PAPER NAME

Trinka Plagiarism Checker - Sample.docx

WORD COUNT

14199 Words

CHARACTER COUNT

77043 Characters

PAGE COUNT

59 Pages

FILE SIZE

346.8KB

SUBMISSION DATE

May 26, 2022 1:04 PM GMT+5:30

REPORT DATE

May 26, 2022 1:06 PM GMT+5:30

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1 INTRODUCTION

The Seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure defines blood pressure for adults aged 18 years. Hypertension is defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg. The classification of hypertension is based on the mean of two or more properly measured seated blood pressure readings. Normal blood pressure ranges below levels <120/80 mmHg. Systolic blood pressure of 120 – 139 mmHg or diastolic blood pressure 80 – 89 mmHg is classified as prehypertension. These patients are at increased risk of progression to hypertension (Gupta, 2003).

Hypertension can be classified into two stages:

- Stage 1 includes patients with systolic blood pressure 140 - 159mmHg or diastolic blood pressure 90 - 99 mmHg.
- Stage 2 includes patients with systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg.

Hypertension is a serious problem throughout the globe due to its high prevalence and its association with increased risk of chronic kidney diseases. High blood pressure may permanently damage the narrow blood vessels in the kidney which play a vital role in filtration of blood. Over time, this damage will keep the kidney from working properly.

Hypertension plays a vital role in accelerating the progression of experimental renal disease. Chronic kidney disease (CKD) is the most occurring form of secondary hypertension and it is also suggests that it is an independent risk factor for cardiovascular morbidity and mortality (Sinclair *et al.*, 2004). The relationship of "benign" (a misnomer) essential hypertension to renal failure is less clear. It was observed that essential hypertension tends to increase in afferent arteriolar resistance, with a lesser increase in efferent resistance, so renal blood flow (RBF) decreases, filtration fraction (FF) increases and glomerular filtration (GFR) tends to be preserved (Birkenhager *et al.*, 1976).

There is evidence both clinically and experimentally that "blood pressure goes with the kidney" (Kuster *et al.*, 1990; Adamczak *et al.*, 2002). The association of hypertension and renal disease was the first recognized by Richard Bright. He observed that the extent of heart damage was at pace with kidney damage (Bright *et al.*, 2009). Sodium retention and activation of the renin-angiotensin system have been considered the most important mechanisms involved in the elevation of blood pressure in subject with kidney disease (Guyton *et al.*, 1990). High blood pressure is almost always present during all stages of chronic kidney disease. A urinalysis may show protein or other change. These changes may appear 6 months to 10 or more years before symptoms appear. The kidney function tests analyse creatinine clearance and blood urea nitrogen levels.

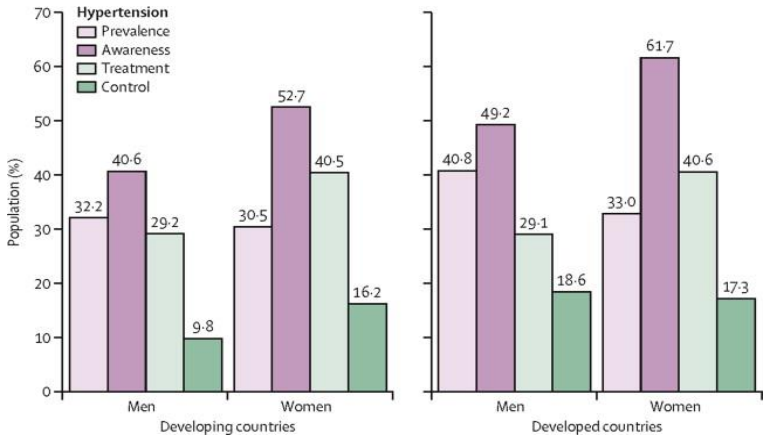

Fig 1 : Frequency of Hypertension among developed and developing countries in terms of its attributes (Ibrahim and Damasceno, 2012)

It is reported that hypertension is the seventh highest contributor to premature death in developing countries (Deepa *et al.*, 2003). The prevalence of hypertension in developed and developing countries is very high and is increasing at an alarming speed (Fig 1). Nearly 26 per cent of the adult population worldwide is affected by hypertension.

Chronic kidney disease affects more than 20 million US adults while more than 79,812 chronic dialysis patients die each year in the United States, with an annual unadjusted mortality rate of 20 to 25% (Coresh *et al.*, 2007). The prevalence of hypertension in patients with chronic kidney disease is estimated to be more than 60%, and more than 90% in patients with advanced renal failure (Stage IV and V) (Levey *et al* ., 2009; Campese *et al* ., 2006). Based on a national survey of representative

samples of non-institutionalized adults in the USA, it is estimated that hypertension occurs in 23.3% of individuals without CKD and 35.8% are in stage 1 CKD, 48.1% of stage 2, 59.9% of stage 3 and 84.1% of stage 4-5 patients (Kearney *et al.* 2005).

As with CKD, awareness of hypertension is low. Hence approaches to control hypertension will play a major role in modification or prevention of chronic kidney diseases. A combination of population-wide and individual health-care interventions is required to make control of the growing epidemiology of hypertension. With this background, present study has been undertaken to study the prevalence of hypertension, its associated factors as well as to increase the awareness on importance of life style modifications among rural dwellers of south India. Studies targeting low socio-economic groups would provide an estimate of the future magnitude of the problem and assist in developing strategies for control of hypertension and chronic kidney diseases (CKD).

1.1 Present Study

To estimate the prevalence of pre-hypertension and hypertension in a rural population from Kancheepuram district of Tamil Nadu, India & their association with risk factors of chronic kidney diseases (CKD)

1.2 Objectives

Until recently hypertension was considered to be one of the important public health problems in the developed and industrialized countries only. In the developing countries, its impact was not fully felt due to presence of rampant communicable diseases. However with control of communicable disease and increased life expectancy with life style changes, hypertension is becoming one of the emerging problems with its implications for concomitant increase in risk of cardiovascular and renal disease.

1.3 Specific Objectives

- To estimate the prevalence of pre-hypertension and hypertension in a rural population from Kancheepuram district of Tamil Nadu.
- To investigate the association of study variables such as age, gender, BMI, waist hip ratio, family history, intake of salts, smoking, alcohol intake, education, social and economic status with the development of pre-hypertension and hypertension in the study population.

- To determine the level of serum electrolytes as well as the status of serum urea, uric acid, creatinine, glucose, triglycerides, cholesterol, HDL and LDL in the study population of pre-hypertensive and hypertensive patients.
- To screen and identify polymorphic or mutational changes at the homocysteine gene loci and its association as a risk factor for chronic kidney diseases in the study population of pre-hypertensive and hypertensive patients by PCR-SSCP-RFLP
- To reduce the incidence of hypertensive diseases through appropriate health awareness and individual health-care interventions.

2 REVIEW OF LITERATURE

2.1 Definition and Current Classification

Arterial hypertension is a pathological condition marked by an increased vascular resistance, which may lead to the failure of the right ventricle. Studies estimate the contribution of hypertension to annual mortality rate to be about 15% (Stephen *et al.*, 2010). In terms of pulmonary arterial pressure, hypertension can be defined quantitatively as a pressure higher than 25mmHg at rest and 30mmHg during strenuous exercise (Aniket *et al.*, 2007).

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, classifies hypertension as:

- Normal: Systolic pressure < 120 mmHg and Diastolic pressure <80 mmHg.
- Prehypertension: Systolic pressure 120-139 mmHg and Diastolic pressure 80-89 mmHg.
- Stage 1 Hypertension: Systolic pressure 140-159 mmHg and Diastolic pressure 90-99 mmHg.
- Stage 2 Hypertension: Systolic pressure ≥160 mmHg and Diastolic pressure ≥100 mmHg.

Hypertension is also classified as Primary and Secondary, based on its underlying cause. Primary or Essential hypertension is often considered to be idiopathic. However, that is not completely valid and its validity is challenged by the dearth of information on its genetic and phenotypic aspects (Luft *et al.*, 1998). Primary or essential hypertension accounts to 95% cases of hypertension across the globe. The disorder is highly heterogeneous. Secondary hypertension develops due to cardiac, renal or other disorders (Luft *et al.*, 1998).

2.2 Epidemiology

Hypertension is gaining grounds at a pace faster than any other disease. This can be attributed to multiple factors, chief of which is altered lifestyle. Hypertension was initially the disease of the urban residents. But with increased migration to cities, and rampant industrialization of the rural side, the disease is claiming the rural population too. This stresses on the significance of various lifestyle associated factors in causing hypertension or maintain it. Therefore, it is imperative to carry

out rigorous demographic studies to understand its epidemiology, which would in turn aid in designing better treatment plans.

Recent studies suggest a tremendous increase in the proportions of populations afflicted with hypertension (Table 1). The World Health Organization's global health statistics reports that 23.10% men and 22.60% women over 25 years suffer from hypertension. Hypertension is the cause of 7.5% of all deaths attributable to cardiovascular diseases in the United States of America.

TABLE 1: Prevalence of Hypertension World-wide (Katharina *et al.*, 2003; Anastase *et al.*, 2012)

Country	Prevalence (%)		
	All	Men	Women
North America	27.6	30.4	24.8
Canada	27.4	31.0	23.8
Europe	44.2	49.7	38.6
Italy	37.7	44.8	30.6
Sweden	38.4	44.8	32.0
England	41.7	46.9	36.5
Spain	46.8	49.0	44.6
Finland	48.7	55.7	41.6
Germany	55.3	60.2	50.3
Sub Saharan Africa	52.4	50.1	44.6

In India, the prevalence of hypertension is reported as ranging from 10 to 30.9% (Padmavati, 2002). Studies conducted in urban localizations reported 25% prevalence and rural epidemiological studies reported 10% prevalence (Kapoor *et al*., 2010).

TABLE 2: Prevalence of Hypertension in India

Reference	Year	Age group	Place	Prevalence (%±S.D)
Urban Population				
Dotto BB	1949	18–50	Calcutta	1.24±0.2
Dubey VD	1954	18–60	Kanpur	4.24±0.4
Sathe RV	1959	20–80	Bombay	3.03±0.3
Mathur KS	1963	20–80	Agra	4.55±0.5
Malhotra SL	1971	20–58	Railways	9.24±0.4
Gupta SP	1978	20–69	Rohtak	6.43±0.5
Dalal PM	1980	20–80	Bombay	15.52±0.5
Sharma BK	1985	20–75	Ludhiana	14.08±1.1
Gupta R	1995	20–80	Jaipur	10.99±0.7
Chadha SL	1998	25–69	Delhi	11.59±1.0
Thakur K	1999	30–80	Chandigarh	13.11±1.0
Rural Population				
Shah VV	1959	30–60	Bombay	0.52±0.1
Padmavati S	1959	20–75	Delhi	1.99±0.4
Gupta SP	1977	20–69	Haryana	3.57±0.4
Wasir HS	1983	20–69	Delhi	5.41±0.8
Baldwa VS	1984	21–60	Rajasthan	5.59±0.8
Sharma BK	1985	20–75	Punjab	2.63±0.3
Kumar V	1991	21–70	Rajasthan	3.83±0.2
Joshi PP	1993	16–60	Maharashtra	4.02±0.9
Jajoo UN	1993	20–69	Maharashtra	3.41±0.3
Gupta R	1994	20–80	Rajasthan	7.08±0.5
Chadha SL	1998	25–69	Delhi	3.58±0.5

2.3 Hypertension Risk Factors

Hypertension can be the outcome of multiple interactions – genetic, environmental, metabolic, lifestyle, and dietary habits. However the studies indicate that these risk factors operate in different combinations in different parts of the world. The chief risk factors associated with hypertension, from which the current study drives its basis, are: age, gender, obesity, diet, alcohol and tobacco consumption, diabetes, renal disease and peripheral vascular disease (WHO/ISH, 2003).

