blood pressure measurement. The results were submitted to multiple regression analysis using the stepdown method until all remaining parameters were significant at a p level below 0.05. Systolic BP and Diastolic BP were used as dependent variables. They concluded that the serum sodium/potassium ratio and the serum calcium/phosphorus ratio both correlated positively with blood pressure, whenever significant.

In the same year, A.R Folsom and his associates measured Serum calcium fractions in patients diagnosed with essential hypertension and matched normotensive candidates in a group of Minnesota population. The hypertensive group comprised 28 subjects whose diastolic blood pressure on both occasions was

greater than 90 mm Hg and who were not taking antihypertensive medication. One normotensive control was matched to each hypertensive subject. Controls were required to be of the same race and sex as the matched hypertensive subject and within 5 years of age. The hypertensive subjects had significantly lower serum ultrafilterable calcium levels ($p = 0.01$) and borderline significantly lower serum ionized calcium levels ($p = 0.09$) compared with levels in normotensive controls. Calculated serum concentrations of complexed calcium were also significantly lower in hypertensive subjects ($p = 0.04$).

In the year 1987, R.M Touyz et al conducted a study on Johhanesburg population among 296 male labourers and measured four parameters namely, serum calcium, serum magnesium, serum sodium and serum potassium. Of the total population studied, 82 were hypertensive, with a diastolic blood pressure (DBP) more than 95 mmHg and/or a systolic blood pressure (SBP) more than 160 mmHg. Mean arterial pressure (MAP) was calculated from the standard formula: MAP = DBP + YJ (SBP - DBP). The hypertensive men were referred to the hypertension clinic at the local hospital for management. Detailed medical history including diet, lifestyle and Quetelet's index was taken. Accuracy of techniques of blood sample collection was established by using eight samples of serum and haemolysed red blood cells, the electrolytes of which, were measured weekly for 9 consecutive weeks. The samples were stored in a refrigerator at 4°C. The data were analysed

with an IBM 4331 computer at the Institute for Biostatistics of the South African Medical Research Council. Partial correlations showed a significant inverse relationship between serum magnesium and MAP (r = 0.575, P value <0.001*), erythrocyte magnesium and MAP (r = 0.329; P value < 0.001*), serum calcium and MAP (r =-0.309; P value < 0.001) and serum potassium and MAP (r = 0.157; P value < 0.001*). This study showed that showed a significant decrease in serum calcium along with serum magnesium and potassium in the hypertensive subjects. They suggested the role of increased calcium influx causing vasoconstriction with a raised peripheral resistance and increased blood pressure.

In 1992, Rachel H et al observed that disturbed calcium metabolism plays an important role in hypertension. They measured the indices of calcium metabolism in male participants who were previously not treated with elevated diastolic blood pressure and male participants matched for age with low diastolic blood pressure .The study population were drawn randomly from a community. They observed that Participants with high diastolic blood pressure had significantly higher level of parathyroid hormone concentrations than those participants with low diastolic blood pressure (p Value <0.01*). The 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol concentrations were comparable in both groups. Pparticipants with high diastolic blood pressure had lower total serum calcium which was statistically significant.(p Value <0.01*). Parathyroid hormone levels

were compared with diastolic pressure. Thus they proved that participants who had higher diastolic blood pressure had lower total serum calcium which was statistically significant.

Roberta M. Ray *et al* of American Heart Association conducted a study on effect of Calcium and Vitamin D supplementation on Blood Pressure in the year 2008. In this study, subjectss were randomly assigned in a double-blind fashion to receive 1000 mg of elemental calcium along with 400 IU of vitamin D3 daily or placebo. Each active tablet provided 500 mg of calcium (taken as calcium carbonate) and 200 IU of vitamin D3. Subjects were instructed to take two tablets daily, preferably in divided doses with meals. BP was measured by trained hospital staff using standardized procedures and instruments, in the right arm, with a conventional manual mercury sphygmomanometer and an appropriately sized cuff, after the participant was seated and resting for 5 minutes. From baseline to the end of follow-up, systolic BP declined by 1 mm Hg during follow-up, while diastolic BP declined by 4 mmHg.

Michael B. Zemel in 2001 opined that regulation of intracellular calcium acts as a key role in hypertension and obesity. Disruption of calcium homeostasis appears to be a fundamental factor linking these conditions. Regulation of intracellular calcium in key disease-related target tissues by calcitropic hormones provides the opportunity to modulate disease risk with oral Calcium in the diet.

Langford and Watson in 1987 were the first one to observe the inverse relationship between serum calcium and hypertension. In a study of 100 black women they found that individuals with low SBP (105mmHg) has consumed more calcium than others with high SBP (125mmHg).This study showed that calcium may be a protective factor in hypertension.

In the year 2007, Abdelmarouf H Mohieldein et al compared the total serum calcium level in women who were pregnant and complicated with pre-eclampsia with those of normotensive control in a case control study in 135 pregnant mothers in Sudan, out of which, 90 women with pregnancy induced hypertension who were admitted after 20th week of pregnancy were taken as the study population. Serum calcium was measured using automated spectrophotometer. The study showed the mean serum calcium of the cases as 8.38 mg/dl), and that of the control as 9.04 mg/dl which was significant statistically. Thus they concluded that decreased serum calcium level may contribute to the onset of pregnancy induced hypertension.

In the year 2004 K. Sudhakar and associates conducted the first study in Indian population to show that calcium acts an important part in the pathogenesis of primary hypertension. The results of this study implied that serum calcium levels were significantly decreased in both males and female participants

diagnosed with primary hypertension and their first-degree relatives as compared with the normotensive individuals. This study proved that essential hypertension is strongly related with altered calcium metabolism.[5]

In the year 2011, Kamlesh Jha and associate examined Serum Calcium in Essential Hypertension and its corelation with severity of the disease using Arsenazo III method. The study showed that the mean serum calcium concentration of control subjects was found to be 2.53 + 0.08 mmol/l with a range of 2.37 to 2.70mmol/l. The females were found to have lower mean serum calcium level (2.5mmol/l + 0.08) than males (2.55 mmol/l + 0.07) but the difference between them was not statistically significant. The mean calcium values were found to be in agreement with recommended normal range of serum calcium concentration in both sexes. The mean serum calcium concentration of grade I hypertension cases was found to be 2.30 + 0.072mmol/l ranging between 2.16 to 2.58 mmol/l which was significantly lower ($p<.0001$) than that of control group but, was significantly higher ($p=.009$) than grade II hypertensive individuals (mean serum calcium level 2.25 + 0.09 mmol/l). Both grade I and Grade II hypertensive subjects of male and female sub-group showed significantly lower values of serum calcium than their respective control counterparts ($p<0.0001$ in all sub-groups). They concluded that both the stages

of hypertension (Stages 1 and 2) showed significantly reduced serum calcium level in hypertensive individuals as compared with normotensive individuals.

In the year 2012, Booloo Sharma and his associates evaluated a total of 345 individuals from north eastern region of India irrespective of age and sex. The study population was divided into three main groups - Normal Control group consisting of 115 normal healthy subjects between 20-50 yrs of age, Experimental / Test group consisting 115 patients between 20 and 50 yrs of age recently diagnosed with essential hypertension, not on medication, and free from any disease that might affect blood pressure or serum electrolyte level under study, First Degree Relative group consisting of 115 first degree relatives of the Test group, who were between 20-50 yrs of age. Detail history including diet, lifestyle, medications, etc and physical examination was done prior to the study. Result values of test group and first degree relative group were compared with normal control group by t test and probability (p) is calculated by observing values of t at a particular degree of freedom . The study showed that Serum Calcium level of test group have a significantly lower values as compared to the control subjects ($p<0.001$). The control group comprising of 115 individuals had a mean serum Calcium level of 9.26 ± 0.44 mg/dl, ranging from 8.4 mg/dl -10.1 mg/dl while the test group has a mean value of 8.23 ± 0.49 mg/dl, ranging from 7.6 mg/dl - 9.5 mg/dl.

In 2012, Azin Alavi et al compared serum calcium level, serum protein level, and blood uric acid level between pregnant females who were hypertensive and normotensive in a cross sectional survey. They evaluated one hundred normotensive and forty eight hypertensive pregnant participants with single live foetus who were in ≥ 28 weeks of pregnancy for serum calcium level, total protein level, and blood uric acid level and compared the values between these two categories. All of the participants were at 28 weeks of pregnancy or above at the time of study and the participants of the two categories were matched for age, parity and Body Mass Index. The Mean standard deviation of serum Calcium levels in normotensive and hypertensive participants were 8.28 (±0.77) mg% and 7.88 (±0.94) mg%, respectively (P value < 0.01*). This study showed that Mean serum Calcium level was significantly lower in hypertensive pregnant women as compared with normotensive participants. They observed that higher serum calcium status in pregnancy favours decrease incidence of pre-eclampsia and particular attention to nutritional diet should be encouraged in pregnant females with hypertension particularly in geographical areas that has high prevalence of malnutrition.

In 2013, Saeed Behradmanesh and associate studied a population comprising of 60 individuals and who were known cases of type 2 diabetes mellitus. Detail history along with anthropometric measurements and insulin doses were done in a cross sectional study. The diagnosis of hypertension in people with diabetes was

confirmed when the mean of at least two readings on minimum two clinic visitations was ≥ 130/80 mmHg. They found a significant negative association of serum calcium with that of diastolic blood pressure.

In the year 2014, Arpita B. Patel et al evaluated serum calcium in 150 individuals, 50 of which, were first degree relatives of hypertensive individuals using an automated analyzer. They observed that the mean Standard deviation of serum total calcium levels in normotensives were 9.53 ± 0.51 and the same was found to be decreased in hypertensive groups (8.35 ± 0.63). They found that there was statistically significant difference between mean total calcium levels in males of hypertensives and normotennsives category. They also found that there was statistically significant difference between mean total calcium levels in females of hypertensives and normotensives category. When the first degree relatives were considered, the first degree relatives of hypertensives had statistically significant lower mean± SD serum total calcium levels (8.81 ±0.59), when compared to the first degree relatives of normotensive (9.33 ±0.45). P value is <0.05. The results confirmed that serum total calcium level and serum albumin level were significantly decreased in both sexes of participants with primary hypertension and their first degree relatives in comparison to their corresponding control while serum ionised calcium levels were not significantly different in hypertensives and their first degree relatives.

