Introduction
1. INTRODUCTION

Hypertension is a disease state in which the arterial blood pressure exceeds established norms, producing an increased morbidity and mortality. It has been called “silent killer” because patients with hypertension generally have no symptoms whatsoever, often for 15 – 20 years. Even without producing any symptoms, the disease insidiously causes cardiovascular disease and premature death. There is a very valid reason for calling it “silent killer”

- 50% of the patients do not know that they are hypertensive
- 50% of them don’t take treatment
- 50% of treated patients don’t have controlled blood pressure

1.1 CLASSIFICATION OF HYPERTENSION BY AETIOLOGY

I. Essential hypertension

II. Secondary hypertension

Renal  Endocrine  Neurogenic  Mechanical  Exogenous  Miscellaneous

Parenchymal  Renovascular

1.2 ESSENTIAL OR PRIMARY HYPERTENSION

Hypertension is defined as having systolic blood pressure 140 mm Hg or greater, and/or having diastolic blood pressure 90 mm Hg or greater, and/or taking antihypertensive medications (WHO, 1996). In the majority of cases of hypertension, so called essential hypertension, the cause of the high blood pressure is not known. There are no diagnostic biochemical features associated with this disease.
Fig. 1.1 Distribution of primary and secondary causes of hypertension

Pie chart in Fig. 1.1 shows the distribution of primary and secondary causes of hypertension. Essential hypertension constitutes 95% of the total (Shapiro and Buchalter, 1992).
A schematic representation of the pathophysiological mechanism that occurs in the development of hypertension (Shapiro and Buchalter, 1992) is given below:

1. Renin angiotensin system
2. Renal retention of salt and water
   - Increased cardiac output
   - Increased sodium in arterial walls
     - Autoregulation
     - Increased arteriolar reactivity
       - Arteriolar vasoconstriction
         - BLOOD PRESSURE INCREASES
### 1.4 Classification of Blood Pressure for Adults Age 18 Years or Older (JNC V, 1993)

<table>
<thead>
<tr>
<th>Category</th>
<th>Systolic BP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>120 - 129</td>
<td>80 – 84</td>
</tr>
<tr>
<td>High normal</td>
<td>130 – 139</td>
<td>85 – 89</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1 (mild)</td>
<td>140 – 159</td>
<td>90 – 99</td>
</tr>
<tr>
<td>Stage 2 (moderate)</td>
<td>160 – 179</td>
<td>100 – 109</td>
</tr>
<tr>
<td>Stage 3 (severe)</td>
<td>180 – 209</td>
<td>110 – 119</td>
</tr>
<tr>
<td>Stage 4 (very severe)</td>
<td>≥ 210</td>
<td>≥ 120</td>
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</tbody>
</table>

### 1.5 Age and Hypertension

With the exception of a few relatively isolated societies, average blood pressure tends to rise progressively with increasing age in almost every population (Whelton *et al.*, 1994). The relationship between age and hypertension has been demonstrated in cross-sectional surveys as well as in cohort studies conducted in western populations (Comoni-Huntley *et al.*, 1989; Dannenberg *et al.*, 1988). The age-related increase in risk of hypertension, however, varies considerably depending on an individual’s stage of life, gender, race, initial level of blood pressure, and exposed to environmental factors.
1.6 GENDER AND HYPERTENSION

Overall the prevalence and incidence of hypertension are slightly higher in men compared to women (Burt et al, 1995). In young adults, the systolic and diastolic blood pressure-tend to be higher in men than women. The age related rise in blood pressure during adulthood is, however, steeper in women than for men. Consequently the prevalence of hypertension is higher in women than in men late in life. In the NHANES I epidemiologic follow up study, hypertension incidence was 11.9 % for men and 8.1 % for women at age 25 to 34 years and 41.8 % for men and 43.3 % for women at age 55 years or older over an average of 9.5 years of follow up. (Comoni-Huntley et al, 1989) Framingham heart study cohort, women had a higher incidence of hypertension compared to men after age 50 years (Dannenberg et al, 1988).