2.4 Genetic Influences and Hypertension

The NHLBI twin study reports a greater incidence of hypertension in monozygotic twins (Feinleib *et al.,* 1977). Also, other demographic studies report a greater intra-familial similarity than between families (Longini *et al.,* 1984). Hypertension also exhibits Mendelian inheritance, reflecting the significance of single genes (Lifton *et al.,* 2001). Genetic mutations in about 10 genes have been associated with Mendelian hypertension (Lifton *et al.*, 2001; Wilson *et al*., 2001).

Newer techniques of genetic analyses have greatly aided in identifying genes responsible for the development of primary hypertension. Remarkably strong associations have been reported between development of hypertension and several chromosomal regions, especially the familial hyperlipidemia region (Hunt *et al.,* 2002). The candidate gene studies on different genotypes at candidate loci in pathways regulating blood pressure, mostly relate to genes of the RAA system; for instance, the M235T in angiotensinogen gene, which is known to cause an upsurge in circulating angiotensin levels and also hypertension, chiefly in men (O' Donnell *et al.*, 1998).

2.5 Hypertension and Chronic Kidney Diseases

Chronic kidney disease is a worldwide public health concern. The US National Kidney Foundation's Work group defines CKD as:

- Kidney damage for ≥3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifest either by:
  - Pathological abnormalities; or
  - Markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests.
- GFR <60 mL/min/1.73m^2 for ≥3 months, with or without kidney damage

Chronic kidney diseases (CKD) have been associated with hypertension and other cardiovascular diseases (CVD) (Fig 2). However, the mechanisms that link CKD and CVD are not well understood yet and further research would be required to see through the innuendo.

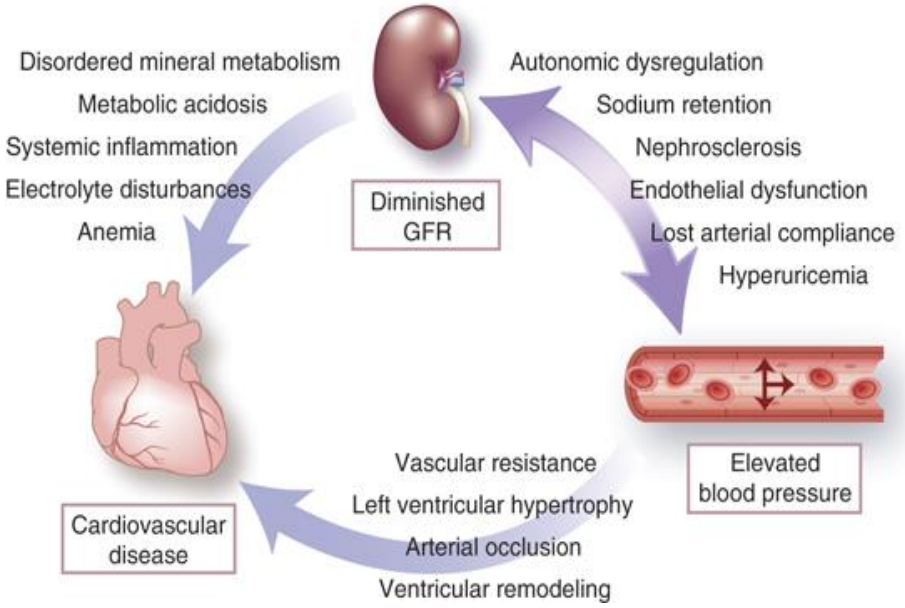

Fig 2 : Pathophysiological relationship between hypertension, CKD and CVD (John & Patrick, 2010).

2.6 Creatinine as an Indicator of Chronic Kidney Diseases

Normal blood creatinine levels [48] range from 0.6 to 1.2 mg/dL in adult males and from 0.5 to 1.1 mg/dL in adult females. Glomerular filtration and proximal tubular secretion in the kidneys filter out creatinine from blood. There is no significant reabsorption. Thus, serum creatinine clearance rate [28] can be used to estimate the glomerular filtration rate. The glomerular filtration rate in turn is an important parameter in analysis of renal function. Any increase in the blood creatinine level indicates an abnormal kidney function (Zhang *et al.*, 2012).

2.7 Homocysteine, Hypertension and Chronic kidney diseases

[13] Homocysteine is a non-protein forming, sulfhydryl containing amino acid and is an intermediate in the remethylation of the amino acid, Methionine. The [13] metabolism of homocysteine occur in two

ways; either using the transsulphuration pathway or the remethylation cycle. Homocysteine can be transsulphurated to cystathionine or remethylated to methionine, and these reactions are controlled by enzyme action. Methionine is regenerated by the remethylation pathway under the action of methionine synthase (MS). These reactions are dependent on the presence of methylenetetrahydrofolate (MTHF) which is controlled by the enzyme methylenetetrahydrofolate reductase (MTHFR) and the co-factors tetrahydrofolate (folate) and methylcobalamine (vitamin B) (Perry, 1999; Dwivedi *et al.*, 2011).

Hypertensive patients with mild renal dysfunction showed higher and pathological levels of plasma homocysteine. Hyperhomocysteinemia appears early in chronic renal failure and continues during the course of the disease. Total plasma homocysteine may also directly induce glomerular pathology that can lead to end stage renal disease (ESRD). Hence it has been suggested that treatment of high Hcy levels in ESRD with associating hypertension prevents deterioration of kidney function (Lim & Cassano, 2002).

Homocysteine may elevate blood pressure through multiple mechanisms, including its effect on vascular endothelial integrity. Homocysteine administration caused direct endothelial cell injury in vitro (Wall *et al.*, 1980) and in animals (Harker *et al.*, 1983). In cell culture studies, homocysteine induced oxidative stress to endothelium (Outinen *et al.*, 1998) and reduced available nitric oxide, a potent vasodilator (Upchurch *et al.*, 1997).

Methylenetetrahydrofolate reductase is an enzyme gene located on the short arm of chromosome 1 at 1p36.3. The cDNA sequence of this gene is 2.2kb long and is composed of 11 exons (103 to 432 bp). Genetic mutations in MTHFR are the most commonly known inherited risk factor for elevated homocysteine levels (Nevin *et al.*, 2007). Many case-control studies have addressed in particular the putative role of C to T transition at nucleotide 677 (C677T) and A to C transition at nucleotide 1298 (A1298C) in the methylenetetrahydrofolate reductase (MTHFR) gene for the elevated levels of homocysteine. About ten percent of the North American population are T-homozygous for this polymorphism. There is ethnic variability in the frequency of the T allele – frequency in Mediterranean/Hispanics is greater than the frequency in Caucasians which, in turn, is greater than in Africans/African-Americans.

2.8 Hyperuricemia and Hypertension

Hyperuricemia is frequently found associated with hypertension. Renal vascular constriction also causes hyperuricemia (Messerli *et al.,* 1980). However, the underlying mechanism of action is largely unknown (Rich, 2000). Excessive accumulation of uric acid may lead to progressive renal insufficiency (Tobian & Binion, 1952). Hyperuricemia is also associated with Diabetes mellitus, Hypertriglyceridemia and Obesity. Mechanistic studies in human subjects associate elevated levels of uric acid with development of hypertension.

Uric acid exerts its harmful effects with the aid of an active renin-angiotensin-aldosterone system (Saito *et al.,* 1978). Hyperuricemia arising out of diuretic therapy can also invoke cardiovascular diseases. The SHEP study reported that subjects, who developed hyperuricemia as a side effect of chlorthalidone administration, stood an equal risk of sustaining cardiovascular diseases as subjects on placebo (Franse *et al.,* 2000). The nephro-degenerative effects of uric acid and its contribution towards development of hypertension need rigorous research. Model studies in rats related hyperuricemia with increased systemic blood pressure, proteinuria, renal dysfunction, vascular disease, and progressive renal scarring. Recent studies point to the possible involvement of hyperuricemia in the activation of the renin-angiotensin and cyclooxygenase-2 (COX2) systems in progressive renal disease (Ross *et al.,* 1956).

Recent *in vitro* and *in vivo* studies speculate that hyperuricemia causes endothelial dysfunction by curbing the proliferation of the endotheliocytes and perturbing nitric acid synthesis. Exaggerated chemokine and cytokine activity, activation of the RAAS system and over expression of C-reactive protein are the deleterious outcomes of hyperuricemia, which in turn lead to hypertension, the chief risk factor of cardiovascular diseases and chronic kidney diseases (Ross *et al.,* 1956).

2.9 Hyperuremia and Hypertension

Urea is the major nitrogenous abundant catabolite of protein catabolism and makes up to 75% of total non-protein nitrogen content (Muhammad & Tabassum *et al.,* 2008). Plasma urea levels are measured for estimating renal function. Urea contributes up to 80% of urinary nitrogen and is inversely related to hypertension. However, there are no studies that explain the underlying mechanism (Holley *et al.,* 1951). There was significant increase in plasma urea with time

independent of gender or the type of hypertension. Studies in animal models report that hyperuremic animals showed high systolic pressures.

2.10 Serum Electrolyte levels as Indicators of Hypertension

Abnormal electrolyte balance is a hallmark of hypertension. Disturbed sodium-calcium ion exchange causes smooth muscle contraction, which leads to constriction of arterioles and hence a higher blood pressure. Studies suggest that Ca^{2+} ion metabolism has a critical role to play in the pathogenesis of hypertension (Cottier *et al.,* 1958). Almost all hypertensive subjects exhibit elevated levels of free intracellular Ca^{2+} · Recent studies highlight the role of parathyroid hypertensive factor in salt sensitive hypertension (Cottier *et al.,* 1958). Calcium is a key component of the secondary signal transduction pathway by which cells react to stimuli. This explains the calcium dependency of cardiac output, vascular resistance and the RAAS.(Albert *et al.,* 1958) first identified lowering of hypertension due to the oral supplementation of calcium. The involvement of calcium homeostasis regulatory mechanisms was elucidated in many studies. Parathyroid hormone, which regulates transmembrane transport of Na^{+}, Ca^{+}, H^{+} and K^{+}, was found to be in abnormal levels in the circulation of hypertensive patients (Weller *et al.,* 1959).

Studies also report increased intra-erythrocytic sodium and decreased potassium in hypertensive patients. This has been attributed to abnormal Na^{+}/K^{+} ATPase activity (Boushey *et al.,* 1995). Altered magnesium metabolism has been associated with the pathogenesis of hypertension (Winer, 1959). Low levels of magnesium and sodium cause hypocalcaemia, which in turn increases the blood pressure. Magnesium modulates the cardiovascular effect of sodium and potassium and it is the co-factor for the sodium potassium ATPase activity (Wall *et al.,* 1980).

Extracellular magnesium concentration can influence calcium metabolism of vasculocytes by altering the inbound membrane flux of Ca^{2+}. Also, decrease in extracellular magnesium content leads to a diminished adenosine triphosphatase and Ca^{2+} ATPase activity (Masser *et al.,* 1994).
Variations were observed between normotensive and hypertensive individuals in terms of salt intake, intracellular electrolytes (Harker *et al.,* 1983), total body electrolytes (Outinen *et al.,* 1998), serum electrolytes (Upchurch GR *et al.,* 1997) and the renal excretion of sodium and water (van der Put *et al.,* 1995). If changes in serum sodium concentration reflected intracellular or metabolic alterations

in electrolyte balance, an easily determined factor would be available for study of electrolyte abnormalities in relation to the development of hypertension. Three investigators have reported a slight increase in serum sodium concentration in patients with essential hypertension, whereas two other studies report no difference (Den Heijler *et al.*, 2005; Homocysteine Studies Collabortion, 2002).

2.11 Treatment of Hypertension

2.11.1 Medication

The overall goal of treating hypertension is to reduce hypertension-associated morbidity and mortality. The selection of specific drug therapy is based on evidence that demonstrates risk reduction. Non-pharmacologic therapy includes modifications in lifestyle, food habits and physical activity. These approaches are recommended by the JNC 7 but provide only minimal control over blood pressure. This mode of treatment chiefly restricts salt intake, alcohol consumptions and focuses on weight reduction in case of overweight and obese patients.