In the year 2013, Takale and his associates studied the level of serum total and ionized calcium concentration in a group of 80 individuals 40 of whom, were normotensives using modified Arsenazo method was used for total calcium estimation . The results were expressed as mean ± S.D. Comparison of control and test group was done by unpaired T test. The results showed both serum total calcium level and serum free calcium level were decreased in hypertensive participants as compared to control thus concluding that decreased serum calcium levels suggest that calcium plays a crucial role in the pathophysiology of hypertension.

In the year 1991, Talat J. Hassan et al studied the serum calcium, blood urea and uric acid levels in 150 female patients who were pregnant to establish the link with pre-ecclampsia. Detail history including diet, lifestyle, exercise, medications and hormones were taken. Out of the total participants, 50 were known cases of pre-ecclampsia and 100 had normal blood pressure. They found the serum calcium level in pre-ecclampsia females to be 1.74 +/- 0.08 mmol/L while that of thatnormotensive females were 1.92 +/- 0.08mmol/L. Foetal age was calculated with ultrasonography. Their study observed low serum calcium was significantly low in pre-ecclampctic females which is associated with both maternal and foetal complications.

In the year 1998, Christina and Martinez noted that low serum calcium level is associated with hypertension and subjects who were supplemented with calcium had significantly reduced blood pressure. They suggested increased calcium supplementation in diet to prevent hypertension related complications, especially in pregnant women.

In the year 2014, Kaushik Kar and his colleagues studied the level of serum total and serum ionised calcium in 47 subjects attending the outpatient department. Detail history of all the participants was taken. They observed that the mean standard deviation of the cases for serum total calcium was 1.032 mmol/L and that of the control was 1.157 mmol/L with p Value <0.001 thus concluding that hypertensive individuals had significantly low serum ionised calcium.

In the year 1990, Zemel and associates observed that during pregnancy there are high risks of various maternal morbidity and complications among which one of the most common is the rise in blood pressure induced by the pregnancy, even though hypertension may not have been present before the pregnancy. The causes of this increase in blood pressure are not exactly known so far. However there has been an observation that reduction in the PGE2 levels is correlated to an increase in the vascular response to angiotensin II in pregnant women who are at risk to develop high blood pressure and large amount of proteins in the urine, most probably because of the rise in intracellular ionic calcium.

In lieu of this, an extensive study was conducted by Julian A. Herrera et al., in the year 2006 to determine if the beneficial effect of oral supplementation of conjugated linoleic acid and calcium in the decrease of the instance of pregnancy-induced hypertension , is related with differences in plasma levels of angiotensin II, renin, calcitropic hormones, prostacyclins, and plasma and intracellular ionized free calcium.

For this particular study, the researchers studied 48 healthy women who were pregnant for the first time, and who also had a family history of female relatives with a disorder during pregnancy characterised by large amount of protein in the urine and high blood pressure and instances of diastolic dip. Their blood samples were taken in a double blind format, with randomization and underwent placebo-controlled trial. The representatives were from two different developing countries and selected from four random outpatient clinics.

Basic calcium and conjugated linoleic acid placebo were orally administered daily in random to the subjects from week 18 to week 22 of gestation until the time of delivery.

After the administration of the supplement and also the placebo, it was found that 8.3% of the women who took the supplement experienced significantly decreased hypertension induced by pregnancy in comparison to 41.7% of women who

received the placebo. Another significant decrease was seen in the supplemented group in concentration of intracellular ionized free calcium from 92.0nmol/L to 62.5nmol/L, from a range of 62.5-220nmol/L to 28-200nmol/L but not in the placebo group. However there were no significant changes seen in the concentration of plasma in ionized calcium, renin, parathomone, prostaglandin E2, angiotensin II and calcitonine. From the participants, 12 women developed high blood pressure induced by pregnancy and showed significant rise in the concentration of intracellular calcium.

Therefore it has been concluded from the research findings and observations that intake of conjugated linoleic acid and calcium supplementation during pregnancy can significantly lower the instances of high blood pressure induced by pregnancy. It also reduces the concentration of intracellular ionized free calcium in peripheral blood lymphocytes.

A comparative study was conducted recently in the year 2013 by Noran Jameel Ibhraheem and Dalia Shaker Obiade from Magazin of Al-Kufa University for Biology on the correlation between serum calcium level and high blood pressure during pregnancy, also termed as Pre-eclampsia. The research was done on healthy pregnant women from the province of Babylon. They were divided into two groups of participants; one included 35 participants who had slightly high blood pressure and the other, 30 participants who had very high blood pressure. The participants

were all from the Babylon maternity and paediatric teaching hospital. The data that were included during the collection were the blood pressure values, body mass index, maternal and gestational ages and also blood samples to analyze serum total calcium, serum albumin and serum ionized calcium.

After the comparison it was observed that the blood pressure value and also the body mass index were much higher in the women with pre-eclampsia than in the women with normal blood pressure. It was also found that ionized calcium levels were much lower in the women with very high blood pressure induced by pregnancy as compared to pregnant patients with normal blood pressure and those pregnant patients with even slightly high blood pressure. The findings also show that the levels of serum total calcium and serum albumin were much lower in the pregnant women with very high blood pressure as compared to pregnant women with normal blood pressure. However it showed that the differences between slightly high blood pressure and normal blood pressure during pregnancy were negligible.

This study confirms also that in order to predict the rise in blood pressure during pregnancy, tests must be done before the second and third trimesters of pregnancy because during the observations it was found that the total ionized calcium and serum albumin levels tested late had not much significance in the prediction of the rise in blood pressure. The researchers have also recommended further tests of other related vitamins along with serum calcium level which may

contribute to this disorder called pre-eclampsia.

In yet another study a representative sample of black women was taken to determine the level of serum calcium in the women who had hypertension induced by pregnancy before and 48 hours after the delivery and the results were compared to the serum calcium levels of black women with normal blood pressure during pregnancy.

The sample included 127 women who were not pregnant and were not taking any medication or contraception, 102 women with normal pregnancies, and a study group with 27 eclamptic patients and 52 severe hypertensive patients. They were also grouped into pre-delivery and post-delivery groups. The blood samples obtained were analyzed to determine the levels of albumin, total protein, total calcium, phosphate, urea and magnesium.

The results show that the mean of the diastolic blood pressure of the women who were not pregnant was much lesser than that of the women with normal pregnancies. Also both the mean of the systolic and diastolic blood pressures of the women with normal pregnancies were again much lesser than that of the study group of eclamptic and severe hypertensive patients.

It was seen that the calcium levels between the women who were not

pregnant and that of the women with normal pregnancy did not have much differences. However, the calcium levels were much lower in those women who had eclampsia and sever hypertension, than as compared to the women who were not pregnant and those with normal pregnancies. Also when the results were compared between those women with eclampsia and those with severe hypertension, the former's level of calcium were significantly lower than the latter's. But not much differences were seen in the calcium levels in both the eclamptic and hypertensive groups when compared before and after delivery.

Those women who had normal pregnancy did not show much differences in their ionized calcium levels before delivery and 48 hours after the delivery. There was also not much difference in the calcium levels between women who were not pregnant, who had normal pregnancies and who were severely hypertensive. After the second trimester, it was seen that the albumin level reduced significantly within those women who had normal pregnancies but the differences seen in the eclamptic and hypertensive after the second trimester women were quite negligible but was definitely lesser in comparison to the normal pregnancy women in each trimester and after delivery. There were also some differences in the levels of phosphorous and urea between these two groups but not in magnesium.

One important observation concluded through this study was that the calculated ionic fraction of calcium was constant throughout the pregnancy but the total calcium level decreased. The calcium level decreased along with serum

albumin which shows that there is a strong correlation with protein-bound fractions. Through this study it has been proposed that the decrease in calcium level during pregnancy is because of physiological hypo-albuminaemia. The researchers have also suggested the further evaluation of ionized fraction of calcium because physiological changes during pregnancy make it hard to interpret the various levels of serum calcium concentration that takes place. The difficulty in interpretation is not only because of the physiological changes but also that serum calcium and albumin declines even more in pre-eclamptic patients, inferring to the increase in protein loss in urine.

This study was also done in keeping with a hypothesis made by Mendlowitz that high blood pressure in pregnant women may be due to defective production of intracellular calcium-transporting protein in both the smooth uterus muscle and vascular muscle. This hypothesis is based on a demonstration on the Okimoto rat where a defect in the transport of calcium in the smooth muscle gave way to hypertension.

The benefit of increase in calcium intake for hypertension can further be supported by another study undertaken by Dickinson Heather O et al., in the year 2008. The ongoing studies on metabolism suggest that calcium plays a role in the regulation of blood pressure. Some studies also have reported that people who take calcium at a higher level have lower blood pressure than others who do not. The debate and

speculation still continues on whether or not supplementation of calcium orally contributes to the decrease in blood pressure. Therefore this study aims to analyze the effects of supplementation of calcium as a form of treatment in high blood pressure in adults. An extensive search for data was carried out in the Cochrane Library, EMBASE, ISI Proceedings, etc.

The participants were all above 18 years of age, whose systolic blood pressure was 140mmHg. Both the systolic and diastolic blood pressure values were monitored before and after the tests and at the end of follow-up. The participants who were pregnant were excluded from the study as well as those who were taking medication for high blood pressure, and also those who took calcium supplements with other medications. The subjects were taken in a randomized controlled trial where comparison was done among supplementation of calcium orally with usual care, no treatment or placebo. The test consisted of treatment and follow-up which took up 8 weeks.

The data that were collected and the quality of the trial were analyzed and assessed separately by reviewers. For the objectivity of the trials, the researchers conducted sensitivity and random effects meta-analyses.