1.7 ETHNICITY AND HYPERTENSION

The incidence of hypertension in India is 5-15% in the adult population against 10-12% in the west (Datey et al, 1982). The prevalence of hypertension in India ranged between 16.89% and 23.7% in rural areas and between 30% and 33% in urban areas (Gupta et al, 1995). African-American have a higher prevalence and incidence of hypertension compared to whites (Bucher et al, 1996 and Hutchinson, 1992). The prevalence and incidence of hypertension is similar or lower in Mexican-American than in non-Hispanic whites (Haffner et al, 1992). Asian-American have a substantially lower prevalence of hypertension compared to other ethnic groups (Klatsky et al, 1991).
1.8 ASSOCIATION BETWEEN HYPERTENSION AND KIDNEY STONE DISEASE

An independent clinical association between hypertension and kidney stone has been recently described (Cappuccio et al, 1990 and Cirillo et al, 1988). In fact studies performed on a general population showed that a history of kidney stone was two or three times more frequent in hypertensive than nonhypertensive subjects. The prevalence of nephrolithiasis has been reported to be 30% (Tibblin, 1967) to 79% (Cappuccio et al, 1990) in hypertensive than in normotensive subjects. More than 85% of kidney stones in men contain calcium; among these, calcium oxalate stones are the most common (Johnson et al, 1979; Coe and Parks, 1984). Alterations in calcium metabolism may play an important role in the pathogenesis of both hypertension and nephrolithiasis and have been suggested as a plausible mechanism linking this two disorder (Strazzullo and Mancini, 1994; Strazzullo et al, 1983). A higher prevalence of hypercalciuria has been reported in patients with essential hypertension (Strazzullo, 1991; McCarron, 1980; Tillman and Semple, 1988; Borghi et al, 1999) and alterations of calcium metabolism such as primary hyperparathyroidism that lead to hypercalciuria have been associated with an increased prevalence of hypertension. Several other mechanisms that may link hypertension and nephrolithiasis have been suggested, which include high dietary intake of sodium, low intake of potassium, and renal damage (Strazzullo and Mancini, 1994; Blauestein, 1977; Kesteloot et al, 1980).

Several studies have indicated that the plasma levels of parathyroid hormone (PTH) are increased in patients with essential hypertension (Nakamura et al, 1991; Young et al, 1990; Papagalanis et al, 1991) and high incidence of hypertension has been found in patients with primary hyperparathyroidism (Sancho et al, 1992). A positive relation between PTH and blood pressure has been demonstrated both in patients with essential hypertension (Young et al, 1990; Duprez et al, 1991; Jespersen et al, 1993) and

Naturally occurring kidney stones are rare in animals, but strains of spontaneously hypertensive rats were found to be more prone to developing kidney stones than normotensive ones. In a trial conducted on a study population of 895 men, aged 50 years at the time of examination, 6.8% were diagnosed as having kidney stones (Tibblin G, 1967). When the participants were classified according to their blood pressure level, the rate of nephrolithiasis increased from 1.1% in the lower blood pressure class to 3.3% in the upper one, which also included treated hypertensive subjects. The relationship between blood pressure and rate of kidney stone disease was found to be statistically significant. Recently it has been reported that in essential hypertensive patients oxalate excretion was increased along with calcium and their urine is supersaturated with calcium oxalate and calcium phosphate (*Borghi et al*, 1999). In their five year followup study Borghi *et al* reported that 19 out of 132 hypertensives and 4 out of 135 normotensive patients had stone episode. Increased 24h calcium (mmol)/sodium (mmol) ratio (x100) was reported in female hypertensive patients (*Tsuda et al*, 2001).
1.9 PRIMARY PREVENTION OF HYPERTENSION

In treating hypertension anti-hypertensive drugs are widely used and their role is well accepted. However, most of these agents may produce adverse events. Hence, an alternative thinking which is getting credence now-a days is an attention directed towards the lifestyle modifications in hypertension. Ideally, the lifestyle modification strategies should be effective, safe, inexpensive and practicable on long-term duration. Such a therapy may control the minimally elevated BP without any risk and be tried initially in most of the patients during the first 3 to 6 months of recognition of hypertension.