Pharmacologic therapy is based on the different classes of anti-hypertensive drugs (Muzaffar, 2011). The important ones are:

- Diuretics
- ACE inhibitors
- Angiotensin II receptor blockers
- Calcium channel blockers
- Alpha blockers
- Beta blockers
- Central alpha agonists
- Vasodilators

The JNC 7 recommends thiazide type diuretics as the first line of treatment. Thiazide diuretics control hypertension by inhibiting Na^+ and Cl^- absorption in the kidneys by blocking the sodium-chloride ion symporter. The ACE inhibitors work by inhibiting the conversion of angiotensin I to angiotensin II, thereby decreasing vascular resistance and increasing their capacity, as

vasoconstriction by angiotensin is curbed. Calcium channel blockers exert their effect by blocking the voltage gated calcium channels in cardiac muscles and blood vessels. This leads to a decrease in intracellular calcium levels. Various drug combinations can be prescribed by the physician based on the stage of hypertension and patient's response to drugs.

2.11.2 Complementary and Alternative therapy

There are many different types of complementary and alternative treatments believed to be effective against high blood pressure (George, 2001). Scientific evidence indicates that a diet that containing low saturated fat and salt and high levels of carbohydrates can be helpful in reducing blood pressure (Pauline & Bernard, 2010). It has been scientifically proved that men and women of all age groups who have a physically active lifestyle, have lower blood pressure or better control over the blood pressure levels (Pauline & Bernard, 2010). Ancient relaxation methods such as yoga, qigong and tai chi are effective in regulating blood pressure levels. Some herbs such as snakeroot, tetrandrine, ginseng and hawthorn have been used in controlling blood pressure. However, due to the potential health risks, their use has not been extensively studied (George, 2001).

3 MATERIALS & METHODS

3.1 Materials

3.1.1 Chemicals and Enzymes

All the chemicals used were of analytical grade purchased from any of the following vendors: Qualigens Fine Chemicals, India, Sisco Research Laboratory (SRL), India, Medox Agencies, Pvt Ltd, India and Synergy Scientific Services Pvt. Ltd, India.

3.1.2 Reagents and Buffers

Buffers for amplification and restriction enzymes supplied by the vendors were used. For the biochemical markers and serum electrolytes, ready to use kits were obtained from Biodiagnostics Pvt. Ltd, India.

3.1.2.1 Reagents for Estimation of Urea

a) Reagent 1 – Buffered enzyme powder
b) Reagent 2 – Hypochlorite
c) Reagent 3 - Standard (40 mg/100mL)

Preparation of working reagent: 25 mL of distilled water was added to reagent 1 and gently swirled to dissolve.

3.1.2.2 Reagents for Estimation of Uric Acid

a) Reagent 1 – Buffered TOOS (1mmol/l)
b) Reagent 2 – Buffered POD, Uricase (>1kU/l, >50U/l)
c) Reagent 3 – Standard (6mg/dl)

3.1.2.3 Reagents for Estimation of Creatinine

a) Reagent 1 – Picric acid (20mmol/l)
b) Reagent 2 – Sodium hydroxide (100mmol/l)
c) Reagent 3 – Standard (2mg/dl)

3.1.2.4 Reagents for Estimation of Glucose

a) Reagent 1 – GOD (>15000U/L), POD (>1000U/L)
b) Reagent 2 – Standard (100mg/dL)

3.1.2.5 Reagents for Estimation of Cholesterol

a) Reagent 1 – Cholesterol esterase (>500U/L), oxidase (>600/L) and and peroxidase (>6000U/L) & 4 Amino Antipyrine
b) Reagent 2 – Standard (200mg/dL)

3.1.2.6 Reagents for Estimation of Triglycerides

a) Reagent 1 – Lipase / GK/GPO Reagent
b) Reagent 2 – Triglycerides buffer
c) Reagent 3 – Standare (200mg/dL)

Preparation of working reagent: The triglycerides and chromogen mix powder (reagent 1) was carefully transferred into the bottle containing 5mL of triglyceride buffer (reagent 2).

3.1.2.7 Reagents for Estimation of High Density Lipoprotein

a) Reagent 1 – HDL-Cholesterol reagent
b) Reagent 2 – HDL Standard (50mg/dL)
c) Precipitating Reagent

3.1.2. 8 Reagents For Estimation of Calcium

a) Reagent 1 – Buffered arsenazo III reagent (≥0.05mmol/L)
b) Reagent 2 – Calcium standard (10mg/dL)

3.1.2.9 Reagents for Estimation of Serum Electrolytes

a) Alfa Wassermann Starlyte III Fluid Pack

3.1.2.10 Reagents for Human Genomic DNA Isolation

a) RBC lysis buffer : 0.155 M NH_4Cl and 0.17 M Tris
b) WBC lysis buffer : 1 M Tris Cl (pH 8.2), 1 M NaCl and

c) 10% SDS (w/v) : 0.5 M Na_2 EDTA (pH 8.2)

d) 6M NaCl

e) Tris – EDTA buffer : 10 mM Tris Cl (pH 8.2) and 1 mM EDTA (pH 8.2)

f) Absolute (99.5%) and 70% ethanol

3.1.2.11 Reagents for Agarose Gel Electrophoresis

a) 5X TBE buffer : 90mM Tris-Borate, 0.01 M EDTA (pH 8.3)

b) 1X TBE

c) 6X Gel loading buffer (Ready to use vials)

d) EtBr : 10mg/ml (w/v)

3.1.2.12 Reagents for Polymerase Chain Reaction (PCR)

a) Primers:

The primers sequences for the amplification of exon 4 of MTHFR gene was obtained from previously published report (Deeparani *et al.*, 2009). The primer details are given in (Table 3). Custom synthesized primers were obtained in lyophilized powder form. All the dilutions were made on ice. The primers were reconstituted using autoclaved double distilled water; working stocks were prepared to a final concentration of 250ng/µl and were stored at –20°C for further analysis.

b) Working dNTPs mix: Each dNTP i.e, dATP, dGTP, dCTP, dTTP was supplied in 10mM concentration. Equal volume of each dNTP was mixed to obtain a final concentration of 2.5 mM each of working dNTPs solution.

c) 10X buffer with 20mM $MgCl_2$: Tris-HCl (pH-8.0), 100mM KCl, 20mM $MgCl_2$; 0.5mM EDTA; 0.1mM DTT, 0.5% Tween 20, 0.05% Nonidet P-40 and 50% glycerol. This was stored at -20^{0}C and used at a working concentration of 1X. $MgCl_2$ was provided at a concentration of 20mM and a final concentration of 1.5mM was used for each reaction.

d) *Taq* Polymerase: *Taq* DNA Polymerase was supplied at a concentration of 5 U /µl and was stored at -20^{0}C. Final concentration used was 0.65 units (0.13µl) / 50µl reaction.

Table 3 : Primer details for the Amplification of Exon 4 of MTHFR gene

Gene / Exon	Primer	Primer Sequences	Amplification Product size (bp)
MTHFR 677 Exon 4	FP	5’ TGA AGG AGA AGG TGT CTG CGG GA3’	198
	RP	5’ AGG ACG GTG CGT GAG AGT G3’	

3.1.2.13 Reagents for Low Ionic Strength – Single Strand Conformation Polymorphsim (LIS – SSCP)

a) Agarose Solution : 0.1%

b) Acrylamide: Bis Acrylamide : 10% (39: 1)

c) 1X TBE buffer (90mM, pH-8.3)

d) 0.5 X TBE Running buffer (45mM, pH-8.3)

e) Ammonium Persulfate (APS) (w/v: 25%

f) SSCP Gel Loading Dye : 10% Sucrose, 0.01% Xylene cyanol, 0.01% Bromophenol blue

g) N, N, N', N' tetramethyl ethylenediamine (TEMED)

3.1.2.14 Reagents for Silver Staining

a) Fixative : 10 % acetic acid, 0.5 % Ethanol

b) Impregnation solution : 0.1 % Silver Nitrate

c) Developer : 1.5 % Sodium Hydroxide, 0.15 % Formaldehyde

d) Stop solution : 0.75 % Sodium Carbonate.

3.1.2.15 Reagents for Restriction Fragment Length Polymorphism (RFLP)

a) Restriction enzymes *Hinf* I

b) 10x Buffer

3.2 Methods

3.2.1 Place of Study

The study was designed to conduct in areas under the jurisdiction of Paiyanoor Village of Kancheepuram District, Tamilnadu. Paiyanoor is a village in the tehsil / mandal of Thiruporur in the Kancheepuram district of Tamil Nadu. Sub Villages in Paiyanoor are Pandithamedu, Ambekarnagar, Koothavakkam & Sambantham Nagar.

Total population (N) = 3686

Nature of land: urban or rural: Rural

Living conditions: BPL

Hospitals available within the study area:

1. Vinayaka Mission's University – Chennai Hospital

   Hospital capacity: 150 bed capacity

   Patient status:

   - Outpatient – 60
   - Inpatient – Daily observation of 5 patients (12 hrs)

3.2.2 Sample Collection

Adults of age ≥18 years residing in and around Paiyanoor, visiting Vinayaka Mission Chennai Hospital for a period of two months (Jan to Feb 2013) was invited to be enrolled for the study. The informed consent (Appendix I) was obtained prior to the enrollment of the participants. They were also given patient information sheet as per the ethical committee guidelines. Those adults who were non cooperative or refuse to provide the necessary information was excluded from the study. Those individuals who are also not in regular checkups were excluded from the study. Children, disabled, acutely ill subjects and pregnant women were also excluded from the study.

Studies have indicated an increasing prevalence of hypertension in rural populations. Based on these reports, we conducted a survey in Pandithamedu sub-village, Paiyanoor village, Thiruporur tahasil, Kancheepuram district of Tamil Nadu. The survey was organized in association with the NSS unit of

AVIT and Vinayaka Missions Chennai Hospitals. BP Values were collected from participants those who attended the camp and the prevalence of hypertension were estimated.

3.2.3 Questionnaire

A structured pretested and predesigned questionnaire was used to assess study subject's self-reported behavioral and lifestyle risk factors, hypertension and anthropometrical parameters (Appendix II). In the questionnaire, all the participants were inquired in detail about their smoking and drinking habits, physical activity, use of medications, and medical history. Alcohol consumption was calculated as grams of absolute alcohol per week and smoking as the number of cigarettes smoked per day.

3.2.4 Physical Examination

For physical examination, standardized calibrated mercury column type sphygmomanometer; stethoscope, common weighing machine and measuring tape were used. Hypertension was diagnosed as per US Seventh Joint National Committee on Detection, Evaluation and Treatment of Hypertension (JNC VII) criteria.

Optimal Blood Pressure	: <115/80
Normal Blood Pressure	: <120/80
Pre-Hypertension	: 120-139/80-89
Stage 1 Hypertension	: 140-159/90-99
Stage 2 Hypertension	: >160/100

Measurement of BP was performed after a 5 min period of rest using a mercury sphygmomanometer, and two BP readings taken from both arms at 30 s intervals. In case if the two readings differed by over 10 mm of Hg, a third reading was obtained, and the three measurements were averaged. The pressure at which sound appeared and disappeared was taken as systolic blood pressure (SBP) and diastolic blood pressure (DBP) respectively.