After the trials the results show that in combining all the trials, subjects who received oral calcium supplements had a much larger significant decrease in their systolic blood pressure in comparison to the control group of subjects but not in the

diastolic blood pressure. The mean difference between the two groups' systolic blood pressure was -2.5mmHg, 12=42%, 95% Cl: -4.5 to -0.6.

This study contributes to some extend to ascertaining the role of serum calcium in maintaining blood pressure in adults by generating a small reduction in the systolic blood pressure but little effect on the diastolic blood pressure after studying the trials and the follow-up done by the researchers. This result is in line with results of a meta-analysis previously conducted by Allender in 1996, Bucher in 1996 and Griffith in 1999.

The DASH Trial conducted in 1999 and in 2000 showed significant decrease in blood pressure after 8 weeks follow-up among participants with high blood pressure, who were on a diet that was high in calcium intake in comparison to those of the control group whose diet was low in calcium.

Sura Sagban et al., in the year 2011 conducted a research to determine the effect of calcium carbonate taken orally by pregnant women with slightly high blood pressure which is also known as pre-eclampsia. As this condition of hypertension is the most common complication during pregnancy which poses a great health risk for both the infant and the mother, various metabolism studies are being done on how to regulate maternal blood pressure and also it is being determined on whether the oral intake of calcium reduces high blood pressure. The study by Sura Sagban et al., was done in order to evaluate and compare the level of calcium in high blood

pressure induced by pregnancy and in healthy pregnant women with normal blood pressure, and also to evaluate the effect of calcium taken orally by pregnant women with slightly high blood pressure, which is also referred to as mild pre-eclampsia. This study also was done to find out if there are any correlation between the effect of orally taken calcium supplements and the change in the concentration of serum calcium.

For this study 45 pregnant women in their third trimester were selected and grouped into 15 healthy pregnant women with normal blood pressure as the control group, 30 pregnant women with slightly high blood pressure out of which 15 of them are untreated and 15 of them are treated with calcium carbonate of 500mg twice daily.

After the test period, the results showed that the concentration of serum calcium in women with slightly high blood pressure was quite low as compared to those women with normal blood pressure. Also it was observed that the level of serum calcium showed significant rise in those pregnant women with slightly high blood pressure who were administered oral supplements of calcium carbonate of 500mg twice daily in comparison to those women with slightly high blood pressure who were not treated any calcium carbonate. The blood pressure value and the SBP and the DBP was observed to have greatly decreased after a month's time of treatment with calcium carbonate of 500mg taken twice daily in contrast to slightly high blood pressure group of pregnant women who were not treated, respectively.

The results that were obtained also showed that the concentration of serum calcium level in those women with slightly high blood pressure is much lesser than those women with normal blood pressure during their pregnancy. This suggests that calcium supplement taken orally can be beneficial in regulating blood pressure by increasing its intake. Also that reducing calcium intake during pregnancy can cause rise in blood pressure which is also called pre-eclampsia and that supplementing this nutrient may prevent pre-eclampsia by regulating the blood pressure.

4. METHODOLOGY

Study population: A total of 100 subjects, of which 50 cases of essential hypertension satisfying inclusion and exclusion criteria visiting medicine OPD and admitted in Govt. Rajaji hospital, Madurai and 50 age and sex matched normotensive controls from 1st June 2014 to 30th November 2014 were taken up for study.

PROFORMA

Name: **OP/IP No.:**

Age: **Hospital: GRH, Madurai**

Sex: **Date of Examination:**

Occupation: **Case / Control No.:**

Address:

Socioeconomic status: Low/Middle/High

Presenting Complaints:

Headache

Palpitation

Chest Pain

Giddiness

Blurring of vision

Oedema

Stroke

Onset and Duration of Hypertension:

Drug Treatment: If any

Symptoms of Target Organ involvement: If any

Past history:

H/o Diabetes, IHD, Tuberculosis, Renal disease, CVA in the past.

Family History :

a) H/o Hypertension Yes/ No

b) H/o Diabetes Mellitus Yes/ No

Personal history:

1. Diet –Veg / Mixed
2. Sleep – Normal / Decreased
3. Bowel and bladder habits – Regular / Disturbed
4. Alcohol consumption – Yes / No; if yes, then

   Duration - Amount - Frequency -
5. Smoking – Yes / No; if yes, then

   Duration - Frequency –

Menstrual History :

General physical examination:

- Height : Weight: BMI:
- Nourishment – Well / Moderate / Poor
- Built – Well / Moderate / Poor
- Pallor / Icterus / Clubbing /Cyanosis/ Lymphadenopathy / Oedema
- Thyroid swelling

Vitals:

- Pulse : Rate, rhythm , volume , character
- Blood pressure : Sitting

  Standing

· Respiratory rate:

Systemic examination :

a) Cardiovascular System:

b) Respiratory System:

c) Per abdomen :

d) Central nervous system:

Clinical diagnosis:

Study profile:

- Serum total calcium
- Serum phosphorus
- Serum calcium / phosphorus ratio

INCLUSION CRITERIA :

- Patients with Newly diagnosed Essential hypertension
- Patients whose age is above 18 years are included
- Both sexes are included

EXCLUSION CRITERIA :

- Patients who are below 18 years
- Patients who are on Vitamin D and Calcium supplementation
- Patients with Primary kidney disease/Chronic Kidney disease
- Chronic Liver disease
- Secondary Hypertension
- Pregnancy
- Drugs influencing Calcium and Phosphorus metabolism

JNC VII criteria was used to define hypertension in the study population. Cut off value for normotensives were taken as Systolic BP <120 mmHg and Diastolic BP <80 mmHg. Informed consent was taken from all the subjects. A detail history including diet, smoking and alcohol habits, lifestyle, drug and treatment history were taken into account.

MEASUREMENT OF BLOOD PRESSURE

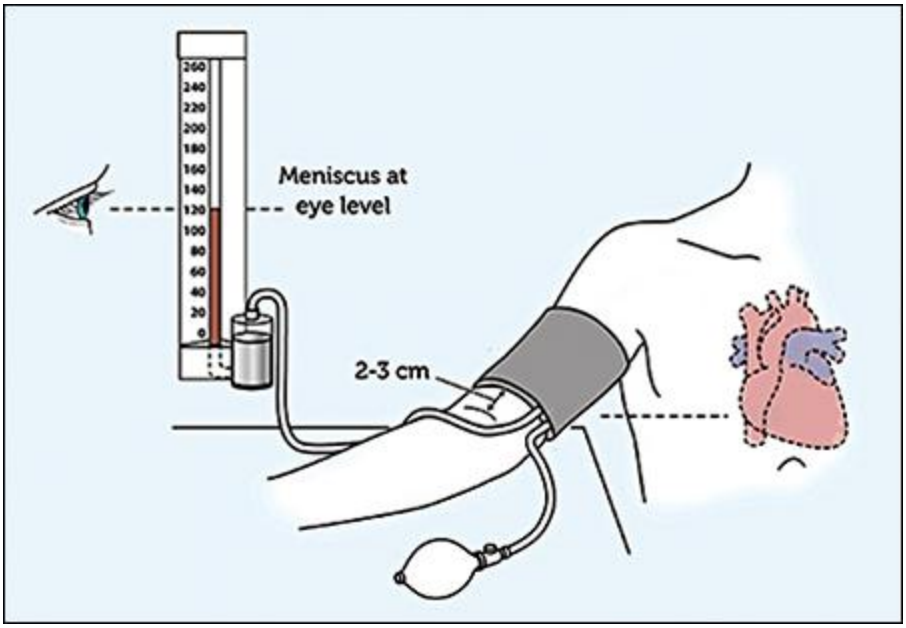


FIG 13: CORRECT TECHNIQUE FOR BP MEASURMENT

Posture: Subject was kept in sitting position for 10 minutes with the back adequately supported, the arms were bared and kept approximately at the level of the heart. No tea, coffee, smoking, alcohol, medications were allowed for the previous 30 minutes.

Environment: Subject was kept in a quiet and warm room with sufficient lighting. Recent meals and bowel / bladder evacuation was asked. Subject was reassured about the procedure to allay any anxiety.

Blood pressure equipment: A mercurial based manual sphygmomanometer with adequate cuff size for adults encircling a minimum area of 80 percent of

the arm and covering two thirds of its length was used. A space of 2.5 cm was kept between the anterior cubital fossa and the lower border of the cuff.

Technique: Cuff pressure was elevated till the radial pulse disappeared. The bladder was deflated 2 mmHg per second and the first Korotkoff sound heard was considered as systolic blood pressure and disappearance of Korotkoff sound phase 5 was considered as diastolic blood pressure. Two readings were taken at 1 and 5 minutes respectively. Both the right and left arm pressures were measured and the mean taken into account. Details of the readings were noted down including the posture, arm used and pulse rate.

Blood sample: 5 ml of non-hemolysed blood was drawn using a syringe from the ante cubital vein without the use of a tourniquet. The sample was immediately centrifuged for tests.

Calcium: Total serum calcium was measured using arsenazo III reagent which is a highly sensitive and stable reagent. Serum calcium reacts with arsenazo III to form a purple coloured complex. Serum calcium was then measured using an automated analyzer. Calibration was done using an aqueous Calcium Standard calibrator.

Principle:

Calcium + Arsenazo →→→→→→→→ Calcium-Arsenazo Complex
(purple colour)

Calculation:

$$\frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times \text{Conc. of Standard} = \text{Calcium in mg/dl}$$

For Example:

If the absorbance of sample = 0.91

And absorbance of standard = 0.80

Concentration of standard = 10mg/dl

Then, $\frac{0.80}{0.80} \times 10 = 10.0$ mg/dl

Phosphorus:

Serum phosphorus was measured using ammonium molybdate reagent in a slightly acidic medium. It reacts with the agent forming a phosphomolybdate complex which absorbs light at 350 nm wavelength. Calibration was done using an appropriate standard calibrator.

Principle:

Phosphorus + H_2SO_4 + Ammonium molybdate →→→→ Unreduced

Phosphomolybdate Molybdate Compound

Calculation:

Absorbance of subject x Conc. of sandardtd = Inorganic Phosphorus in mg/dL

Absorbance of Std.