1.9.1 Life style modifications on prevention of hypertension (He and Whelton, 1997)

<table>
<thead>
<tr>
<th>Documented efficacy</th>
<th>Limited or unproven efficacy</th>
</tr>
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<tbody>
<tr>
<td>Weight loss</td>
<td>Calcium supplementation</td>
</tr>
<tr>
<td>Dietary sodium reduction</td>
<td>Magnesium supplementation</td>
</tr>
<tr>
<td>Alcohol moderation</td>
<td>Fish oil supplementation</td>
</tr>
<tr>
<td>Potassium supplementation</td>
<td>Dietary fiber supplementation</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Reduced dietary saturated-to-polyunsaturated fat ratio</td>
</tr>
<tr>
<td></td>
<td>Increased vegetable protein intake</td>
</tr>
<tr>
<td></td>
<td>Caffeine consumption moderation</td>
</tr>
<tr>
<td></td>
<td>Stress management</td>
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</table>


1.9.2 DIETARY INTERVENTIONS

**Weight reduction** – An increased body weight or obesity has a substantial impact on BP. The caloric restriction may influence in reversing this process. Ramsay et al demonstrated a decrease of 2.5/1.5 (SBP/DBP) mm Hg of BP for every one kg loss of the body weight. The mechanism of decreased BP during weight loss may relate to several hormonal changes. The weight reduction also causes a significant enhancement of antihypertensive effects of the drugs (chlorthalidone and atenolol) and impressive reduction in hyperinsulinemia, a likely major cardiovascular risk factor and promotor of hypertension (Kaplan, 1989). The reduction in insulin may lead to lowered sodium retention as well as sympathetic nervous system activation.

**Alcohol consumption** – Alcohol can induce hypertension and its incidence is higher in people who consume more than three drinks (1.5 ounces) per day as compared to those who consume less than three drinks (Shaper et al, 1988). The prevalence of alcohol-associated hypertension is about 1 % in the general population and 7 % in men. The incidence rises to 13 % in men who consume more than 50 drinks per week (Bulpitt et al, 1987). Reducing the alcohol consumption of hypertensives, who are heavy drinker, lowers their BP and the requirement for antihypertensive medications (Chockalingam et al, 1990). A reduction of weekly alcohol intake from 440 ml to 66 ml was associated with the fall in BP of 4.8/3.3 (SBP/DBP) mm Hg. The fall of BP was 10.2/7.5 (SBP/DBP) mm Hg if an average weight loss of 7.5 kg was also accomplished (Puddey et al, 1992).

**Dietary sodium reduction** – Because of documented efficacy of sodium restriction and volume contraction in lowering BP, patients are instructed to curtail the salt intake
drastically. It has also been reported that there is a decreased incidence of coronary
disease by about 16 % and stroke by 22 % with sodium intake of 50 mM a day (Law et

**Potassium supplementation** – Results from both observational epidemiologic studies
and randomized controlled trials suggest an inverse relationship between potassium
intake and blood pressure in human populations (Puddey et al, 1985).

**Calcium supplementation** – Even though there exists a controversy over calcium intake
and hypertension, there are few reports on relating the low calcium intake and incidence

**Magnesium supplementation** – There are contradictory results in the literature from two
research groups on magnesium intake and blood pressure (Widman et al, 1993; Whelton
and Klag, 1989).

**Dietary fiber** – The diet rich in plant fibers either alone or with low fat, low sodium
could lower the BP by about 5mm Hg in hypertensive patients but not in normotensives
(Swain et al, 1990).

**Vitamin C** – There is an inverse relationship between BP and plasma vitamin C level
suggesting that a deficiency of vitamin C might lead to hypertension (Ness et al, 1996).
The intravenous injection of vitamin C also lowered the BP in hypertensives and
diabetics (Ceriello et al, 1991). The protective effect of vitamin C could be due to its
antioxidant properties.

**Vitamin E** – Combination of oral antioxidant supplementation reduces blood pressure in
hypertensive patients via increased availability of nitric oxide. Antioxidant
supplementation increases the circulating levels of Vitamin E, beta-carotene and nitric
oxide in all subjects (Galley et al, 1991). In another study, alpha tocopherol acetate in combination therapy promoted normalization of lipid per-oxidation processes in erythrocyte membrane and reduction of blood plasma content of the lipid per-oxidation products (Kuznetsov et al, 1994). Dietary supplementation of vitamin E significantly lowers elevated tissue aldehydes, vascular cytosolic free calcium and blood pressure in spontaneously hypertensive rats (Vasdev et al, 2001; Newaz and Nawal, 1998). Our present study highlighted the antioxidant properties of vitamin E on hypertensive patients.

1.10 RISK FACTORS AND STONE DISEASE

Major risk factors that contribute to calcium stone formation are hypercalciuria, hypocitraturia, hyperoxaluria, hyperuricosuria, hypomagnesuria, pH, urinary volume and renal tubular acidification defect.