Anthropometric measurements viz. height, weight, waist circumference and hip circumference were recorded as per standard guidelines laid down by World Health Organization (WHO). Body weight was measured (to the nearest 0.5kg) with the subject standing motionless on the weighing scale, feet about 15cm apart and weight equally distributed on each leg. Subjects were instructed to wear

minimum outwear (as culturally appropriate) and no footwear while their weight was being measured. Height was measured (to the nearest 0.5cm) with the subject standing in an erect position against a vertical surface, and the head positioned so that the top of the external auditory meatus was level with the inferior margin of the bony orbit (Frankfurt's plain). Body Mass Index was calculated as weight in kilograms divided by height in meters squared. Based on their BMI, individuals were classified into four groups: thin (BMI <18.5), normal (BMI=18.5-24.9), overweight (BMI = 25.0-29.9) and obese (BMI > 30.0) as per WHO. Waist circumference was measured with a standard measuring tape, while subjects with lightly clothed, at a level midway between the lower margin of the last rib and iliac crest in centimeters (to the nearest 0.1cm). Waist circumference (WC) cut-offs was taken as 90 cms for males and 80 cms for females to define abdominal obesity using South Asia Pacific Guidelines. Hip circumference (HC) was measured at the maximum circumference over the buttocks in centimeters (to the nearest 0.1cm) with the subject in standing position. Waist hip ratio was calculated as waist circumference divided by hip circumference. The maximum circumference over the buttocks in centimeters (to the nearest 0.1cm) with the subject in standing position cut-off used for the waist-hip ratio (WHR) for males was 0.9 and for females 0.8 to define obesity. Physical activity of subjects was assessed by taking into consideration the occupational as well as non-occupational physical activity.

3.2.5 Biochemical Analysis

Laboratory assessment was performed for the subjects who gave written consent for the laboratory evaluation during interviews. Blood samples were drawn from these subjects for the estimation of serum electrolytes, glucose, serum triglycerides, LDL, HDL-cholesterol, CK total and CK-MB. The mentioned parameters were analyzed as per the kit protocols.

3.2.5.1 Estimation of Urea

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. 1000 µL of the working reagent was taken in a clean labeled test tube. 10 µL of the test sample was added to the test tube containing working reagent. The test tube was incubated at 37°C for 3 min. After incubation, 1000 µL of the reagent 2 (hypochlorite solution) was added to the test tube. The final reaction mixture was incubated at 37°C for 5 min. The reaction mixture was

automatically introduced into the analyzer and absorbance was taken at 578nm through a preset program. The readings were noted down for further analysis.

3.2.5.2 Estimation of Uric Acid

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. 800µL of phosphate buffered TOOS (reagent 1) was allowed to react with 20µL of the sample in a clean labeled test tube. The test tube was incubated at 37°C for 5 minutes. 200 µL of phosphate buffered peroxidase-uricase solution (reagent 2) was added to the test tube. The final reaction mixture was incubated at 37°C for 5 minutes. The reaction mixture was automatically introduced into the analyzer and absorbance was taken at 550nm through a preset programme. The readings were noted down for further analysis.

3.2.5.3 Estimation of creatinine

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. 1mL of picric acid reagent and sodium hydroxide reagent each, provided in the diagnostic kit, were allowed to react with 100µL of sample in a clean labeled test tube. The mode of estimation was kinetic. The reaction mixture was hence immediately fed to the analyzer. The reaction mixture was automatically introduced into the analyzer and absorbance was taken at 510nm through a preset program. The readings were noted down for further analysis.

3.2.5.4 Estimation of Glucose

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. The estimation of glucose (FBS & PPBS) was done by GOD/POD method. 1mL of reagent I (glucose oxidase) was allowed to react with 10µL of the serum sample in a clean labeled test tube. The reaction mixture was automatically introduced into the Reckon – Biochemical Analyzer and absorbance was taken at 505nm through a preset program. The readings were noted down for further analysis.

3.2.5.5 Estimation of Cholesterol

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. For estimation of cholesterol, CE/CO method was followed. 1mL of the reagent I (buffered cholesterol oxidase/esterase mix) was allowed to react with 10µL of the sample in a clean labeled test tube. The reaction mixture was incubated at 37°C for 10 minutes. The reaction mixture was automatically introduced into the Reckon – Biochemical Analyzer and absorbance was taken at 505nm through a preset program. The readings were noted down for further analysis.

3.2.5.6 Estimation of Triglycerides

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. The estimation of triglycerides was carried out by GPO method. 1mL of the working reagent was allowed to react with 20µL of the sample in a clean labeled test tube. The reaction mixture was incubated at 37°C for 5 minutes. The reaction mixture was automatically introduced into the Reckon – Biochemical Analyzer and absorbance was taken at 520nm through a preset program. The readings were noted down for further analysis.

3.2.5.7 Estimation of High Density Lipoproteins

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. For estimation of high density lipoproteins, PTA method was followed. 100µL of the precipitating reagent provided was mixed with an equal volume of the sample taken in a clean labeled test tube. The mix was centrifuged at 3000rpm for 10 minutes. 50µL of the obtained supernatant was mixed with 1mL of HDL-Cholesterol reagent in another clean labeled test tube. The final reaction mixture was incubated at 37°C for 10 minutes. The reaction mixture was automatically introduced into the Reckon – Biochemical Analyzer and absorbance was taken at 505nm through a preset program. The readings were noted down for further analysis.

3.2.5.8 Estimation of Very Low Density Lipoproteins

Very low density lipoprotein levels were calculated from the estimated triglyceride concentration, using the formula:

$$VLDL = \frac{TGL\ Concentration}{5}$$

3.2.5.9 Estimation of Low Density Lipoproteins

Low density lipoprotein levels were calculated from estimated total cholesterol and HDL cholesterol and calculated VLDL levels, using the formula:

LDL= Total cholesterol – HDL cholesterol – VLDL

3.2.5.10 Estimation of Calcium

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. The estimation of calcium was done by Arsenazo method. The kit included single use vials containing buffered arsenazo III reagent. 10µL of the sample was added to the contents in the vial. The reaction mixture was incubated at room temperature for 5 minutes. The reaction mixture was automatically introduced into the Reckon – Biochemical Analyzer and absorbance was taken at 630nm through a preset program. The readings were noted down for further analysis.

3.2.5.11 Estimation of Serum Electrolytes – Sodium, Potassium and Chloride

2mL of peripheral venous blood was drawn using a BDII disposable syringe. The blood sample was transferred to a serum clot activator vacuette and centrifuged at 37°C for 10 minutes to separate the serum. The sampling probe was submersed into the serum in vacuette. The readings obtained were noted for further analysis.

3.3 Molecular Analysis

3.3.1 Isolation of DNA

Miller's method (1988) was followed for human genomic DNA extraction from blood lymphocytes. 5ml of peripheral blood was collected in a sterile tube containing Na_2EDTA and centrifuged for 30 minutes at 3000 rpm. The buffy coat was transferred into a sterile tube. Twice the volume of RBC lysis buffer was added and incubated at 37°C for 10 minutes. This was centrifuged at 3000 rpm for 20 minutes and the supernatant discarded. The steps were repeated till a clear white pellet was obtained. To the pellet, 3ml of WBC lysis buffer was added and aspirated well. 0.2 ml of 10% SDS was added, mixed well and incubated at 37°C overnight. After overnight incubation, 1ml of 6M NaCl was added and vigorously vortexed for 15 seconds prior to centrifugation at 3000rpm for 20 minutes. The supernatant with DNA was transferred to another sterile tube to which twice the volume of cold absolute ethanol was added. DNA was precipitated by gently swirling the tube. It was then centrifuged at 3000 rpm at 4°C. The supernatant was removed and DNA is washed in 70% ethanol, pelleted down, dried and dissolved in TE buffer for further use. The samples were stored at -20^{o}C.

3.3.2 UV Spectrophotometric Analysis of DNA Sample (Sambrook and Russel, 2001)

The concentration and purity of the DNA samples was determined using UV spectrophotometer (Schimadzu UV-vis 300). To 980 µl of sterile distilled water, 20 µl of genomic DNA solution was added and mixed uniformly. Using autoclaved TE buffer as reference, the DNA was scanned in 1 cm path length quartz cuvettes at 260 nm and 280 nm. The purity of the DNA sample was checked by OD_{260}/OD_{280} ratio. A ratio of 1.8 – 2 indicates absence of protein contamination.

The concentration of the DNA was quantitated using the formula.

At OD_{260} = 1, concentration = 50 µg/ml, the double stranded DNA concentration is

$$= \frac{\text{Measured } OD_{260} \times 50 \times \text{Dilution factor}}{1(OD_{260})} \ (\mu g/ml)$$

3.3.3 Agarose Gel Electrophoresis of DNA Samples (Sambrook and Russel, 2001)

5µl of the DNA samples along with 2µl of loading dye were electrophoresed on agarose gel with 1X TBE buffer. Electrophoresis was performed in a horizontal slab gel (10 x 6cm or 20 x 15cm) apparatus with a constant voltage of 5 v/cm at RT. EtBr (0.5µg/ml) was added to gel solution prior to casting. After run, the gel was viewed under Gel documentation (Biotech R&D Laboratories) and documented using Nikon 4500 Cool Piz.

3.3.4 Polymerase Chain Reaction (PCR)

The exon 4 of MTHFR gene was amplified by modifying the existing protocols (Deeparani *et al.*, 2009). All the reactions were carried out in sterile conditions. Total reaction volume was 50µl. The reaction mixture is given in (Table 4). The amplification was performed using Eppendorf Mastercycler gradient (Germany). The reaction conditions for MTHFR gene amplification is given in the (Table 5)**.**

Table 4: PCR Reaction Mixture

S. No.	PCR Kit Components added	Volume added (µL)
1.	Double Distilled Water	38.0
2.	10 x *Taq* Buffer	5.0
3.	dNTP mix	3.0
4.	Forward Primer	1.0
5.	Reverse Primer	1.0
6.	Template DNA	1.0
7.	*Taq* DNA Polymerase	1.0
Total Reaction volume		50.0

Table 5: PCR Reaction Condition for MTHFR Exon 4

S. No.	Steps	Temperature	Reaction Time	No of cycles
1.	Initial Denaturation	94°C	5 minutes	N = 1
2.	Denaturation	94 °C	1 minute	N = 40
3.	Annealing	60 °C	1 minute	
4.	Extension	72 °C	2 minute	
5.	Final Extension	72 °C	7 minutes	N = 1
	Hold @ 4°C until use			

After a brief spin the PCR tube was kept in the thermocycler and the reaction started. After completion of the reaction, the MTHFR PCR products (198bp) were checked on a 3% agarose gel. Electrophoresis was carried out in 1X TBE buffer at 25°C for 2 ½ hrs at 50v/ 40mA. 100bp DNA ladder was electrophoresed along with the PCR products to identify the product size. The bands were visualized under UV Transilluminator and documented using gel documentation system. The amplified products were stored at –20^{0}C for further analysis.

3.3.5 Low Ionic Strength- Single Stranded Conformation Polymorphism (LIS-SSCP)

15mg of agarose (0.1%) was melted in 1x TBE. On cooling to room temperature, it was added to acrylamide - bis acrylamide solution and mixed well. The standardized acrylamide - bis acrylamide gel solution was prepared accordingly. To this 25% APS and TEMED were added. The solution was mixed well and at once poured vertically between 2 glass plates separated by 0.1cm spacer. The gel was allowed to set.

4µl PCR amplicon and 10µl of LIS-SSCP loading dye were mixed and heat denatured for 3 minutes. The denatured samples were immediately plunged into ice, spun and electrophoresed on composite gel with 1.0X TBE as running buffer. 1µl of double stranded DNA was treated in the same way and loaded as a control. Electrophoresis was performed in a vertical slab gel (10 x 6cm or 20 x 15cm) apparatus. Running voltages of 50 to 150V was examined. The gel was pre run for about 15 minutes

at a constant voltage of 150V. The gel was initially run at 150V for 15 minutes, followed by 100V till the xylene cyanol dye migrated to the bottom of the gel. After the run, gel plates were removed from the unit and the gel was transferred to the tray for silver staining.