For Example:

If Absorbance of Subject = 0.25

Absorbance of Standard = 0.29

Concentration of Standard = 5 mg/dl

then, $\frac{0.25}{0.29} \times 5 = 4.3$ mg/dl

5. RESULTS

The mean, median and standard deviation of the study population on various parameters are given below in the table.

	BMI	AGE	CALCIUM (mg/dl)	PHOSPHORUS (mg/dl)	CP RATIO
Mean	22.54	44.06	8.88	3.17	2.9182
Median	21.9	43	8.8	3.2	2.71
Standard Deviation	2.784	11.748	0.573	0.6472	0.62272
Minimum	18	18	8	1.9	2.11
Maximum	31	78	10	4.3	4.59

TABLE 5: **Mean, median and standard deviation of the participants**

The age distribution of the participants is given below in the table. 2. 36% of the participants were in the age group 18 – 39 years, 55% of the population was in the age group 40 – 59 years, while 9% of the population was in age group of 60 years and above.

TABLE 6: AGE DISTRIBUTION OF THE PARTICIPANTS

		Frequency	Percent
AGE (Years)	18-39	36	36
	40-59	55	55
	>=60	9	9
	Total	100	100

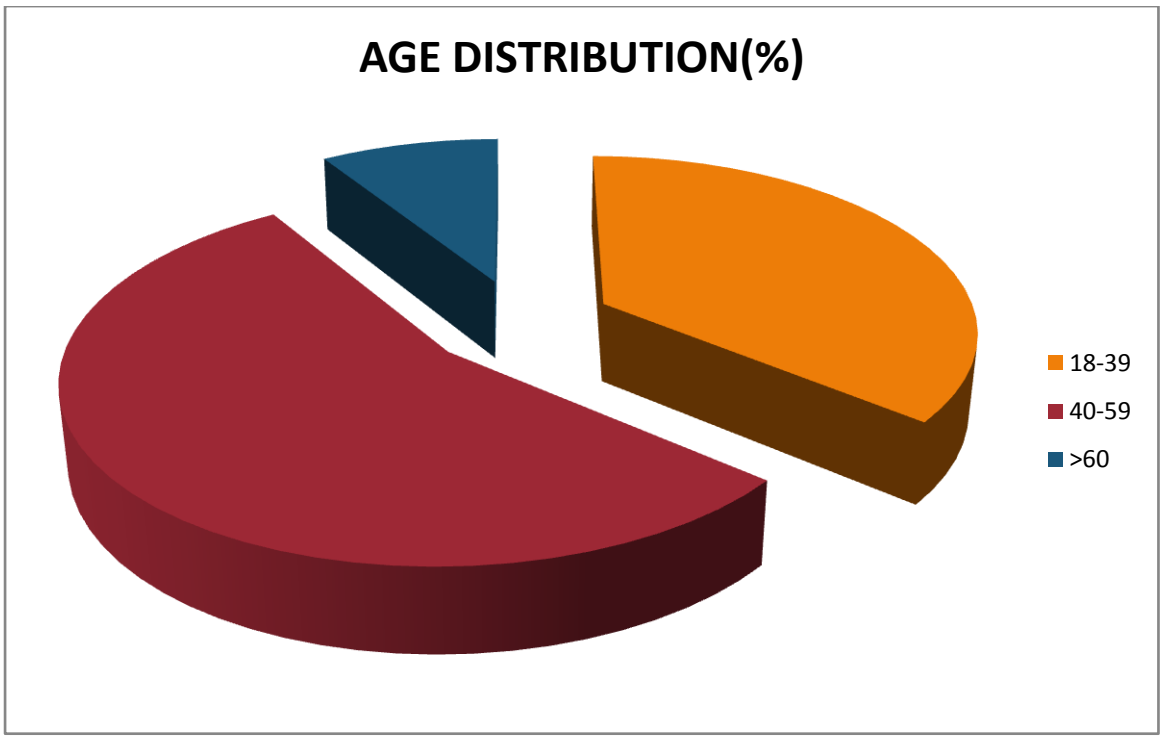


FIG 14: AGE DISTRIBUTION OF THE PARTICIPANTS

The sex distribution of cases and control is given in the table 3 below. Among the cases, 24 participants were females while 26 participants were male which was statistically significant (p value 0.04). Among the controls, both the males and females were equal with 25 participants each.

		CASES	CONTROLS	
SEX	FEMALE	24	25	49
		48.00%	50.00%	49.00%
	MALE	26	25	51
		52.00%	50.00%	51.00%

Pearson Chi-Square: 0.04 ; p Value: 0.841

TABLE 6: SEX DISTRIBUTION OF THE STUDY POPULATION

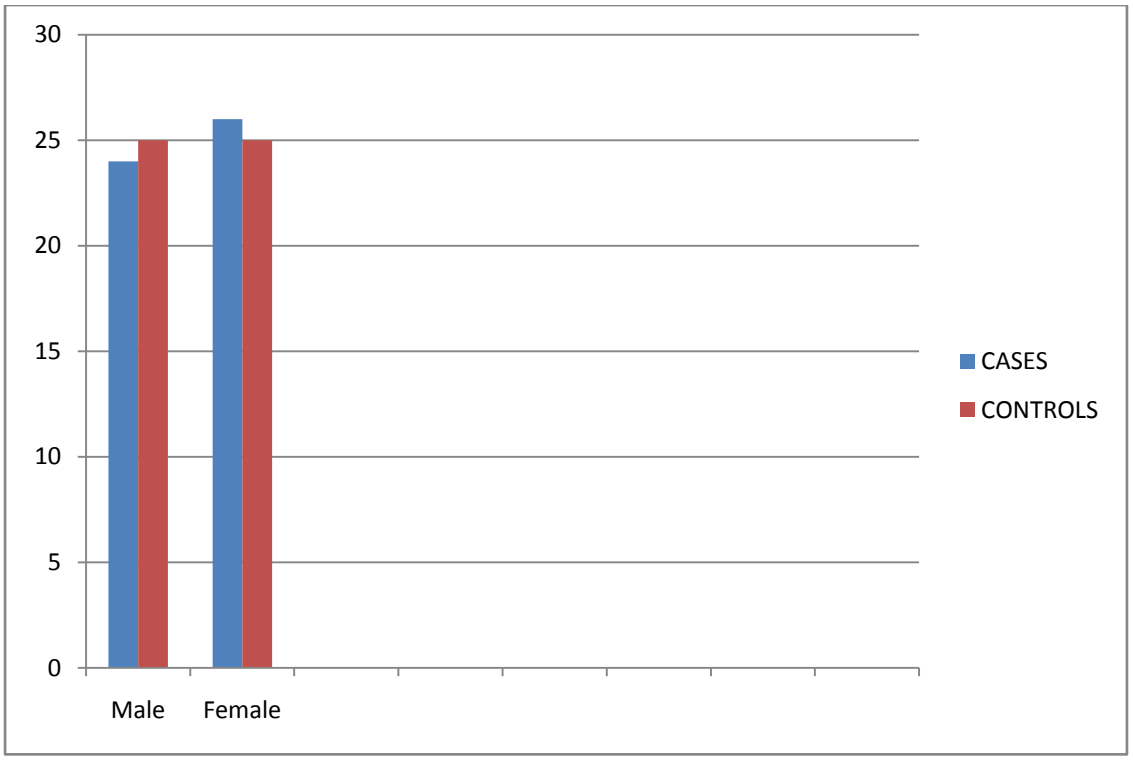


FIG 15: SEX DISTRIBUTION OF THE STUDY POPULATION

The median age of the study population was 44. 62% of the cases were less than the median, while 36% of the control were less than median. On the other hand, 38% of the cases and 64% of the control were above the median.

		CASES	CONTROLS	TOTAL
AGE(Years)	>=44	31	18	49
		62.00%	36.00%	49.00%
	<44	19	32	51
		38.00%	64.00%	51.00%
		50	50	100

CUT OFF USING MEDIAN AGE

Pearson Chi-Square: 6.763; ODDS RATIO: 2.901; 95% CI: 1.288 – 6.534; p Value: 0.009*

TABLE 7: MEDIAN AGE OF THE PARTICIPANTS

Out of the total participants, 20 % of the cases were under "overweight" category and 4.40 % were under "obese" category, while 10.2 % of the controls were overweight and 2.1% were obese, the p value being 0.06.

		CASES	CONTROLS	TOTAL
BMI	<18.49	1	0	1
		2.20%	0.00%	1.10%
	18.5-24.49	33	44	77
		73.30%	89.80%	81.90%
	25-29.99	9	5	14
		20.00%	10.20%	14.90%
	30-34.99	2	0	2
		4.40%	0.00%	2.10%
		45	49	94