1.10.1 Hypercalciuria

It is the most commonly associated abnormality with idiopathic stone disease (Pak et al, 1980). The urinary excretion of more than 4 mg/kg/24h is observed in approximately 50% of patients with calcium oxalate/apatite nephrolithiasis. Hypercalciuria is a frequent finding in patients with calcium oxalate nephrolithiasis. It contributes to urinary supersaturation with respect of calcium oxalate (Pak and Halt, 1976).

Hypercalciuria can be of resorptive, absorptive or of renal type. The resorptive hypercalciuria is generally characterized by primary hyperparathyroidism, which causes excessive resorption of bone and increased intestinal absorption of calcium by parathyroid stimulated renal synthesis of 1,25 (OH)₂ vitamin D2 (Kaplan et al, 1977). In absorptive hypercalciuria, there is increased intestinal absorption of calcium leading
to increased renal filtration load and suppressed parathyroid function. In renal hypercalciuria there is increased circulating calcium and decreased tubular reabsorption, which leads to calcium leak in urine resulting in hypocalcemia, which in turn stimulates PTH and the production of vitamin D3.


1.10.2 Hypocitraturia

Hypocitraturia has been reported in 50 % of the patients with calcium oxalate nephrolithiasis. It occurs as an isolated abnormality, or more commonly, with other abnormalities such as hyperoxaluria or hypercalciuria. Recently our research group reported hypocitraturia in hypertensive patients (Latha et al, 1999). Citrate retards the crystallization of stone-forming calcium salts by two broad means. First, it complexes calcium and reduces ionic calcium concentration in the urine (Meyer and Smith, 1975; Pak et al, 1982). Secondly, citrate directly inhibits crystallization of calcium oxalate and calcium phosphate (Nicar et al, 1987). Citrate is a potent inhibitor of calcium phosphate precipitation (Bisaz et al, 1978) and growth of calcium oxalate crystals (Tiselius, 1981).
1.10.3 Hyperuricosuria

It is a common feature in calcium oxalate stone formers (Coe and Kavalach, 1974). Uric acid is believed to act as nidus for calcium oxalate monohydrate crystallization and to reduce urinary inhibition activity by removing large molecular weight polyanions from solutions (Coe and Parks, 1981). Patients with hyperuricosuric nephrolithiasis excrete more than 600 mg uric acid / day and have a urinary pH greater than 5.5. Purine gluttony is a most common cause of hyperuricosuria in patients with nephrolithiasis. In such patients, dietary purine restriction corrects hyperuricosuria. Similar results has been observed in hypertensive patients (Borghi et al, 1999).

1.10.4 Hyperoxaluria

Hyperoxaluria is a condition associated with increased excretion of oxalate in urine. Robertson et al, (1978) have proposed hyperoxaluria to be a more important risk factor in forming stones than calcium. The importance of oxalate in urinary saturation of calcium oxalate was demonstrated by Finlayon (1977). Recently hyperoxaluria has been reported in hypertensives (Borghi et al, 1999 and Latha et al, 1999).

1.10.5 Hypomagnesuria

Hypomagnesuria has been reported in hypertensive patients (Tillman and Semple, 1988) and stone disease (del Valle et al 1999). Magnesium is found to increase the solubility of calcium oxalate in vitro and it has been used in the prevention of kidney stones (Hammarsten, 1929). It has been reported that magnesium deficiency develops kidney stones in rats within a short period of time (Bunce and King, 1978; Gershoff and Andrus, 1961). Following five years of observation, Prien and Gershoff
(1974) have found that 60% of the stone patients who have received 300 mg of magnesium oxide and 10 mg of pyridoxine have been completely free of stone recurrence. Magnesium binds oxalate in the intestine by which oxalate absorption is reduced. Magnesium also binds oxalate in the urine and acts as inhibitor of calcium phosphate crystallization in the urine (Robertson and Peacock, 1972).

1.10.6 Urinary volume

Changes in urinary volume are expected to influence the concentration of stone forming constituents and inhibitors/promotors in the urine. The effect of urinary dilution on the crystallisation of calcium salts was quantitatively assessed by Pak et al (1980). Both invivo and invitro studies have shown that urinary dilution significantly reduces the urinary activity product ratio (state of saturation) with respect to calcium phosphate, calcium oxalate or monosodium urate.