3.3.6 Silver Staining (modified from Bassam *et al.*, 1991; Peng *et al.*, 1995; Wallace, 1997)

After electrophoresis the DNA bands on the gel was visualized by silver staining. All solutions were prepared in double distilled water. On completion of electrophoresis, the glass plates were removed and the gel was placed in the tray containing the fixative. Staining was carried out and consisted of the following steps: fixation, developing and photography. The gel was fixed with 10% acetic acid and given 2 changes, 2 minutes each. The fixative was discarded and the gel was impregnated with 0.1% silver nitrate solution. After impregnation, the gel was washed with double distilled water for 2 times, for a time interval of 20 seconds each. The developer was added and gently shaken until the bands were visible. Once the bands were developed, the reaction was arrested by adding stop solution and the gel was documented.

3.3.7 Restriction Fragement Length Polymorphism (RFLP)

The amplified MTHFR PCR products (198bp) were subjected to *Hinf* I restriction enzyme digestion. The restriction conditions for MTHFR PCR products is given in Table 6.

Table 6: Restriction Digestion Reaction Mixture for MTHFR PCR product

S. No.	Components Added	Volume Added (µL)
1.	Double Distilled Water	38.0
2.	2X Assay Buffer	25.0
3.	MTHFR PCR product	20.0
4.	*Hinf* I	3.0
Total Reaction Volume		50.0

The restricted products were visualized on 3% agarose gel stained with ethidium bromide and documented using gel documentation system. For MTHFR 677, the PCR will yield a 198 bp product, which on digestion with *Hinf* I produces a 175 and 23 bp fragments for TT condition (homozygous

polymorphic) and a 198,175 and 23 bp fragments for CT condition (heterozygous polymorphic). An undigested product length of 198 bp was retained by the wild types (CC homozygous).

3.4 Statistical Analysis

Data entry and statistical analysis were performed using the Microsoft Excel and SPSS windows version 15.0 software. Statistical differences between groups were performed by one-way analysis of variance (ANOVA) for continuous variables and the chi-square test for categorical variables. The baseline characteristics of subjects were expressed as means ± standard deviations for continuous variables. Independent variables tested were age, gender, smoking, family history of hypertension, physical activity, BMI, WHR, variations in serum markers and genetic markers. Variables were age and sex adjusted while performing the analysis. Values of $P < 0.05$ was considered to be statistically significant.

4 RESULTS

4.1 Study Sample

The study was conducted at Vinayaka Missions Chennai Hospital for a period of 2 months (January to February 2013). A total of 68 subjects were recruited for the study. The subjects were divided into three groups: Normotensive, Pre-hypertensive & Hypertensive. The prevalence & risk of pre-hypertension and hypertension for the development of cardiovascular diseases and chronic kidney diseases was analyzed among the three groups through biochemical, anthropometric, socio-economic & genetic markers.

4.2 Gender & Age

Out of 68 registered, 36 (52.94%) are females and 32 (47.05%) are males (p = 0.628). The mean age ± S.D for males is 51.43 ± 18.10 yrs and for females 53.19 ± 13.73yrs (p= 0.651). Out of 68 registered, 10 (14.70%) are pre-hypertensive and 46 (67.64%) are hypertensive (p< 0.01). The prevalence of pre-hypertension is higher in females (60%) than males (40%). The prevalence of hypertension is also higher in females (52.17%) than males (47.82%) (p = 0.881).

Table 7 : Groups with respect to Age in Years

Group	Size (N)	Mean ± SD	F value	P value
Normotensive	12	39.00[a] ± 17.97	5.978	0. 004**
Pre-hypertensive	10	56.00[b] ± 14.97		
Hypertensive	46	55.07[b] ± 13.84		

Note: ** denotes significance @ 1%. Different alphabets between group denotes significance at 5% level using Duncan Multiple Range Test (DMRT)

The mean age ± S.D for normotensive is 39.00 ± 17.97yrs, for pre-hypertensive, 56.00 ± 14.97yrs and hypertensive 55.07± 13.84 yrs (P=<0.004**), there is significant difference between the three groups with respect to age. Based on DMRT, the normality calculated is significantly different with respect to pre-hypertensive and hypertensive groups at 5% but not significant between pre-hypertensive and hypertensive groups.

Table 8 : Sample Size under different Age Groups

Age Group	Frequency (N)	Chi-square value	P value
20 -35	10 (14.7)	19.647	**<0.001****
36-50	21 (30.88)		
51-75	30 (44.11)		
≥ 75	7 (10.29)		

Note: ** denotes significance @ 1%. The value within bracket refers to row percentage.

The study sample was categorized into different groups according to their age. There are 4 groups: 20 – 35yrs, 36 – 50 yrs, 51 – 75yrs and above 75yrs. The maximum number of participants is in the age group of 51 – 75 yrs (30) followed by the age group of 36 – 50 yrs (21). The frequency among the different age groups is found to show a P value less than 0.01. It indicates a significant association between sample size and different age groups (p=<0.001**). The proportion of gender among different age group was calculated. The p value is greater than 0.05 and indicates that the proportion of the two genders within different age groups is not significant (p = 0.668).

Table 9 : Prevalence of Pre-Hypertension and Hypertension among different Age Groups

Age Group in years	Group			Total	Chi-square value	P value
	Normotensive	Pre-Hypertensive	Hypertensive			
20-35	7 (70.0%)	1 (10.0%)	2 (20.0%)	10 (100.0%)	24.137	<0.001**
36-50	2 (9.5%)	2 (9.5%)	17 (81.0%)	21 (100.0%)		
51-75	3 (10.0%)	5 (16.7%)	22 (73.3%)	30 (100.0%)		
Above 75	0 (.0%)	2 (28.6%)	5 (71.4%)	7 (100.0%)		
Total	12 (17.6%)	10 (14.7%)	46 (67.6%)	68 (100.0%)		

Note: ** denotes significance @ 1%. The value within bracket refers to row percentage.

Pre-hypertension is highest in the age group of ≥ 75. Hypertension is highest in the age group of 36 – 50 yrs with a similar percentage in the age group of 51 – 75 yrs. It can be seen from Table 3 that the prevalence rate of pre-hypertension and hypertension shows an upward trend as age advances in the study population. The prevalence of hypertension increases with age till the age group of 51 – 75 years and then dipped at the age group of above 75yrs. All the differences are statistically significant ($p<0.01$), which indicates that in the present study, age has a greater association with hypertension.

4.3 Systolic (SBP) and Diastolic Blood (DBP) Pressure

Table 10 : Systolic (SBP) and Diastolic blood (DBP) pressure among the Groups

Groups	Systolic Blood Pressure ± SD	Diastolic Blood Pressure± SD
Normotensive	114.75[a] ± 6.57	74.92[a] ± 6.68
Pre-Hypertensive	129.50[b] ± 1.08	77.30[b] ± 5.12
Hypertensive	145.13[c] ± 12.52	93.61[b] ± 9.68
F value	41.062	30.526
P value	<0.001**	<0.001**

Note: ** denotes significance @ 1%

Overall, the mean systolic and diastolic blood pressure of normotensive group is 114.75 ± 6.57 / 74.92 ± 6.68mmHg respectively. The mean systolic and diastolic blood pressure for pre-hypertensive is 129.50 ± 1.08 / 77.30 ± 5.12mmHg respectively. Hypertensive group has a mean blood pressure of 145.13 ± 12.52 / 93.61 ± 9.68mmHg respectively. Since P value is less than 0.01, there is significant difference between the groups with respect to SBP and DBP (p=<0.001**). This indicates that SBP and DBP are closely associated with pre-hypertension and hypertension.

Based on DMRT for the variable systolic blood pressure, the normality is significantly different with respect to pre-hypertensive and hypertensive groups at 5%. The difference is also significant between pre-hypertensive and hypertensive groups. However, DMRT for diastolic blood pressure showed a significant difference between normotensive and hypertensive but not between pre-hypertensive and hypertensive.

4.4 Anthropometric Characteristics (BMI, WC, HC and WHR)

Table 11 : WC, HC and WHR among the Groups

Groups	BMI	WC	HC	WHR
Normotensive	26.81 ± 6.21	88.69 ± 9.88	94.74 ± 11.03	0.94 ± .08
Pre-Hypertensive	25.10 ± 3.56	91.44 ± 9.73	98.42 ± 8.40	0.93 ± .11
Hypertensive	26.34 ± 4.11	86.67 ± 12.46	90.75 ± 15.11	0.96 ± .14
F Value	0.435	0.732	1.459	0.329
P Value	0.649	0.485	0.240	0.721

4 There is no significant difference between normotensive, pre-hypertensive and hypertensive groups with respect to mean BMI (p= 0.649). Subjects with hypertension have a comparably high waist-hip ratio (.96 ± .14) than the other two groups; normotensive (.94 ± .08) and pre-hypertensive (.93 ± .11). Since P value is more than 0.05% in all the three variables, there is no difference among the groups with respect to WC, HC and WH.

4.5 Hypertension and Risk Factors for Chronic Kidney Diseases

Table 12 : Distribution of Chronic Kidney Disease markers among the Groups

Variables	Group			F Value	P Value
	Normotensive	Pre Hypertensive	Hypertensive		
Glucose FBS	93.08 ± 17.02	94.60 ± 17.17	104.09 ± 27.65	1.289	0.283
Glucose PPBS	119.17 ± 36.94	129.90 ± 18.65	151.24 ± 63.72	1.882	0.160
Urea	25.33 ± 6.60	32.70 ± 10.78	30.65 ± 9.66	2.008	0.142
Creatinine	1.02 ± .34	1.02 ± .29	1.02 ± .29	.000	1.000
Uric Acid	3.99 ± .94	5.04 ± 1.45	4.14 ± 1.52	1.869	0.163
Cholesterol	129.25^{a} ± 35.84	153.00^{b} ± 27.46	156.89^{b} ± 20.40	5.987	.004**
TGL	123.83 ± 43.91	163.60 ± 66.31	142.70 ± 65.09	1.116	0.334
HDL	37.92 ± 6.47	38.30 ± 2.98	38.07 ± 3.71	0.023	0.978
LDL	66.58^{a} ± 33.47	81.90^{ab} ± 25.29	90.10^{b} ± 23.22	4.110	.021*
VLDL	24.75 ± 8.78	32.80 ± 13.35	28.71 ± 13.11	1.132	0.329
Sodium	136.83 ± 1.34	137.10 ± 1.73	136.76 ± 2.40	0.101	0.904
Potassium	4.16 ± .43	3.93 ± .37	4.09 ± .48	0.719	0.491
Chloride	107.83 ± 2.33	108.90 ± 2.73	107.15 ± 2.64	1.951	0.150
Calcium	8.96 ± .65	9.19 ± .33	9.27 ± .47	1.867	0.163

Note: ** denotes significance @ 1%, * denotes significance @ 5%. Different alphabets between group denotes Significance at 5% level using Duncan Multiple Range Test (DMRT)

There is a high prevalence of cardiovascular risk factors in the study population. Significant differences of LDL and cholesterol levels are noted among the pre-hypertensive and hypertensive groups when compared with the normotensive groups. These differences are highly significant for cholesterol at 1% and for LDL at 5%. There are differences in TGL, FBS and PPBS among the groups. However it is not significant. Based on DMRT for the variable cholesterol, the normality is significantly different with respect to pre-hypertensive and hypertensive groups at 5% but not significant between pre-hypertensive and hypertensive groups with respect to cholesterol levels. However, DMRT for LDL among the three groups showed that there is a significant difference at 5% between normotensive and hypertensive but not between normotensive and pre-hypertensive.