TABLE 8: BMI OF CASES AND CONTROLS

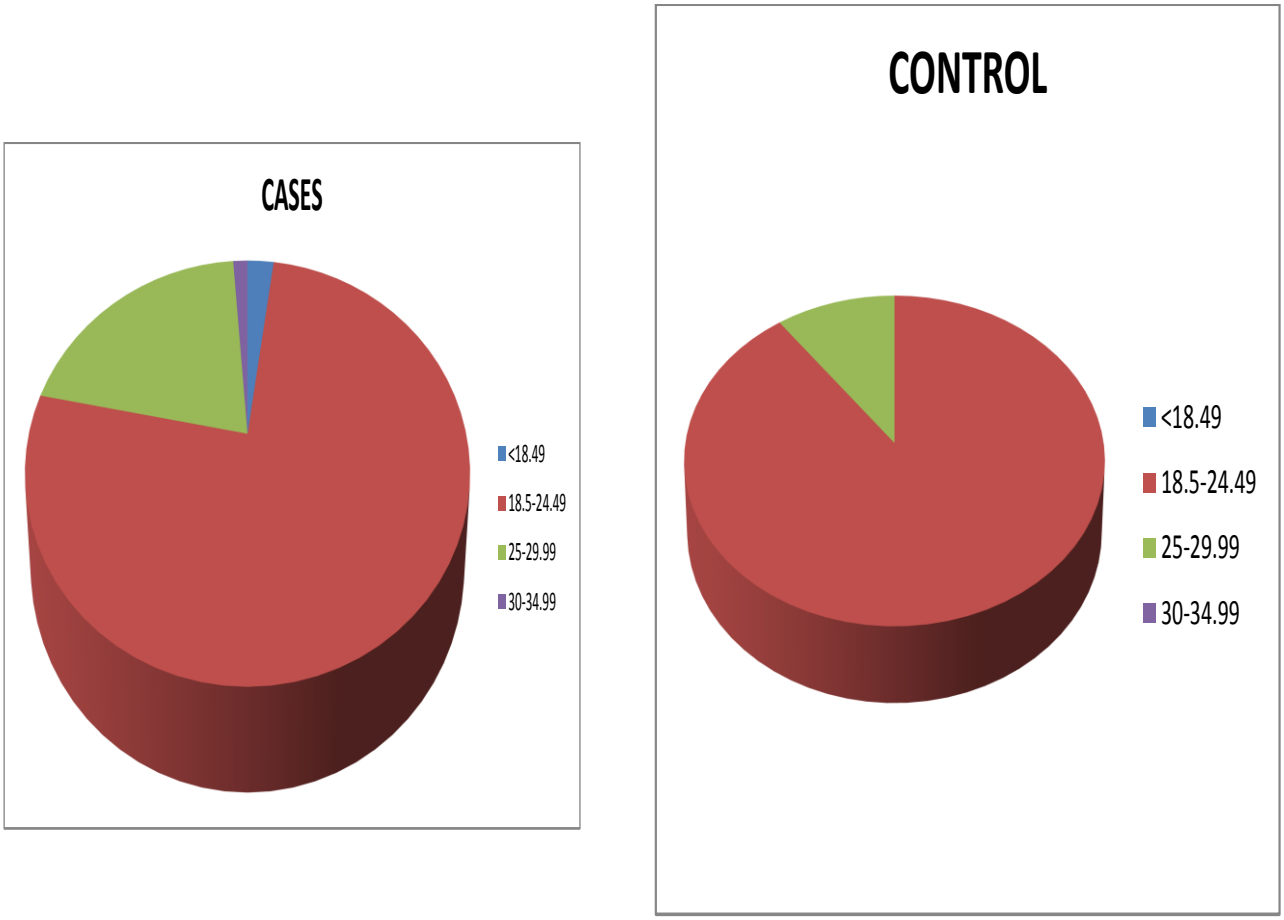


FIG 16: BMI OF STUDY POPULATION

		CASES	CONTROLS	TOTAL
BMI	>=25	11	5	16
		24.40%	10.20%	17.00%
	<25	34	44	78
		75.60%	89.80%	83.00%
		45	49	94

Pearson Chi-Square: 3.368; OR: 2.847; 95% CI: 0.903 - 8.973; p Value: 0.066

TABLE 9: NORMAL AND OBESE/OVERWEIGHT OF THE PARTICIPANTS

Out of all the participants, 60 % of the cases showed decreased serum level while 24 % of the controls showed decreased serum calcium level with p value <0.001. Only 40% of the cases had normal serum calcium level while 76 % of the controls had normal serum calcium level. (Pearson Chi-Square: 13.3, OR: 4.75; 95%: 2.008 – 11.236; p Value: <0.001)

		CASES	CONTROLS	TOTAL
SERUM CALCIUM (mg/dl)	<8.07	30	12	42
		60.00%	24.00%	42.00%
	>=8.07	20	38	58
		40.00%	76.00%	58.00%
		50	50	100