1.10.7 Renal tubular acidification defect

Stone formers secrete more oxalate in the renal tubule despite of the same or even less amount of plasma oxalate levels (Manoharan et al, 1989). This supports the view that renal leak of oxalate due to tubular handling defect is present in patients with CaOx nephrolithiasis. One of the consequences of defective acidification is alkaline urine. This alkaline urine increases the risk of formation of calcium phosphate stone. Beckman et al, (1976) have shown increased evidence of these defects in patients with idiopathic renal lithiasis. These are caused mainly by the failure of the distal collecting tubule to efficiently exchange protons for sodium ions.
1.11 LIPID PEROXIDATION AND ITS BIOCHEMICAL CONSEQUENCES

Lipid peroxidation is a reaction of oxidative degeneration of polyunsaturated fatty acids. It is initiated by any chemical species (R•) that has sufficient energy to abstract a hydrogen atom from the methylene carbon atom of polyunsaturated fatty acid (LH) present in the phospholipid side chains.

\[ R^\cdot + LH \rightarrow L^\cdot + RH \]

Super oxide anions, hydrogen peroxide and hydroxyl radicals are the three important reactive oxygen species that are capable of abstracting a hydrogen atom. Once initiated, lipid per-oxidation proceeds as a free radical chain reaction forming lipid radicals, lipid peroxides and peroxide breakdown products. These may damage lipid themselves, besides damaging proteins and nucleic acid.

Phospholipids, with their unsaturated fatty acid side chains, are major constituents of all biological membranes and are therefore, potential targets for oxygen radical attack. Damage to the membranes may be subtle and involve only small changes in the composition of fatty acids. Yet this is often sufficient to increase greatly the susceptibility of the membrane to oxidative damage (Gutteridge, 1978; Halliwell and Gutteridge, 1985).

Proteins and enzymes in aqueous solutions undergo major reactions of polymerisation, polypeptide chain scission and chemical changes in individual amino acids when subjected to lipid per-oxidation. Sulhydryl enzymes are also inactivated by products of lipid peroxidation. Chio and Tappel (1969) have shown that malondialdehyde reacts with amino group of the lysine residues of ribonuclease A and albumin.
1.12 LIPID PER-OXIDATION AND HYPERTENSION

Research from various laboratories shows that lipid per-oxidation plays a major role in hypertension. Increased lipid peroxide formation has been reported in hypertensive patients (Uysal et al, 1986; Uotila et al, 1993; Kuznetsov et al, 1994; Lacka et al, 1997; Digiesi et al, 1997; Koska et al, 1999; Liu et al, 1999; Latha et al, 1999; Srinivas et al, 2000; Chaninova et al, 2000; Kumar and Das, 1993; 2000) and animals (Newaz and Nawal, 1998).

Decreased catalase, superoxide dismutase and glutathione peroxidase have also been reported in essential hypertension (Iarema et al, 1992; Jain and wise, 1995; Wen et al, 1996; Lacka et al, 1997; Liu et al, 1999; Latha et al, 1999; Channinova et al, 2000; Pedro-Botet et al, 2000; Chaves et al, 2002) by researchers. Few authors reported increased GPX activity in hypertensive patients (Uotila et al, 1993; Russo et al, 1998).

Sagar et al, (1992) reported that essential hypertensive patients had lower level of superoxide dismutase and reduced glutathione in neutrophils in their blood.

Low levels of vitamin E, C and reduced glutathione have been observed in hypertensive patients (Tse et al, 1994; Wen et al, 1996; Kumar and Das, 1993 and 2000; Wen et al, 1996; Srinivas et al, 2000; Block, 2001; Galvan et al, 2001).

Similar changes have been reported in vitamin B6 deficient rats, urolithic rats (Selvam and Ravichandran, 1991; Malini et al, 2000) and stone patients (Anuradha and Selvam, 1989).
These observations have suggested that lipid per-oxidation plays an important role in hypertension. The following reports confirm that vitamin E prevents lipid per-oxidation processes in hypertension patients.

Combination of oral antioxidant supplementation reduces blood pressure in hypertensive patients via increased availability of nitric oxide. The combination of antioxidant consist of 200 mg of zinc sulfate, 500 mg of ascorbic acid, 600mg of alpha tocopherol and 30 mg beta carotene daily for 8 weeks to hypertensive patients results circulating levels of vitamin E, beta carote ne and nitric oxide increased in all subjects (Galley *et al*, 1991).