Table 13 : Distribution of Chronic Kidney Disease markers among different Age Groups

Variable	Age Group in years								F Value	P Value
	20-35		36-50		51-75		Above 75			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
BMI	26.49	6.73	26.97	3.66	25.26	3.79	27.92	5.24	1.026	0.387
WC	88.44	10.70	88.94	11.53	86.74	12.40	87.27	12.20	0.157	0.925
HC	94.64	8.23	92.27	14.94	90.97	13.51	97.49	18.89	0.495	0.687
WHR	0.93	0.08	0.97	0.09	0.96	0.16	0.91	0.13	0.482	0.696
SBP	120.40 [a]	14.24	137.90[b]	11.48	141.70[b]	16.48	142.43[b]	13.59	5.755	0.002**
DBP	77.70[a]	11.04	90.67[b]	10.68	89.90[b]	11.58	85.71 [a,b]	12.72	3.508	0.020*
Glucose FBS	91.80	17.99	95.57	14.60	105.33	32.24	109.43	18.24	1.368	0.260
Glucose PPBS	123.70	53.66	124.43	36.28	155.47	67.44	167.43	37.79	2.190	0.098
Urea	28.00	8.03	27.19	6.31	33.03	11.95	28.43	3.21	1.908	0.137
Creatinine	1.04	0.31	0.98	0.22	1.07	0.35	0.89	0.07	0.963	0.416
Uric Acid	4.13	1.11	4.11	1.59	4.65	1.38	3.09	1.12	2.556	0.063
Cholesterol	136.80	37.31	156.10	27.17	154.60	22.28	144.86	18.45	1.562	0.207
TGL	133.60	52.33	138.76	57.93	151.80	73.92	126.00	26.63	0.468	0.705
HDL	37.80	2.30	38.24	4.75	38.20	4.49	37.43	3.46	0.087	0.967
LDL	72.30	33.65	90.14	29.13	85.81	23.43	81.71	19.87	1.061	0.372
VLDL	26.70	10.37	27.71	11.64	30.59	14.91	25.57	5.53	0.487	0.693
Sodium	136.60	1.51	137.24	1.67	137.03	2.22	135.00	3.11	2.209	0.096
Potassium	4.16	0.37	4.13	0.42	4.01	0.54	4.10	0.32	0.410	0.746
Chloride	108.00	1.83	107.48	2.68	107.73	2.64	106.14	3.53	0.805	0.496
Calcium	9.00	0.68	9.26	0.47	9.19	0.45	9.34	0.50	0.841	0.477

The mean systolic as well as diastolic blood pressures are found to steadily increase with age, lowest being in age group 20 – 35yrs (120.40 ± 14.24 / 77.70 ± 11.04) and highest in age group of above 75yrs (142.43 ± 13.59 / 85.71 ± 12.72). Since P value for the distribution of mean systolic as well as

diastolic blood pressures among different age groups is less than 0.01, increasing age is significantly associated with difference in the SBP and DBP among the study population.

Based on DMRT for the variable mean systolic blood pressure, the normality is significantly different at 5% with respect to the age group of 20 – 35 yrs but not significant between 36 – 50yrs, 51 – 75 yrs and above 75 yrs. However, DMRT for mean diastolic blood pressure showed that there is a significant difference at 5% between 20 – 35 yrs, 36 – 50yrs and 51 – 75 yrs hypertensive but not between 20 – 35 yrs and above 75 yrs.

Discriminant Test (Tests of Equality of Group Means)

Discriminant analysis is used to distinguish between normotensive, pre-hypertensive and hypertensive groups based on the predicted risk factors. The association among the groups is measured using 20 variables. The tests of equality of group means measure each independent variable's potential before the model is created. Wilks' lambda, the F statistic and its significance level are presented in the following table.

Table 14 : Discriminant Test for the Study Sample

Variables	Wilks' Lambda	F value	P value
Gender	0.996	0.121	0.886
Age in years	0.845	5.978	< 0.001**
BMI	0.987	0.435	0.649
Waist/HIP Ratio	0.990	0.329	0.721
Systolic Pressure	0.442	41.062	< 0.001**
Diastolic Pressure	0.516	30.526	< 0.001**
Glucose FBS	0.962	1.289	0.283
Glucose PPBS	0.945	1.882	0.160
Urea	0.942	2.008	0.142
Creatinine	1.000	0.000	1.000
Uric Acid	0.946	1.869	0.163
Cholesterol	0.844	5.987	< 0.001**
TGL	0.967	1.116	0.334
HDL	0.999	0.023	0.978
LDL	0.888	4.110	0.021*
VLDL	0.966	1.132	0.329
Sodium	0.997	0.101	0.904
Potassium	0.978	0.719	0.491
Chloride	0.943	1.951	0.150
Calcium	0.946	1.867	0.163

The above test displays the results of ANOVA for the independent variables using the grouping variables as the factor. According to the results in the table, out of 20 variables, only 4 variables in discriminant model are significant (p = <0.05).

Table 15 : Standardized Canonical Discriminant Function Coefficients

Variables	Function	
	1	2
Gender	.237	-.028
Age in years	.027	.269
BMI	-.045	-.149
Waist/HIP Ratio	.246	-.298
Systolic Pressure	-.766	.339
Diastolic Pressure	-.659	-.674
Glucose FBS	.544	.561
Glucose PPBS	-.253	-.172
Urea	-.653	.736
Creatinine	.500	-.911
Uric Acid	.785	.653
Cholesterol	-4.672	-3.175
TGL	1.838	1.984
HDL	.487	.696
LDL	4.357	3.574
Sodium	.178	.061
Potassium	-.240	-.018
Chloride	.116	.353
Calcium	.329	.036

Standardized Canonical Discriminant Function Coefficients indicate a high value for Cholesterol (-4.672), followed by LDL (4.357) and TGL (1.838) for function 1. The values were higher for LDL (3.574) in function 2, followed by cholesterol (-3.175) and TGL (1.984). So classification based on these independent variables is justified.

Table 16 : Classification Results(a)

Original Membership	Predicted Group Membership			Total
	Normotensive	Pre Hypertensive	Hypertensive	
Normotensive	12 (100%)	0 (0.0%)	0 (0.0%)	12
Pre Hypertensive	0 (0.0%)	10 (100%)	0 (0.0%)	10
Hypertensive	0 (0.0%)	3 (6.5%)	43 (93.5%)	46

Note: a: 95.6% of original grouped cases correctly classified. The value within bracket refers to row percentage.

The classification table shows the practical results of using the discriminant model. Out of 12 samples in normotensive, all the 12 are correctly classified. For pre-hypertensive, 100% correct classification is obtained. However, in hypertensive, out of 46 samples, only 3 (6.5%) are misclassified. The overall correct classification of the model is 95.6% (i.e. out of 68 samples, 65 samples were correctly classified. So the group classification is justified by the Standardized Canonical Discriminant model.

4.6 Socio-demographic characteristics

Table 17 : Prevalence of modifiable risk factors among the Groups

Study variable		Normal (Sample Size: 12)	Pre-hypertensive (Sample Size: 10)	Hypertensive (Sample Size: 46)	Chi-square value	P value
Family status	Married	7	9	45	21.970	**< .001****
	Divorced	0	0	0		
	Widow/Widower	1	1	1		
	Single	4	0	0		
Socio-economic status	Lower class	7	7	27	1.810	0.771
	Middle class	3	3	12		
	Upper class	2	0	7		
Education status	Post high school/college/ university	7	4	21	0.849	0.654
	Others	5	6	25		
Intake of salts	Two pinches per meal	6	3	15	1.404	0.496
	More than two pinches per meal	6	7	31		
Diet	Vegetarian	4	6	15	2.725	0.256
	Non-vegetarian	8	4	31		
Occupation	Skilled worker	0	0	13	15.900	**0.014***
	Unskilled worker	4	3	8		
	Professional	6	2	6		
	Unemployed	2	5	19		
Smoking	Current smoker	2	1	2	4.447	0.349
	Non-smoker	10	8	43		
	Ex-smoker	0	1	1		
Alcohol intake	Current alcoholic	3	1	7	1.468	0.832
	Non-alcoholic	9	9	38		
	Ex-alcoholic	0	0	1		
Tobacco chewing	Current tobacco chewer	0	0	6	7.131	0.129
	Non-tobacco chewer	11	9	40		
	Ex-tobacco chewer	1	1	0		
Family history of hypertension	Yes	0	2	10	3.140	0.298
	No	12	8	36		

Overall 52.94% of the population is illiterate. The percentage of hypertensive among the illiterate respondents is higher as compared to the literate ones. However there was is no significant association with education in the present study. 38.23% of the screened population is unemployed. The unemployed mostly included housewives. Among the unemployed, unskilled or manual labour is 22.05% and skilled workers 19.11%. Hence this population occupationally represents a typical agricultural rural population. Greater prevalence of hypertension is observed in unemployed (41.30%) and skilled (28.26%) category of occupation followed by unskilled (17.39%) and professional (13.04%). The higher prevalence in the unemployed and skilled category is statistically significant as compared to the other two categories of the study population ($p = 0.014^*$).

Socioeconomic status is not significantly associated with hypertension in our study. 41 (60.29 %) subjects belonged to lower socioeconomic class. 18 (26.47%) are in the middle class and 9 (13.23%) are reported to be in the upper class. The maximum number of subjects with hypertension is in the lower class (89.13%). The maximum number of subjects with hypertension is also in the lower class (17.07%). Among the study subjects, 44 (64.70%) are in the group of "additional dietary salt intake". The prevalence of pre-hypertension and hypertension among additional salt takers is observed to be 7 (15.90%) and 31 (70.45%) respectively. The prevalence of pre-hypertension and hypertension among non-additional salt takers is observed to be 15 (62.5%) and 3 (12.5%) respectively. This prevalence between the two variable is not statistically significant among the study groups ($p=0.496$). The prevalence of hypertension did not differ significantly between vegetarians (36.76%) and non-vegetarians (63.23%).

Among the study population, 7.3% are current smokers, 89.7% are non-smokers and 2.9% are ex-smokers. All the current and ex-smokers are males. Since the majority of the population consisted of females, there is no significant association of smoking with pre-hypertension and hypertension. In terms of alcoholism, current alcoholics constituted 16.177%, non-alcoholic 82.35% and ex-alcoholic 1.47%. Similar to smoking, all the current and ex-alcoholics are males. Since the majority of the population consisted of females, there is no significant association of alcoholism with pre-hypertension and hypertension. Among the study population, 8.8% are current tobacco chewers, 88.23% are non-tobacco chewer and 2.94% are ex-tobacco chewer. There is a lower prevalence of pre-hypertension and hypertension among all the categories.

4.7 Compliance in terms of Hypertension Preventive Measures

Of the 46 hypertension patients 39 (84.78%) are aware of their disease. Only 27 (58.69%) are taking regular treatment and 15 (32.60%) have their BP under control. 17.64% have a history of hypertension in their family, where as 82.35% did not have a previous history of hypertension. Out of 46 patients who knew to have hypertension 31.7% made some life style modifications, dietary modifications and compliance for taking medicines. This calls for a stringent implementation of awareness and other preventive measures to bring down the menace of hypertension.

4.8 Screening of Mutations / Polymorphisms at MTHFR Exon 4

Blood samples were collected and high molecular weight genomic DNA was isolated from 68 subjects. The genomic DNA isolated show good yield and less of purity (Fig 3). The OD 260 / 280 nm is in the range of 1.8 – 2.0 indicating less protein contamination. PCR amplification of MTHFR exon 4 produced amplicons of 198bps (Fig 4). The amplicons spanned the region of interest. The LIS-SSCP analysis indicates an absence of mobility variation among the samples analyzed (Fig 5). The MTHFR 677 PCR product (198bp) on digestion with *Hinf* I showed same mobilty as that of the unrestricted DNA (Control) in the gel (Fig 6). The restrictin pattern indicated the presence of CC genotype (homozygous).