CUTOFF USING LOWER LIMIT OF RANGE

Pearson Chi-Square: 13.3, OR: 4.75; 95%: 2.008 – 11.236; p Value: <0.001

TABLE 10: SERUM TOTAL CALCIUM OF THE PARTICIPANTS

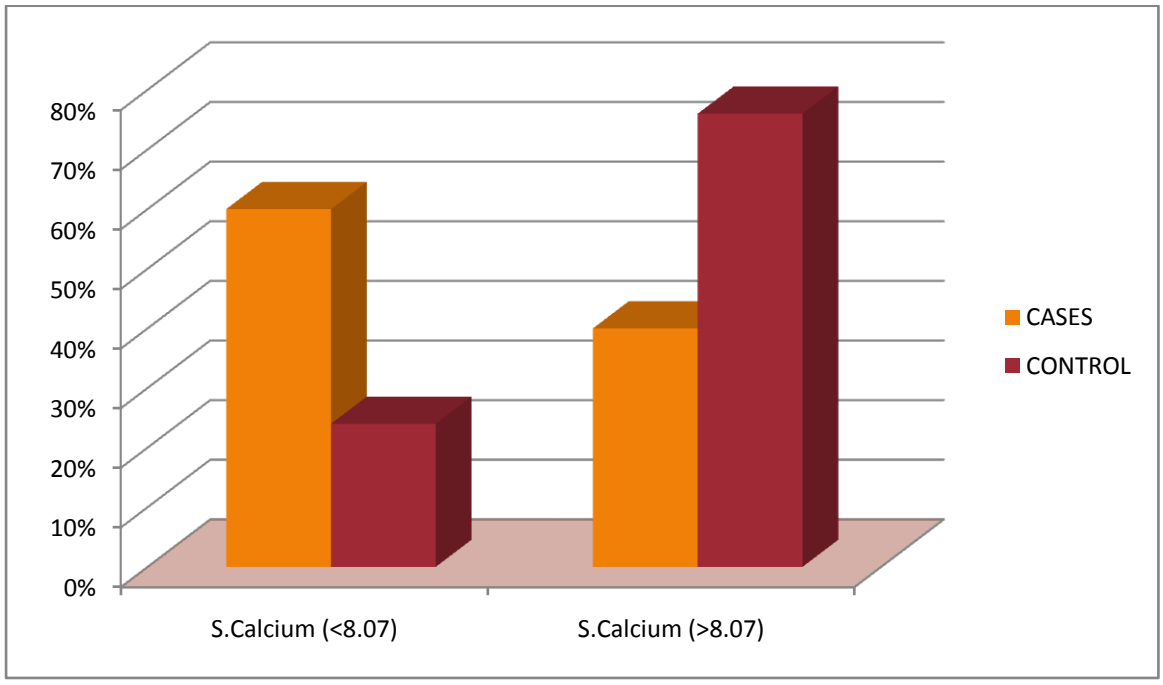


FIG 17: SERUM TOTAL CALCIUM IN CASES AND CONTROL

		CASES	CONTROLS	TOTAL
CP RATIO	≥ 4.09	11	0	11
		22.00%	0.00%	11.00%
	<4.09	39	50	89
		78.00%	100.00%	89.00%
		50	50	100

CUTOFF USING UPPER LIMIT OF RANGE

Pearson Chi-Square: 12.36; p Value: <0.001

TABLE 11: CALCIUM PHOSPHORUS RATIO IN CASES AND CONTROLS

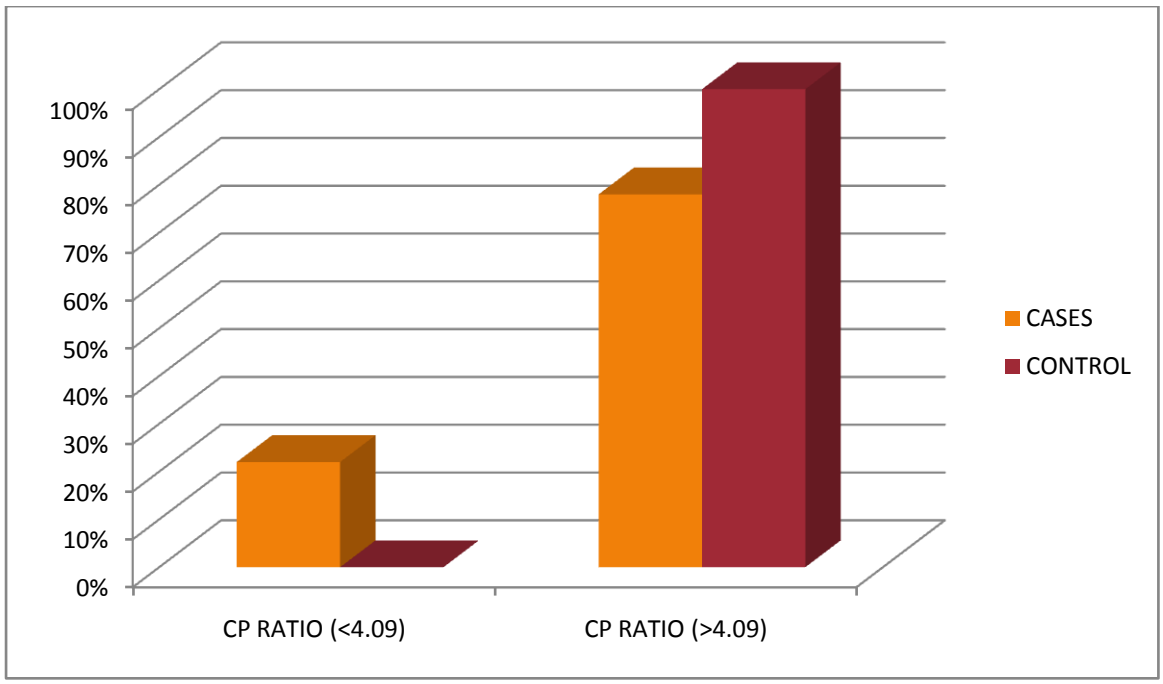


FIG 18: GRAPH SHOWING CALCIUM PHOSPHORUS RATIO IN CASES AND CONTROLS

		CASES	CONTROLS	TOTAL
CP RATIO	>2.7	35	17	52
		70.00%	34.00%	52.00%
	<2.7	15	33	48
		30.00%	66.00%	48.00%
		50	50	100

CUTOFF USING MEDIAN

Pearson Chi-Square:12.981; OR: 4.529; 95% CI: 1.952 – 10.508; p Value: <0.001*

TABLE 12: CALCIUM PHOSPHORUS RATIO USING LOWER LIMIT AS CUT OFF RANGE

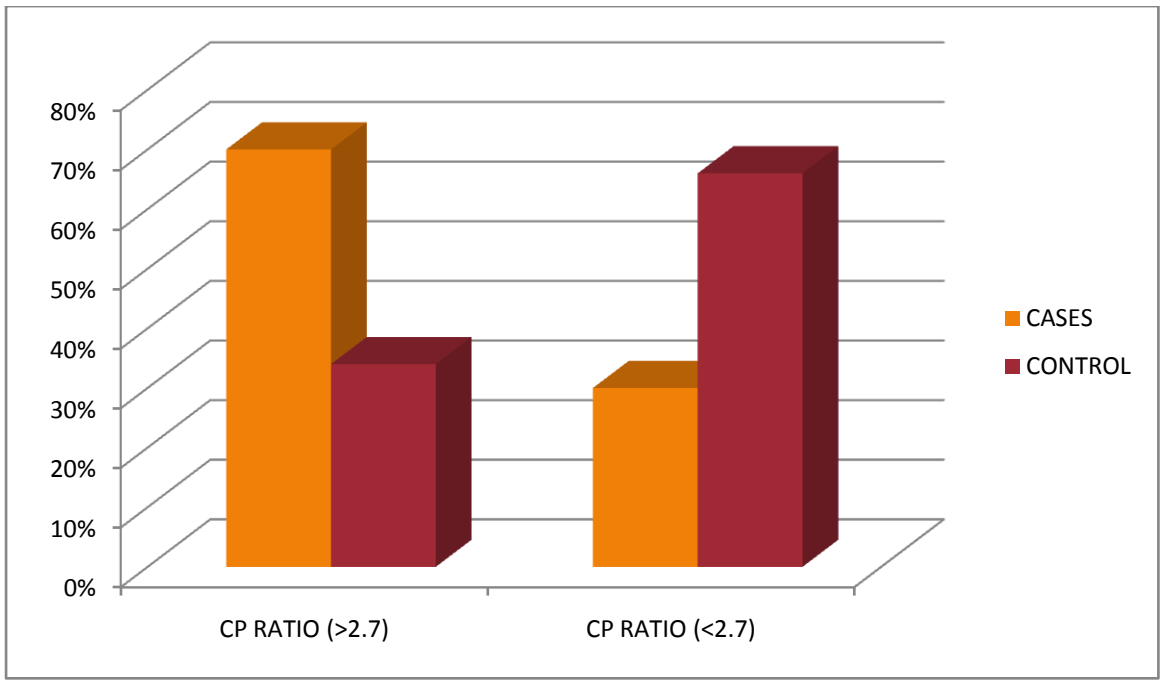


FIG 19:GRAPH SHOWING CP RATIO BETWEEN CASES AND CONTROLS

28% of the cases had a positive family history of hypertension while 72% did not have any family history. Among the control, only 12% had family history of hypertension while 88% of them did not have any positive family history. Although, the association of positive family history of hypertension was slightly stronger with cases, we did not find it statistically significant (p Value: 0.046)

		CASES	CONTROLS	TOTAL
FAMILY HISTORY	YES	14	6	20
		28.00%	12.00%	20.00%
	NO	36	44	80
		72.00%	88.00%	80.00%
		50	50	100

Pearson Chi-Square: 4; OR: 2.582; 95% CI: 0.995 – 8.173, p Value: 0.046*

TABLE 13: POSITIVE FAMILY HISTORY BETWEEN CASES AND CONTROLS

36% of the cases were smokers and 64% were non smokers. On the other hand, 32% of the control were smokers and 68% did not have any history of smoking. Similarly, smoking has only a slightly stronger association with Cases but we did not find it statistically significant (p Value: 0.673).

		CASES	CONTROLS	TOTAL
SMOKER	YES	14	6	20
		36.00%	32.00%	34.00%
	NO	32	34	66
		64.00%	68.00%	66.00%
		50	50	100

Pearson Chi-Square: 0.178; OR: 1.195; 95% CI: O.522 – 2.737, p Value: 0.673

TABLE 14: SMOKING HABITS IN CASES AND CONTROLS

In our study population, 28% of both cases and control were alcoholics while 72% of them were non-alcoholics. The association of alcohol in cases and control was equal and statistically not significant.

		CASES	CONTROLS	TOTAL
ALCOHOL	YES	14	14	28
		28.00%	28.00%	28.00%
	NO	36	36	72
		72.00%	72.00%	72.00%
		50	50	100

Pearson Chi-Square: 0; p Value: 1.0

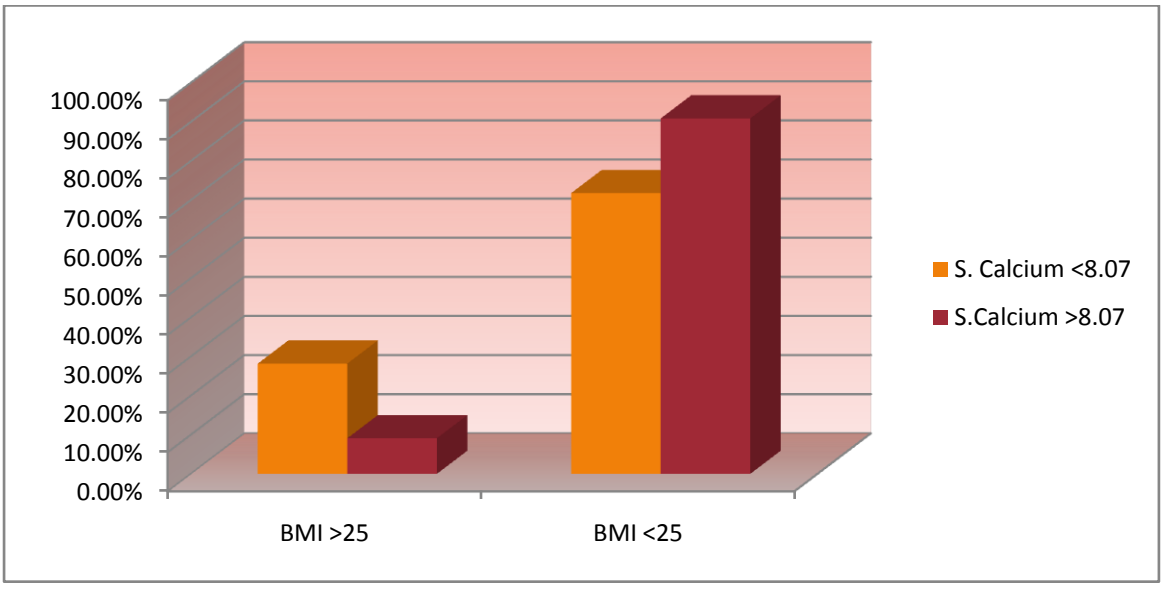
TABLE 15: ALCOHOL HABIT IN CASES AND CONTROLS

28% of the participants who had BMI > 25 had serum calcium less than 8.07 mg/dl while only 9.10 % with normal serum calcium level had BMI more than 25. 71% of the participants who had BMI < 25 had decreased serum calcium while 90% of the participants with BMI less than 25 had normal serum calium. (p Value: 0.015*). In the participants, we observed that a higher percentage of participants who are obese or overweight had lower calcium level rather than normal serum calcium which was statistically significant.

		CALCIUM (mg/dl)		
		<8.07	**>8.07**	TOTAL
BMI	>25	11	5	16
		28.20%	9.10%	17.00%
	<25	28	50	78
		71.80%	90.90%	83.00%
		39	55	94

Pearson Chi-Square: 5.903; OR: 3.929; 95% CI: 1.239 - 12.456; p Value:0.015*

TABLE 16: ASSOCIATION OF BMI AND SERUM TOTAL CALCIUM



In our study, 64.30% of the participants who were above 44 years had low serum calcium while 37.90% had normal serum calcium level. 35.70% of the participants who were below 44 years had low serum calcium and 62.10% of the participants in this category had normal serum calcium. This shows that older people are more likely to have low serum total calcium as compared to younger age group. (p Value: 0.009*)

		CALCIUM (mg/dL)		
		<8.07	**>8.07**	TOTAL
AGE	>44	27	22	49
		64.30%	37.90%	49.00%
	<44	15	36	51
		35.70%	62.10%	51.00%
		42	58	100

Pearson Chi-Square: 6.771; OR: 2.945; 95%: 1.292 – 6.717; p Value: 0.009*

TABLE 17: AGE DISTRIBUTION WITH SERUM TOTAL CALCIUM

In our study, 50% of the obese/overweight individuals had serum calcium phosphorus ratio more than 4.09 and 13.10% had serum calcium phosphorus ratio less than 4.09. 50% of the participants with normal weight had serum calcium phosphorus ratio more than 4.09 while 86% of the participants in this category had serum calcium phosphorus less than 4.09. This shows that subjects who are obese/overweight are more strongly associated with an increased C:P