According to Barbagallo *et al* (1999) vitamin E (600 mg/day for 4weeks) supplementation increases the vitamin E, magnesium and glutathione levels in blood and decreases blood pressure of hypertensive patients.

Accumulation of lipid per-oxidation products (LPO) in erythrocyte membrane (EM) and rise in total lipid content in cellular membrane and blood plasma (BP) has been reported in hypertensive patients. The use of alpha tocopherol acetate in combination therapy promoted normalization of LPO processes in EM and reduction of BP content of the LPO products (Kuznetsov *et al*, 1994).

According to Newaz and Nawal, (1998) vitamin E may prevent development of increased blood pressure, reduce lipid peroxides in plasma and blood vessels, and enhance the total antioxidant status, including SOD activity in hypertensive rats.

### 1.13 ANTIOXIDANT ENZYMES AND ANTIOXIDANTS

Reduction of oxygen in the respiratory chain is often incomplete. Thus, some amounts of free radicals and other reactive oxygen species are continuously produced
even under normal conditions, in all organisms. In view of the disturbances that can be caused by free radicals, organisms have evolved not only antioxidant defense systems to protect against them, but also repair systems that prevent the accumulation of oxidatively damaging molecules (Halliwell and Gutteridge, 1989).

### 1.13.1 Superoxide dismutase (SOD)

Superoxide dismutases are a family of metalloproteins that catalyse dismutation of superoxide radicals.

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{(ground state)}
\]

They are present in cytosol and mitochondria of mammalian cells and provide the first line of defence against free radical damage. In the presence of superoxide dismutase, superoxide radicals cannot react with potential substrates (other than itself or its protonated form) or drive O\(_2\) dependent Haber – Weissman reaction to form OH or O\(_2\).

The cytosolic enzyme (SOD) is composed of two similar subunits, each one containing one equivalent of Cu\(^{2+}\) and Zn\(^{2+}\), whereas the mitochondrial enzyme contain Mn\(^{2+}\). Decreased SOD activity has been reported in hypertensive patients (Iarema et al, 1992; Shi, 1993; Latha et al, 1999; Koska et al, 1999; Srinivas et al, 2000; Pedro-Botet et al, 2000; Chaves et al, 2002).

### 1.13.2 Catalase

Catalase is a heme containing protein present in all animal tissues, high activity being found in liver and red blood cells. It catalyses the following reaction:

\[
2H_2O_2 \rightarrow 2H_2O + O_2
\]
In liver, catalase is mainly localised in microsomes and microperoxisomes. Its presence in cytosol and mitochondria is still doubted. It has been emphasized that because of its relatively low affinity for hydrogen peroxide, catalase plays an insignificant role in hydrogen peroxide degradation under physiological conditions (Aebi and Suter, 1966).

### 1.13.3 Glutathione peroxidase

In erythrocytes and other tissues the enzyme glutathione peroxidase, containing selenium as a prosthetic group catalyses the reduction of H$_2$O$_2$, in the presence of glutathione, to form water and oxidized glutathione. Glutathione peroxidase also reduces a vast array of other hydroperoxides including fatty acid hydroperoxides (Little and Brien, 1968).

\[
2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O}
\]

\[
2\text{GSH} + \text{ROOH} \rightarrow \text{GSSG} + \text{ROH} + \text{H}_2\text{O}
\]

GPX is found at high activity in liver, moderate activity in heart, lung, brain and erythrocytes and also activity in muscle. The ratio of GSH/GSSG in normal cells is kept high by GSSG to GSH. This is achieved by the enzyme glutathione reductase which catalyses the reaction.

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+
\]

As glutathione reductase operates and lowers the NADPH/NADP ratio, the HMP speeds up to replace the NADPH. Therefore, a decrease in reduced glutathione, resulting from a deficiency in glutathione synthetase, a decrease in glutathione reductase, or a
decrease in the enzymes of HMP shunt, will impair the function of GPX and potentially result in oxidative damage to the membrane.