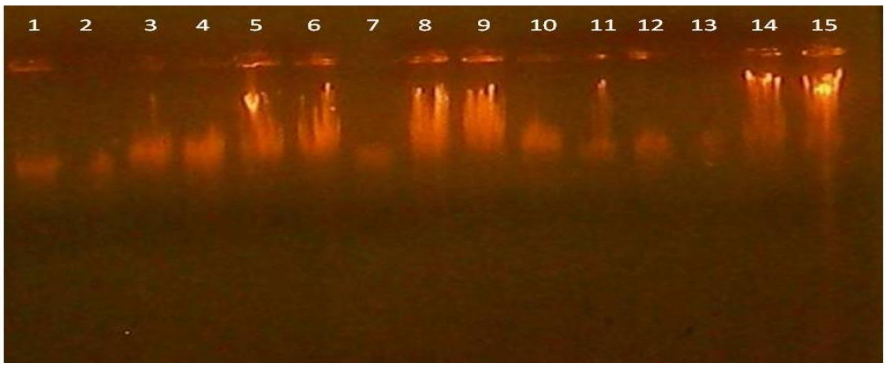

Fig 3: 0.7% Agarose gel electrophoregram of Human Genomic DNA

Lane 1 – 15 : Human Genomic DNA

Run Voltage : 50V

Run Buffer : 1x TBE

Run Time : 1 ½ -2 hrs

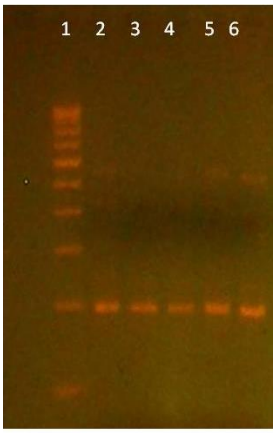

Fig 4 : PCR of MTHFR Exon 4 checked on a 3 % agarose gel

Lane 1	: 100bp Ladder
Lane 2 -6	: PCR Products (198bp)
Run Voltage	: 50V
Run Buffer	: 1x TBE
Run Time	: 2 - 2 ½

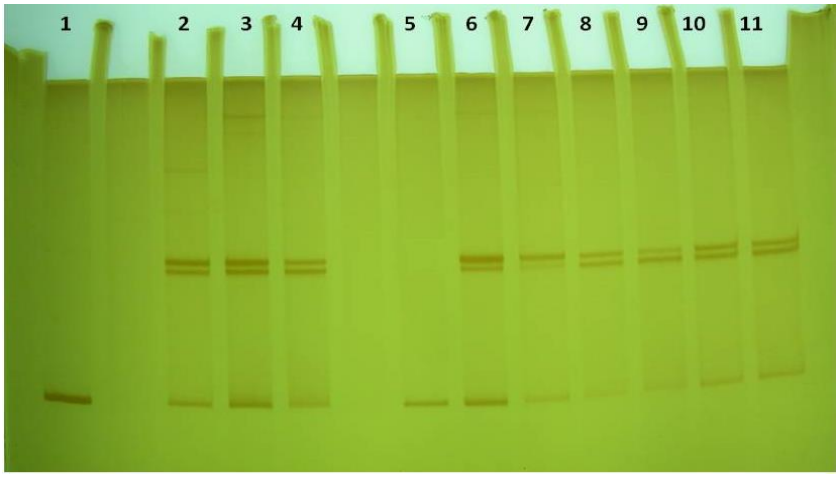

Fig 5 : Silver stained Composite gel (10%) showing LIS-SSCP patterns of 198bp PCR products.

Lane 1 : ds DNA control
Lane 2 : Sample No.02
Lane 3 : Sample No.03
Lane 4 : Sample No.04
Lane 5 : ds DNA control
Lane 6 : Sample No.04
Lane 6 : Sample No.06
Lane 7 : Sample No.07
Lane 8 : Sample No.08
Lane 9 : Sample No.08
Lane10 : Sample No.08
Lane 11 : Sample No.08

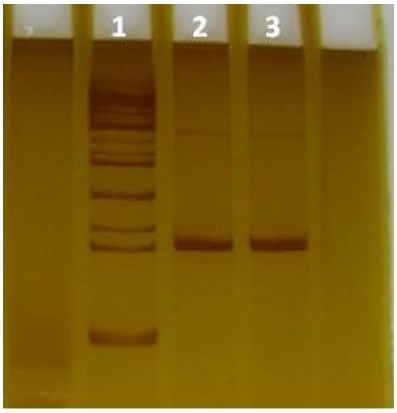

Fig 6 : Silver stained Composite gel (10%) showing RFLP patterns of 198bp PCR products.

Lane 1	: 100bp Ladder
Lane 2	: Restricted PCR Product (198bp)
Lane 2	: Unrestricted PCR Product (198bp)
Run Voltage	: 50V
Run Buffer	: 1x TBE
Run Time	: 2 - 2 ½

4.9 Survey at Pandithamedu village, Kancheepuram Dt, Tamil Nadu

4.9.1 Study sample

Studies have indicated an increasing prevalence of hypertension in rural populations. Based on these reports, we conducted a survey at Pandithamedu, a sub-village under Paiyanoor village, Thiruporur tehasil Kancheepuram district of Tamil Nadu. The survey was done in association with the NSS unit of AVIT and Vinayaka Missions Chennai Hospitals. Systolic and Diastolic pressure of participants those who attended the camp were recorded and the prevalence of hypertension was estimated. Through this camp we also gave general awareness on hypertension, its significance and association with various diseases such as cardiovascular and kidney diseases.

4.9.2 Gender and Age

A total of 55 subjects participated in the camp. The subjects were divided into three groups: Normotensive, Pre-hypertensive & Hypertensive. The prevalence of pre-hypertension and hypertension was estimated based on the systolic and diastolic blood pressure values. The proportion of pre-hypertension (26) is high when compared to normotensive and hypertensive groups. There is significant proportion between the three groups (p = <0.001**). Females (42, 76.36%) formed the maximum number of subjects in this study. The number of subjects under the male gender is 13 (23.635%). Since P value is greater than 0.01, the overall proportion was significant with respect to gender (p = <0.001**). The mean age ± S.D for males is 49.15±17.69 yrs and for females 46.57 ± 15.198 yrs. The proportion between the two genders with respect to age is not significant (p = 0.447)

Table 18: Groups with respect to Age in Years

Group	Size (N)	Mean	SD	F value	P Value
Normotensive	18	33.39[a]	6.78	38.96	<0.001**
Pre-hypertensive	26	48.15[b]	12.54		
Hypertensive	11	67.45[c]	7.76		

Note: ** denotes significance @ 1%.

Since P value is greater than 0.01, the proportion between the three groups with respect to age is highly significant (p = <0.001**). Based on DMRT for the variable age, the normality is

significantly different with respect to pre-hypertensive and hypertensive groups at 5%. The difference is also significant between pre-hypertensive and hypertensive groups

Table 19 : Sample Size under different Age Groups

Age Group	Frequency (N)	Chi-square value	P Value
20 -35	16 (29.1)	1.127	0.569
36-50	17 (30.9)		
51-75	22 (40)		

The study population was categorized into different groups according to their age. There were 3 groups: 20 – 35yrs, 36 – 50 yrs, 51 – 75yrs. The maximum number of participants are in the age group of 51 – 75 yrs (22). The frequency among the different age groups has a P value greater than 0.05. It indicates a less significant association between sample size and different age groups (p = 0.569). The proportion of gender among different age group was calculated. The difference was compared for significance by Chi-square test. The p value is greater than 0.05 and indicates that the proportion of the two genders within different age groups is not significant (p = 0.650).

Table 20 : Prevalence of Pre-Hypertension and Hypertension among different Age Groups

Age Group in years	Group			Total	Chi-square value	P value
	Normotensive	Pre Hypertensive	Hypertensive			
20-35	12 (75.0%)	4 (25.0%)	0 (.0%)	16 (100.0%)	35.277	0.000**
36-50	6 (35.3%)	11 (64.7%)	0 (.0%)	17 (100.0%)		
51-75	0 (.0%)	11 (50.0%)	11 (50.0%)	22 (100.0%)		
Total	18 (32.7%)	26 (47.3%)	11 (20.0%)	55 (100.0%)		

Note: The value within bracket refers to row percentage

Pre-hypertension is highest in the age group of 36 – 50yrs. All the hypertension subjects are in the group of 51 – 75 yrs. The differences is found to be statistically significant ($p<0.001$), which indicates that in the present study, age has a strong association with hypertension.

4.9.3 Systolic (SBP) and diastolic blood (DBP) pressure

Table 21 : Systolic (SBP) and Diastolic blood (DBP) pressure among the Groups

Variables	Groups	N	Mean ± Std. Deviation	F value	P Value
SBP	Normotensive	18	115.56^{a} ± 7.374	44.141	.000**
	Pre Hypertensive	26	129.62^{b} ± 4.691		
	Hypertensive	11	137.36^{c} ± 8.418		
DBP	Normotensive	18	76.39^{a} ± 4.984	38.983	.000**
	Pre Hypertensive	26	81.04^{b} ± 4.686		
	Hypertensive	11	91.09^{c} ± 1.446		

Since p value is less than 0.01, there are significant differences between the groups with respect to SBP and DBP. This indicates that SBP and DBP are closely associated with pre-hypertension and hypertension. Based on DMRT for the variable systolic blood pressure, the normality is significantly different with respect to pre-hypertensive and hypertensive groups at 5%. The difference is also significant between pre-hypertensive and hypertensive groups. DMRT for diastolic blood pressure show a significant difference between normotensive and hypertensive but not between pre-hypertensive and hypertensive.

Table 22 : Systolic (SBP) and Diastolic blood (DBP) pressure among the Age Groups

Variables	Age groups	N	Mean	Std. Deviation	F value	**P Value**
SBP	20-35	16	117.50[a]	7.815	24.394	**0.000****
	36-50	17	124.53[b]	8.133		
	51-75	22	134.73[c]	7.119		
DBP	20-35	16	77.50 [a]	3.706	22.712	**0.000****
	36-50	17	78.06 [a]	6.057		
	51-75	22	87.14 [b]	5.027		

Note: ** denotes significance at 1%. Different alphabets between groups denotes significance at 5% level using Duncan Multiple Range Test (DMRT)

The mean systolic and diastolic blood pressure is found to increase steadily with age, being lowest in the age group of 20 – 35 yrs and highest in the age group of 51 – 75 yrs. Since P value is less than 0.01, there are significant differences between the groups with respect to SBP and DBP (p= <0.001**). This indicates that SBP and DBP are closely associated with pre-hypertension and hypertension. Based on DMRT for the variable systolic blood pressure, the normality is significantly different with respect to pre-hypertensive and hypertensive groups at 5%. The difference is also significant between pre-hypertensive and hypertensive groups. DMRI for diastolic blood pressure show a significant difference between normotensive and hypertensive but not between pre-hypertensive and hypertensive.

5 DISCUSSION

Hypertension is considered to be one of the most rampant non-communicable diseases. Previous studies in various geographical locations have reported the prevalence of hypertension to be about 25% in urban population and 10% in rural population (Kannan and Satyamoorthy, 2009). Prevalence of hypertension in India has been increasing. This can be credited to increased urbanization, lifestyle changes and changes in food habits and increased life expectancy (Gupta, 2004).

Chronic Kidney Disease is multifactorial, associated with chronic co-morbid conditions such as diabetes mellitus, cardiovascular disease, obesity and hypertension. Since all of these conditions bring about vascular inflammation, CKD is considered as an inflammatory condition. Elevated blood pressure (BP) is closely associated with CKD progression, and lowering of BP may slow down GFR decline and in improving kidney function (Kim *et al.,* 2012).

Several risk factors have been implicated in the etiology of hypertension. This includes geographic considerations, genetic socio-economic, socio-cultural and dietary, nutritional status etc. While the risk factors and their impact on hypertension is documented by well designed studies in the Western countries, systematically conducted studies using rigorous epidemiological techniques are lacking in India (Murray and Lopez, 1996; Pradeepa and Mohan, 2008; Madhukumar *et al.,* 2012).