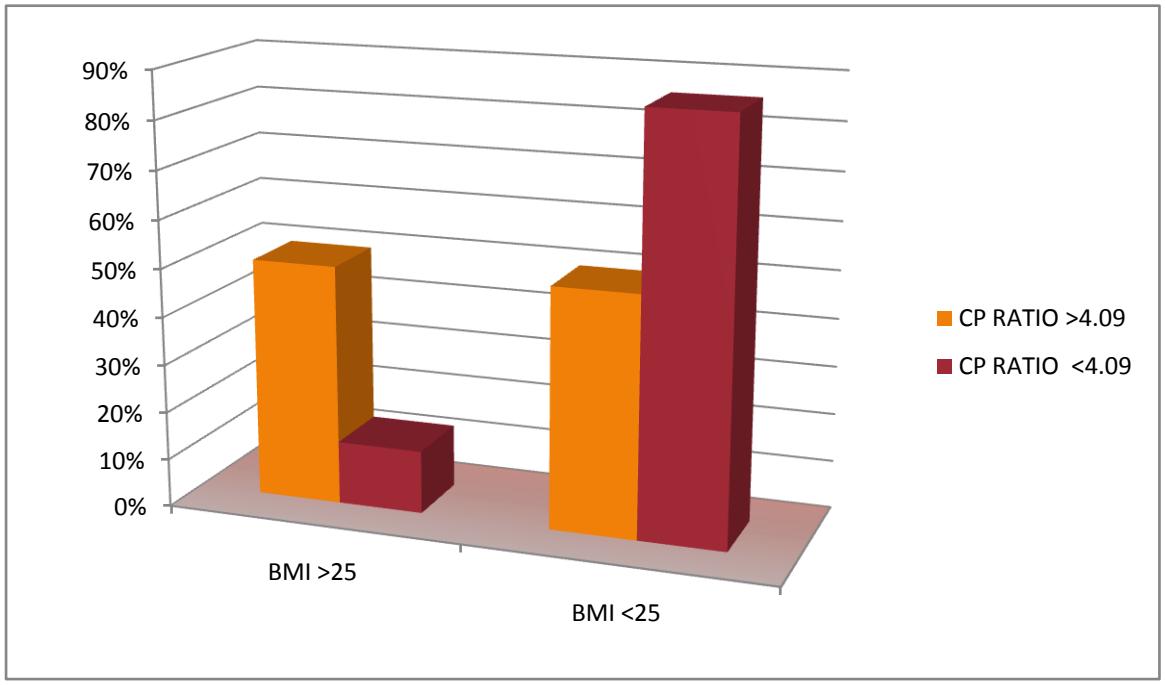
ratio as compared to subjects with normal body weight which was statistically significant. (p Value: 0.003*)

TABLE 18: ASSOCIATION OF BMI AND CALCIUM PHOSPHORUS RATIO

		CALCIUM PHOSPHORUS(CP) RATIO		
		>4.09	**<4.09**	TOTAL
BMI	>25	5	11	16
		50.00%	13.10%	17.00%
	<25	5	73	78
		50.00%	86.90%	83.00%
		10	84	94

Pearson Chi-Square: 8.617; OR: 6.636; 95% CI: 1.649 – 26.705; p Value: 0.003*

FIG 21: GRAPH SHOWING ASSOCIATION OF BMI WITH CP RATIO



Multivariate analysis by logistic regression

TABLE 19**: Logistic regression model for factors associated with hypertension**

MULTIVARIATE ANALYSIS							
Variable	OR (unadjusted)	95% C.I (unadjusted)		Chi Sq.P-value	OR (Adjusted)	95% C.I. (adjusted)	
		Lower	Upper			Lower	Upper
Female	0.923	0.421	2.022	0.81	0.88	0.33	2.4
Serum calcium <8.07 mg/dl	4.75	2.008	11.236	**0.003***	4.61	1.66	12.81
CP ratio > 2.7	4.529	1.952	10.508	**0.002***	5.26	1.89	14.7
Smoker	1.195	0.522	2.737	0.06	3.39	0.96	11.93
BMI >=25	2.847	0.903	8.973	0.33	2.02	0.5	8.21
Age >=44yrs	2.901	1.288	6.534	0.11	2.32	0.83	6.51

To adjust for potential confounders, selected variables from the Univariate analysis were analyzed. A logistic regression model was derived by entering the following variables: Sex, serum calcium <8.07 mg/dl, CP ratio >2.7, smoking history. BMI>=25 and Age >=44years. In this regression model Serum calcium <8.07 mg/dl [Adjusted OR 4.61 (95%CI 1.66-12.81] and CP ratio >2.7. Adjusted OR 5.26 (95%CI 1.89-14.7] are significantly associated with

hypertension after adjusting for confounding factors.

In other words the odds of a patient with hypertension is 4.61 times more likely to have a serum calcium level less than 8.07 mg/dl as compared to a non hypertensive and the odds of a patient with hypertension to have a CP ratio>=2.7 is 5.26 more than that of a normotensive individual.

7. DISCUSSION

In our study, the age distribution of the participants were grouped into three categories. 2. 36% of the study population was in the age group 18 – 39 years, 55% of the population was in the age group 40 – 59 years, while 9% of the population was in age group of 60 years and above. Among the three groups, age group 18-39 % had the lowest number of participants. This may be attributed to the fact that most hypertensive patients remain asymptomatic for many years and they seek medical attention only when complication sets in. As expected, participants from age group 40-59 were the highest as they began to develop hypertension related problems like end organ damage during this age.

The sex distribution of cases and control is given in the table 3 below. Among the cases, 24 participants were females while 26 participants were male which was statistically not significant (p value 0.04). Among the controls, both the males and females were equal with 25 participants each. In our study, we observed a slightly increased number of male participants but it was not significant. Therefore, our observation is that both males and females are almost equally affected, especially in the middle age.

The median age of the study population was 44.62% of the cases were less than the median, while 36% of the control were less than median. On the other hand, 38% of the cases and 64% of the control were above the median.

Out of the total participants, 20 % of the cases were under "overweight" category and 4.40 % were under "obese" category, while 10.2 % of the controls were overweight and 2.1% were obese, the p value being 0.06 which was statistically not significant in relation to hypertension. However we observed that over weight/obese individuals tend to have decreased serum calcium level which was statistically significant. (p Value: 0.015*). Thus BMI may be an independent risk factor for low serum calcium.

In our study, 86% of the participants with C:P ratio in normal range had normal BMI, while only 13.10% of the overweight/obese participants had C:P ratio in the normal range which was statistically significant. (p Value:0.003*)

In the study population, 60 % of the cases showed decreased serum level while 24 % of the controls showed decreased serum calcium level with p value <0.001. Only 40% of the cases had normal serum calcium level while 76 % of the controls had normal serum calcium level. (Pearson Chi-Square: 13.3, OR: 4.75; 95%: 2.008 – 11.236; p Value: <0.001). This is in agreement with studies done various authors like Jha et al[7] where they concluded that serum total calcium is

decreased in hypertensive subjects as compared to normotensives.

Touyz et al [2] also observed a significantly decreased level of serum calcium in hypertensive individuals along with decreased serum potassium and decreased serum magnesium. Yogesh et al [13] mentioned that serum ionised calcium calcium was decreased in hypertension but no significant correlation exists between serum total calcium and hypertension. Our finding is also in accordance with studies done by Stern et al [5], Takale et al [3], Sharma et al[9], Sudhakar et al [4]. However, in a study done by Hazari and associates [10], they could not find any significant association of both serum calcium and serum ionized calcium with hypertension.

36% of the cases were smokers and 64% were non smokers. On the other hand, 32% of the control were smokers and 68% did not have any history of smoking. Similarly, smoking has only a slightly stronger association with Cases but we did not find it statistically significant (p Value: 0.673)

In our study population, 28% of both cases and control were alcoholics while 72% of them were non-alcoholics. The association of alcohol in cases and control was equal and statistically not significant (p Value: 1). This is in contrast to the observations made by Danni and associates[29], Klatsy [30] who opined that alcohol may serve as an independent risk factor for hypertension.

28% of the cases had a positive family history of hypertension while 72% did not have any family history. Among the control, only 12% had family history of hypertension while 88% of them did not have any positive family history. Although, Sudhakar et al [4], and Sharma [18] concluded that serum calcium was decreased in the first degree relatives of hypertensive individuals as well, we observed that the association of positive family history of hypertension was only slightly stronger with cases, and we did not find it statistically significant (p Value: 0.046)

Using the multivariate analysis, we observed that participants with low serum total calcium are significantly associated with hypertension after adjusting for confounding factors.

In other words the odds of a patient with hypertension is 4.61 times more likely to have a serum calcium level less than 8.07 mg/dl as compared to a non hypertensive individual and the odds of a patient with hypertension to have a CP ratio >2.7 is 5.26 more than that of a normotensive individual.

7. SUMMARY

- Majority of our patients were in the age group of 40 – 55.
- Males outnumbered females slightly.
- Most of the patients had normal body mass index.
- Positive family history had only a slight association with essential hypertension.
- Obesity may be an independent risk factor for low serum calcium.
- Smoking and alcohol habits did not show any relationship with essential hypertension.
- There was a significant association between serum total calcium, serum calcium / phosphorus ratio with essential hypertension.

8. CONCLUSION

. Hypertension is an emerging health problem in India. Hypertension significantly increases the risk of mortality and morbidity of cerebrovascular accidents (both ischaemic and haemorrhagic), coronary artery disease, congestive heart failure, chronic kidney failure, and peripheral vascular diseases. When majority of people come to know that they have hypertension they have already advanced into a stage with target organ damage - a fatal stroke or myocardial infarction or irreversible renal failure. In addition to a primary increase in cardiac function propelled by increased activity in the sympathetic nervous system, primary retention of cations and water by kidney, other factors contributing to hypertension are genetic predisposition, low serum calcium level, high Sodium and low excretion, low Potassium and low calcium intake and increased excretion. Many recent studies have implicated serum calcium level in the role of development of hypertension and increasing evidence are emerging supporting this theory. This tendency has been observed even in pregnant women. Thus, in addition to the routine dietary restriction salt in hypertensive individuals, estimation of serum calcium level and calcium / phosphorus

ratio may be considered in patients with essential hypertension when planning for a treatment strategy and addition of oral calcium supplement in diet may offer a favourable outcome.

9. LIMITATIONS

- In this study, Although patients with associated comorbidities which are likely to affect serum albumin were excluded, however, serum total calcium level was not corrected for serum albumin.
- Small sample size of the study.