1.13.4 Ascorbic acid

Ascorbic acid is characterized by the presence of a labile-hydrogen and is a two electron/hydrogen reduc-tant. It reacts with the per-oxy radical according to the following reaction:

$$\text{LOO}^\cdot + \text{Ascorbate} \rightarrow \text{LOOH} + \text{Monohydroascorbate}$$

This property of ascorbic acid makes it a good antioxidant (Packer et al, 1980). A second antioxidant role for ascorbic acid arises from its reaction with oxygen derived free radicals in presence of transition metal ions (Porter, 1980).

$$\text{O}_2^\cdot - + 2\text{H}^+ + \text{Ascorbate} \rightarrow \text{H}_2\text{O}_2 + \text{dehydroascorbate}$$

$$2\text{OH}^\cdot + \text{Ascorbate} \rightarrow 2\text{H}_2\text{O} + \text{dehydroascorbate}$$

Enzyme system exist in vivo to reduce these dehydroascorbates back to ascorbate, namely NADH – monodehydroascorbate reductase (Bendich et al, 1984).

1.13.5 Vitamin E

Vitamin E is the major chain breaking, fat soluable antioxidant is found in intercalated cellular membranes containing polyunsaturated fatty acids and is considered the first line of defense against lipid per-oxidation (Maclin, 1980), protecting cell membranes at an early stage of free radical attack through its free radical quenching activity. Vitamin E reacts directly with a variety of oxy radicals, including the peroxy radical ($\text{LOO}^\cdot$), trichloromethyl radical ($\text{CCL}_3^\cdot$) and hydroxy radical ($\text{OH}^\cdot$) (Burton et al,
1985; McCay, 1985). It also scavenges superoxide anion (O$_2$) (Foote, 1976). Reaction of $\alpha$-tocopherol radical to $\alpha$-tocopherol is achieved by the reaction with ascorbic acid (Niki et al, 1984).

Low levels of Vitamin E have been reported in hypertension (Pierdomenico et al, 1998), malabsorption syndrome such as celiac disease, biliary atresia, cystic fibrosis, cancer (Dasgupta et al, 1993), coronary heart disease (Singh et al, 1995) and abetalipoproteinemia (Fritsma, 1983). Hemolysis and a shortened lifespan of red blood cells have been reported at vitamin E plasma levels of 0.5 mg/dl, which is at the low end of the normal range (Machlin, 1984).

Low levels of Vitamin E and C are often found in patients with sustained high blood pressure, which increases the risk of coronary heart disease. It is believed that sustained hypertension forces LDL into the arterial wall and increases its risk of oxidation. Vitamin E and C are known to protect against LDL oxidation.
1.14 REVIEW OF LITERATURE

Plasma lipid peroxide, an product of lipid per-oxidation processes has been reported to be increased in hypertensive patients (Uysal et al, 1986; Shi et al, 1993; Uotila et al, 1993; Kuznetsov et al, 1994; Lacka et al, 1997; Liu et al, 1999; Latha et al, 1999; Srinivas et al, 2000; Kumar and Das, 1993; 2000) and also in animals (Newaz and Nawal, 1998), which determines the oxidative damage.

Low levels of antioxidant enzymes SOD, GPX and catalase have been reported in hypertensive patients ((Iarema et al, 1992; Jain and wise, 1995; Liu et al, 1999; Latha et al, 1999; Channinova et al, 2000; Pedro-Botet et al, 2000; Chaves et al, 2002) and animals (Newaz and Nawal, 1998).


Balta et al (1999) reported lower levels of glutathione-S-transferase in arterial hypertension.

Low levels of antioxidants such as vitamin E, C and reduced glutathione have been reported in hypertensive patients (Tse et al, 1994; Wen et al, 1996; Kumar and Das, 1993 and 2000; Wen et al, 1996; Russo et al, 1998; Srinivas et al, 2000; Block, 2001; Galvan et al, 2001).

Recently urinary 8-hydroxy-2’-deoxyguanosine (8-OhdG) has been recognized as a sensitive biomarker of oxidative DNA damage and also of oxidative stress in hypertensive patients and hypertensive rats (Negishi et al, 2000).
Patients with sustained hypertension had lower blood level of vitamin E and C, and their LDL cholesterol was more prone toward oxidation, which increases the risk of coronary heart disease (Pierdomenico et al, 1998).

Sagar et al, 1992 reported that essential hypertensive patients had lower level of superoxide dismutase and reduced glutathione in their neutrophils isolated from blood.