There is an increasing trend of hypertension as age advances (Joshi *et al.,* 2000). Using the JNC VII criteria, we found hypertension to be of 67.6% prevalence among the study sample. Pre-hypertension was found to be less prevalent at a 10%. Prashant *et al*., (2012) reported a prevalence of 19.04% in central Indian rural population. Another epidemiological study set in Puducherry, reported a prevalence of 26.3% (Bharathi *et al.,* 2012). A high prevalence value obtained from our study indicates alarming increase in the prevalence of hypertension in the rural population. We found a high significance between frequencies of hypertension among the study sample groups which conforms to the trends reported in similar epidemiological studies.

The CURES study reported hypertension to be 49.6% prevalent among males. We found hypertension to be 68.8% prevalent among males and pre-hypertension to 12.5% prevalent, while for females it stood at 66.7% and 16.7% respectively. Bharathi *et al*., (2012) report hypertension to be 28% and 26.9% prevalent among females and males respectively. They also reported pre-hypertension to be 56% and 58.8% prevalent among females and males respectively. Our study

shows a higher prevalence of hypertension. This is in agreement with the trends observed in other studies set in the rural south Indian backdrop.

Mean age of hypertensive patients, as reported by the CURES study, was 44.9 ± 12.9 for males. We found the mean age of male subjects to be 51.44 ± 18.100. We found the mean age to be 53.19 ± 18.100 for females. Our findings are in accordance with the prior studies (Ref). However, the correlation of age with gender was not significant, which is in contrast with the reviewed studies (Ref). This could be credited to the nature of the study. The study employed random population sampling on a limited sample size and was not age-adjusted.

The mean age of hypertensive patients was found to be 55.07 ± 13.84 and for pre-hypertensive subjects, 56.00 ± 14.97. Prashant *et al.*, (Ref) reported the mean age to be 53.11 ± 12.46. Radhika *et al.*, (2007) reported the mean age to be 44.9 ± 12.9. The findings of our study are in accordance with prior studies (Ref). A significant variation was observed between the classes- normal and pre-hypertension with respect to age in years, validated by Duncan's post-hoc test. However, the variance between the classes - pre-hypertension and hypertension, was not significant. This again is in accordance with the prior studies. Age specific distribution shows that hypertension was most prevalent in the age-group 51-75. Bharati *et al.*, (2012) have reported similar findings. This could be attributed to the sedentary lifestyle at this age group and the lack of physical activity. The age group classes did not show significant variance in a compounded age-gender distribution.

The majority of current studies agree that waist circumference (WC), body mass index (BMI) or waist-to-hip ratio (WHR), high systolic and diastolic blood pressure as positive prognostic indicators for hypertension. In the present study, the systolic and diastolic pressures were found to have high significance of class variance and the mean systolic and diastolic pressures for pre-hypertensive and hypertensive groups was 129.50 ± 1.08, 77.30 ± 5.12 and 145.13 ± 12.52 and 93.61 ± 9.68. The mean BMI for the hypertensive class was 26.34 ± 4.11 and 25.10 ± 3.56 for pre-hypertensive.

Community based analysis from central India showed an overall prevalence of isolated systolic and isolated diastolic hypertension (Kokiwar *et al.*, 2012). In our study, the prevalence of isolated systolic and isolated diastolic hypertension was very less. Prashanth *et al.*, (2012) reported it to be 23.5 ± 4.7 in their study. A higher BMI for hypertensive patients in the present study is possibly the consequence of less physical activity and high fat consumption. The mean waist circumference of hypertensive patients was found to be 86.67 ± 12.46 and 91.44 ± 9.73 for pre-hypertensive patients

in our study. Study from a rural community of central India showed age, increased BMI and WHR associated with hypertension (Kokiwar *et al.*, 2012). Radhika *et al* (2007) reported mean waist circumference to be 88.2 ± 11.2 for hypertensive patients. Similar epidemiological studies reported high correlation between hip general and central obesity and hypertension. However we couldn't establish any significance. This could be credited to the limited sample size.

Accumulation of lipids is one of the most important causes of essential hypertension, especially age-associated hypertension. Accumulation of lipids causes arteriosclerosis and narrowing of vessels. This in turn causes vascular stiffening and also attracts inflammatory responses. We found a strong correlation between hypercholesterolemia,pre-hypertension and hypertension in the present study. The considerable increase in the mean value among pre-hypertensive and hypertensive class shows the prominent contribution of cholesterol and LDL to the genesis and establishment of hypertension in the present study sample.

Diabetes was significantly associated with hypertension in studies across different India populations (Malhotra *et al.*, 1999; Mohan *et al.*, 2007; Yadav *et al.*, 2008). However in our study we did not observe an association of hypertension with pre-hypertensive and hypertensive subjects.

Epidemiological studies have recently shown that uric acid may be negative prognostic marker for mortality in subjects with pre-existing kidney failure. Elevated serum uric acid is highly predictive of mortality in patients with abnormal kidney functions or other renal disease (Ochiai *et al.*, 2005). However in our study we did not observe any variations in the uric acid, urea and creatinine levels among pre-hypertensive and hypertensive subjects as predicted by other studies (Tobian and Binion, 1952).

Studies have reported differences between normotensive and hypertensive individuals in terms of salt intake, intracellular electrolytes, total body electrolytes, serum electrolytes and the renal excretion of sodium and water in-take. However in the present study we didn't find any variations in serum electrolyte levels among the three groups.

Kannan and Satyamoorthy (2009) observed occupational status associated with hypertension in their study from kancheepuram. On the contrary few other studies (Padmavathi and Guptha, 1959; Gosh *et al.*, 1983) found that hypertension was more common in professional group as compared to unskilled and semiskilled groups. Miall *et al.*, (1962) did not find any significant association

between occupational status and hypertension. In our study we observed a stronger association of hypertension with skilled and unemployed subjects.

Vedapriya *et al* (2012) have reported a significant correlation between income and hypertension. Hypertension was found to be more prevalent in low income groups. However our study couldn't establish any significant relationship between the two classes. Studies also reported significant relationship between family history of hypertension (Vedapriya *et al.,* 2012). However our study couldn't establish any considerable significance. This could be the implication of a limited sample size. The study conducted by Vedapriya *et al.* reports a significant association between alcohol consumption and hypertension prevalence. We couldn't deduce such relationship, which could possibly be the consequence of random sampling on a small sample pool.

Experimental studies have established casual links between homocysteine and blood pressure. Studies have reported increased blood pressure upon inducing hyperhomocysteinemia (Rolland *et al.,* 1995; Miller *et al.,* 2000). The present study attempted to screen the MTHFR gene for the mutation C677T in exon 4. Heterozygous individuals of this mutation suffer mild hyperhomocysteinemia. We did not obtain any polymorphisms in the study sample. However, these observations cannot be extrapolated to the whole population in the study localization, given the rarity of single nucleotide polymorphisms and the limited sample size of the study.

Our study documented high prevalence of both pre-hypertension and hypertension. Final statistical analysis showed that the important correlates of hypertension were age, systolic and diastolic pressures, serum cholesterol levels, serum LDL levels, family status and occupation.

A greater understanding of the risk factors that account for the increase in hypertension could potentially contribute to its future prevention by addressing its root causes. Public health efforts to reduce the prevalence of hypertension have rightly focused on non-pharmacological approaches that lower blood pressure. Current guidelines on Hypertension recommend lifestyle modifications, including salt reduction, as one of the most effective approaches to prevent hypertension and indeed as first line treatment for mild hypertension (WHO, 2003).

Epidemiological studies to assess the prevalence of Hypertension are urgently needed in developing countries like India to determine the baseline against which future trends in risk factor levels can be assessed and preventive strategies planned to promote health (Madhukumar et al., 2012). Most of the

studies are conducted on the urban population and do not report about risk factors in rural population since the life style differs vastly in the two groups. Hence studies are needed in rural areas to have a base line data about the prevalence of hypertension and its association with the risk factors. Preventive strategies have to be planned for the rural areas. Simple life style modifications in the rural areas will change the epidemic scenario.

6 SUMMARY AND CONCLUSION

- ✓ Hypertension is a chronic condition of concern due to its role in the causation of coronary heart disease, stroke and other vascular complications. It is the commonest cardiovascular disorder, posing a major public health challenge to population in socioeconomic and epidemiological transition. It is one of the major risk factors for cardiovascular mortality, which accounts for 20 – 50% of all deaths (Gupta, 1997). Many factors like alcohol consumption and smoking also increase the risk. High fatty diet and body mass index have a positive correlation and physical activity is negatively related with hypertension (Malhotra *et al.*, 1999; Mohan *et al.*, 2007; Yadav *et al.*, 2008).
- ✓ Prevalence of hypertension in India as reported by various literatures has been on an increasing trend for the last three decades. The increase has been reported as about 30 times among urban residents and about 10 times among rural residents. The average prevalence of hypertension in India is 25% in urban areas to 10% in rural areas (Kearney *et al.*, 2005; Gupta, 2006). Most of the studies are reported from urban population. More studies are needed based on rural Indian scenario.
- ✓ With this background, the present study was conducted from a rural population of Tamil Nadu to study the prevalence of hypertension, its associated factors as well as to increase the awareness on importance of life style modifications. Such studies targeting low socio-economic groups provides an estimate of the future magnitude of the problem and assist in developing strategies for control of hypertension and chronic kidney diseases (CKD).
- ✓ In our study population, prevalence of pre-hypertension among males was 12.5% and 16.7% in females. Hypertension showed 68.8% prevalence among males and 66.7% prevalence among females. A marginally higher prevalence of pre-hypertension and hypertension was observed among males. However it was not significant. Age was identified as a determining factor for the prevalence of hypertension in the present study. A significant association in the age group of 50's with high systolic and diastolic values was noted in the present study. The BP values among the groups were significantly high in the pre-hypertensive & Hypertensive groups when compared with the control group. These variables were found to increase with increasing age.

- ✓ There was difference in the waist hip ratio among the groups. However our study couldn't establish a significant relationship with hypertension. Family status & Occupation was found to be significant among the groups. Of all the hypertensive subjects, 41.3% were unemployed, 45.6% belonged to the working class and only 13% were professionals. We could not find any significant association between other anthropometric & socio-economic factors analyzed for hypertension or pre-hypertension.
- ✓ We found a strong correlation between hypercholesterolemia and hypertension. Cholesterol and LDL values were significantly different among the groups and were high in the Hypertensive group. This indicates the presence of compounding risk factors in addition to Hypertension in the study population. This also supports reports from other studies that high blood pressure is closely associated with coronary heart disease, stroke, congestive heart failure and impaired renal function. There was difference in TGL, FBS, PPBS, urea & uric acid among the groups. However our study couldn't establish a significant relationship with pre-hypertension & hypertension. The creatinine, urea & uric acid levels were not significantly associated with pre-hypertension & hypertension in the study population and indicate an absence of chronic kidney diseases. However the increased cholesterol & LDL Levels may lead to chronic kidney diseases in addition to cardiovascular complications in future if not treated properly.
- ✓ Molecular analysis of exon 4 of MTHFR gene revealed an absence of variation in the DNA sequence among the study population. PCR-SSCP-RFLP analysis revealed the presence of 677 CC, the wild type genotype in the study population. Individual with two copies of 677C (677CC) have the "normal" or "wildtype" genotype. 677TT individuals (homozygous) are said to have mild MTHFR deficiency and 677TT are predisposed to mild hyperhomocysteinemia (high blood homocysteine levels). Mild elevations in serum homocysteine may contribute to elevations in blood pressure which may be one of the important causes for CVD. The present study indicates an absence of hyperhomocysteinemia among the study populaiton.
- ✓ A high correlation between age, systolic and diastolic values with hypertension was observed in the subjects at the Manampathy camp. However, gender bias was insignificant. The survey indicates a high prevalence of Hypertension in Manampathy village and hence calls for preventive measures especially applicable to rural population.

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