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MASTER CHART

CASES

SR. NO.	NAME	AGE	SEX	BMI	BP (mmHg)	SR. CALCIUM (mg/dl)	SR. PHOSPHORUS (mg/dl)	C : P RATIO	FAMILY HISTORY	SMOKER	ALCOHOLIC
1	PANCHAVARNAM	45	F	21.14	152 / 108	9.5	3	3.1	NO	NO	NO
2	KATHIRASEN	58	M	22.1	166 / 106	8.1	3.8	2.23	NO	YES	YES
3	RAMASMY	54	M	23.14	192 / 112	8.6	2.1	4.09	YES	YES	YES
4	MARRIAMAL	50	F	19.3	154 / 106	8.9	2.3	3.86	YES	NO	NO
5	CHELLAMAL	55	F	20.1	148 / 96	9.5	3.2	2.96	NO	NO	NO
6	RAKKAMAL	41	F	20.71	158 / 102	8.6	2.1	4.09	YES	NO	NO
7	VEERAMMAL	55	F	21	156 / 100	9.2	3.4	2.7	NO	NO	NO
8	ARUL	32	M	23.63	210 / 116	8.2	2.6	3.1	NO	YES	YES
9	NANDHINI	18	F	22.1	164 / 104	9.6	3.9	2.46	NO	NO	NO
10	MUTHU	40	M	21.1	166 / 110	8.1	2.2	3.68	YES	YES	NO
11	BASKARN	61	M	22.14	140 / 102	9	3	3	NO	NO	NO
12	NATARAJAN	78	M	20.8	210 / 120	8.3	2	4.15	NO	NO	NO
13	KRISHNA	62	M	22.12	142 / 98	8.2	2.4	3.41	YES	YES	YES
14	DURAI RAM	41	M	19.88	154 / 106	8.5	2	4.3	NO	YES	YES
15	ALEX	36	M	21.12	160 / 100	8.3	2.4	3.45	NO	YES	YES

16	PONNUTHAI	39	F	22.21	142 / 96	8	2.3	3.47	NO	NO	NO
17	KALIAMMAL	44	F	27.12	150 / 108	8.4	2.8	3	NO	NO	NO
18	POOCHANDU	31	F	26.13	162 / 112	8.5	2	4.25	NO	NO	NO
19	SURESH	40	M	20.1	144 / 100	10.1	3.8	2.65	NO	YES	YES
20	MD. YUSUF	34	M	29.12	166 / 104	9.1	2.7	3.37	NO	YES	NO
21	SARITHA	45	F	28.34	146 / 100	9.7	4	2.4	NO	NO	NO
22	ROSEMARY	25	F	19.23	152 / 100	8.4	2.6	3.23	NO	NO	NO
23	MUTHUSAMY	43	M	30 . 12	162 / 102	8.8	2.1	4.19	YES	YES	NO
24	SEETHALAKSHMI	38	F	22.12	150 / 110	9	2.7	3.33	NO	NO	NO
25	SHARADHA	66	F	31.43	168 / 116	8	1.9	4.21	NO	NO	NO
26	REVATHI	42	F	21.4	148 / 106	9.2	3.9	2.35	NO	NO	NO
27	PRABHU	44	M	18. 98	144 / 100	8.9	2.9	3.06	YES	NO	NO
28	GOVINDARAJ	50	M	28.34	180 / 114	8.6	2	4.3	NO	YES	YES
29	LAKSMI	33	F	21.1	162 / 108	8.9	3.1	2.87	YES	NO	NO
30	MOOVINDRAN	55	M	27.13	156 / 106	8.6	3.7	2.32	YES	YES	YES
31	KARUPASAMY	48	M	18.14	148 / 100	8.8	4	2.32	YES	NO	NO
32	KARTHIK	30	M	21.46	152 / 98	10.1	2.2	4.59	NO	YES	YES
33	BOSE	45	M	22	164 / 100	8.4	3.8	2.21	NO	NO	YES
34	LENIN	54	M	23. 64	170 / 110	8.5	2.4	3.5	NO	NO	NO
35	KARTHIKEYAN	44	M	22.4	162 / 104	8.4	2.1	4	NO	YES	YES
36	SUJATHA	48	F	31. 4	168 / 102	8.8	3.9	2.25	NO	NO	NO
37	NIVEDHA	61	F	29.63	158 / 108	8.9	3.4	2.61	NO	NO	NO
38	TAMILARASI	49	F	19.4	156 /98	8.3	2.7	3.07	NO	NO	NO
39	SUNANTHA	55	F	24.3	144 / 102	8.3	3.8	2.18	YES	NO	NO
40	INDUMATHI	43	F	21	150 / 104	8.7	3.2	2.71	NO	NO	NO

41	JYOTHI	38	F	22.42	154 / 108	9.3	3	3.1	YES	NO	NO
42	SUBRAMANIAM	54	M	28.4	176 / 124	8	2.1	3.8	NO	YES	YES
43	SUBBULAKSHMI	67	F	27.6	170 / 120	8.4	2	4.2	NO	NO	NO
44	JAGADISH	54	M	20.4	150 / 104	9	4.1	2.19	YES	YES	YES
45	ALAGAR	56	M	19.4	148 / 102	8.7	3.9	2.23	NO	YES	NO
46	PANDIYAN	49	M	22.46	156 / 110	8.6	2.4	3.58	NO	NO	NO
47	RAMU	50	M	21.3	150 / 110	8.3	3.5	2.37	NO	YES	NO
48	HASEENA BEGUM	54	F	31.4	164 / 112	8.3	2	4.15	NO	NO	NO
49	JEYA	42	F	20.83	160 / 110	8.1	2.8	2.89	YES	NO	NO
50	SHEIKH	56	M	21. 68	154 / 100	8.5	3.3	2.57	NO	NO	NO

CONTROLS

SR. NO.	NAME	AGE	SEX	BMI	BP (mmHg)	SR. CALCIUM (mg/dl)	SR. PHOSPHORUS (mg/dl)	C : P RATIO	FAMILY HISTORY	SMOKER	ALCOHOLIC
1	A	31	M	21.6	118 / 78	9.3	3.1	3	NO	NO	YES
2	AA	42	M	22.1	112 / 70	9.5	3.5	2.71	NO	NO	NO
3	B	33	F	19.64	110 / 70	9	3.5	2.57	NO	NO	NO
4	BB	40	M	20.44	106 / 72	8.9	3	2.96	NO	YES	NO
5	C	51	M	21.7	114 / 70	9.7	3.5	2.77	NO	YES	YES
6	CC	45	M	22 . 4	118 / 74	8.4	3.2	2.65	NO	YES	YES
7	D	54	M	19.8	110 / 70	8.3	3.1	2.67	NO	NO	NO
8	DD	57	M	22.6	116 / 74	9.1	3.9	2.33	NO	NO	NO
9	E	28	M	20.4	104 / 68	9.4	2.9	3.24	NO	NO	NO
10	EE	30	F	21.5	100 / 68	10.1	3.8	2.65	NO	NO	YES

11	F	43	F	22.8	110 / 70	10.3	4	2.57	NO	NO	NO
12	FF	41	F	20.6	116 / 76	8.2	3.6	2.27	YES	NO	NO
13	G	56	M	25.2	118 / 76	8.9	4.2	2.11	NO	NO	NO
14	GG	62	F	26.2	118 / 78	8.2	3.7	2.21	NO	NO	NO
15	H	34	F	23.4	110 / 70	8.6	3.6	2.38	NO	NO	NO
16	HH	43	F	20.6	102 / 74	9.3	2.9	3.2	NO	NO	NO
17	I	50	M	22.4	108 / 70	9.8	3	3.26	NO	YES	YES
18	II	23	M	21	100 / 64	9.1	3.7	2.45	NO	YES	NO
19	J	34	F	19.8	96 / 64	8.7	3.3	2.63	NO	NO	NO
20	JJ	36	M	20.8	102 / 68	9.6	3.8	2.52	YES	YES	YES
21	K	31	F	24.8	108 / 74	8.5	3.9	2.17	NO	YES	NO
22	KK	43	F	22	110 / 70	8.8	3.2	2.75	NO	NO	NO
23	L	27	F	19.8	96 / 70	9.3	3.5	2.65	NO	NO	NO
24	LL	29	M	21.8	100 / 72	9.6	3.1	3.09	YES	YES	NO
25	M	32	M	22.6	106 / 76	8.9	3.6	2.47	NO	YES	YES
26	MM	43	F	21.6	106 / 70	8.8	3.8	2.31	NO	NO	NO
27	N	33	F	20.8	100 / 70	9.4	4	2.35	NO	NO	NO
28	NN	36	F	21.4	92 / 66	9.2	3.7	2.48	NO	NO	NO
29	O	60	M	26.4	118 / 78	8.2	3.1	2.64	NO	YES	YES
30	OO	68	F	23.6	100 / 70	8.6	3.8	2.26	NO	NO	NO
31	P	45	M	21.4	104 / 74	9.1	3.8	2.39	NO	YES	YES
32	PP	24	F	19.2	98 / 70	9.4	3.5	2.68	NO	NO	NO
33	Q	36	F	25.6	100 / 72	8.2	3.5	2.34	NO	NO	NO
34	QQ	52	F	23.1	112 / 74	8.9	3.6	2.47	NO	NO	NO
35	R	44	M	22	108 / 70	9.2	2.8	3.28	NO	YES	YES
36	RR	34	M	23.4	110 / 78	9.3	3.5	2.65	NO	YES	NO
37	S	22	M	22.6	118 / 78	10.3	4.3	2.39	YES	NO	YES

38	SS	33	F	22.5	108 / 74	9.2	3.2	2.87	NO	NO	NO
39	T	55	M	21.8	110 / 78	8.2	2.5	3.28	NO	NO	NO
40	TT	54	F	20.4	106 / 72	8.8	3.1	2.83	NO	NO	NO
41	U	36	F	22.06	108 / 72	9	3.2	2.81	YES	NO	NO
42	UU	57	F	21	110 /76	8.1	2.9	2.79	NO	NO	NO
43	V	46	M	27.08	116 / 78	8.7	3.6	2.41	YES	YES	YES
44	VV	25	M	19.7	106 / 70	10.1	3.9	2.58	NO	YES	YES
45	W	59	F	23.7	116 / 76	8.9	3	2.96	NO	NO	NO
46	WW	34	M	21.09	104 / 76	9.4	3.7	2.54	NO	YES	YES
47	X	35	M	23.11	106 / 72	8.3	3.4	2.44	NO	NO	NO
48	XX	57	F	20.8	110 / 78	8.8	3.6	2.44	NO	NO	NO
49	Z	39	M	21.6	114 / 70	9.8	3.9	2.51	NO	NO	NO
50	ZZ	32	F	22.8	116 / 72	9.6	3.5	2.74	NO	NO	NO
						443.4					