Increased lipid peroxidation product, conjugated dienes and malondialdehyde and increased glutathione peroxidase activity in plasma and erythrocytes were reported in hypertension complication of pregnancy by Uotila et al 1993. According to them lipid per-oxidation is an important factor in the pathogenesis of pre-eclampsia.

According to Barbagallo et al (1999) Vitmin E (600 mg/day for 4weeks) supplementation increases the vitamin E, magnesium and glutathione levels in blood of hypertensive patients.

According to Newaz and Nawal (1998) vitamin E may prevent development of increased blood pressure, reduce lipid peroxides in plasma and blood vessels, and enhance the total antioxidant status, including SOD activity in hypertensive rats.

The use of alpha tocopherol acetate in combination therapy promoted normalisation of LPO processes in EM and reduction of BP content of the LPO products (Kuznetsov et al, 1994).

Lipoprotein disorders in patients with essential hypertension are characterized by hypertriglyceridemia, especially increased VLDL, LDL and decreased HDL levels (Castelli and Anderson, 1986; Catalano et al, 1994; Haffner et al, 1992; Lemne et al, 1994; Srinivas et al, 2000).

Similarly hypomagnesuria (Tillman and Semple, 1988), hyperoxaluria and hyperuricosuria (Borghi et al, 1999), and hypocitraturia by our research group (Latha et al, 1999) have been reported in hypertensive patients.

24 hour Glycosaminoglycan and albumin reported to be increased and creatinine clearance was decreased in hypertensive patients (Francisco et al, 1999).

Measurement of serum cystatin C concentration is a more reliable marker in the assessment of glomerular filtration rate than inulin clearance in hypertensive patients reported by Seco et al, 1999.

According to Resnick et al (1997) serum total magnesium and ionised magnesium were low in hypertensive patients. Kisters et al (1998) reported that erythrocyte magnesium concentration was low and serum magnesium concentration was normal in hypertensive patients when compared with normal subjects.

Patients with hypertension reported to have decreased serum ionized calcium (McCarron, 1982; Young et al, 1992), elevated parathyroid hormone (strazzullo et al, 1983; McCarron et al, 1980; Young et al, 1990; Jesperson et al, 1997) and low serum 1,25(OH)₂ vitamin D (Young et al, 1990).
More recently, Weber et al (2001) reported that the hypomagnesemia and hypercalciuria in patients with hypertension due to impaired tubular re-absorption of magnesium and calcium in the ascending limb of Henle’s loop.

It was reported in the literature that red cell membrane calcium content was significantly increased and magnesium was decreased compared to control (Kosch et al, 2001).

Recently Calmilletti et al (2001) reported that decreased HDL cholesterol in serum and decreased nitric oxide and increased calcium in platelets of hypertensive patients.

Elevated NAG activity has been reported in essential hypertension, renovascular hypertension and hypertension with renal parenchimal disease (Kunin et al, 1978; Price, 1982; 1992; Alderman et al, 1983; Johnston et al, 1983; Simon et al, 1984).

1.15 SCOPE OF THE PRESENT INVESTIGATION

In India very little study has been done on the antioxidant status and urinary stone risk factors in the essential hypertensive patients. An association between arterial hypertension and kidney stone disease was described by Cappuccio et al (1990) and Cirillo and Laurenzi (1988). In fact, studies conducted on a general population showed that a history of kidney stone was approximately 2 or 3 times more frequent in hypertensive patients than in nonhypertensive subjects. The hypercalciuria and hyperoxaluria were regarded as the pathogenic link between hypertension and urolithiasis. More recently Selvam (2002) reported that membrane injury by free radicals facilitated the fixation of calcium oxalate crystals and subsequent growth into kidney stones.
The present investigation is aimed to study the role of Vitamin E on antioxidant status and urinary stone risk factors in essential hypertensive patients. Low levels of antioxidants (Vitamin E, C and reduced glutathione), antioxidant enzymes (SOD and GPX), increased lipid peroxide, hypercalciuria, hypocitraturia, hypomagnesuria, hyperproteinuria, elevated oxalate in 24 hrs urine and decreased creatinine clearance are monitored in hypertensive patients. Following the administration of vitamin E restore the antioxidants, antioxidant enzymes and lipid peroxides levels to near normal level. Vitamin E supplementation also restores urinary oxalate, magnesium, protein and creatinine clearance to near normal. The results are presented and discussed in the light of relevant literature.