1. INTRODUCTION AND REVIEW OF LITERATURE

1.1. UROLITHIASIS

Urolithiasis, the process of formation of stones in the kidney, bladder and/or urethra is a complex phenomenon not yet thoroughly understood. It is one of the most common urological disorders and has afflicted humans since time immemorial. It is a longstanding medical illness and still a common public health problem. A large portion of the world population suffers from urinary tract and kidney stones, formed due to deposition of calcium, phosphates and oxalates. In this process, the chemicals start accumulating over a nucleus, which ultimately takes the shape of a stone. These stones may persist for an indefinite period of time, leading to secondary complications causing serious consequences to patient’s life. It is very painful and a proper cure is very much needed to get rid of the problem (Kshetrimayum BS et al., 2013). Many parts of the world including India are now suffering from the stone diseases. It affects 20% of the general population worldwide. In the United States, up to 12% of men and 6% of women will develop urolithiasis at some point in their life. In Thailand, the highest prevalence (16.9%) was reported in the Northeast provinces, while in Middle Eastern countries, the lifetime prevalence of kidney stone is even higher. Urolithiasis usually recurs and it poses difficulty in the management and burdensome medical costs. Recurrence rates as high as 50% in 10 years have been documented (Ramello et al., 2000; Stamatelou et al., 2003). Epidemiological studies indicate many factors like age, sex, industrialization, socioeconomic status, diet and the environment influence urolithiasis. In urolithiasis, calcareous stone is the most common type of kidney stone disease. It accounts for more than 80% of all stones. The primary chemical complexes are calcium oxalate (CaOx) and calcium phosphate (CaP). Urinary stones contain both crystalloid and colloid components. The crystalloid components are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium-ammonium phosphate, uric acid and creatinin. Uric acid (UA)
stone represents about 4.5–23% and the other less frequent types of kidney stones are magnesium ammonium phosphate (MAP) or struvite stones, ammonium urate stones, cystine stones, xanthine and other miscellaneous stones (Pak et al., 1997).

In urolithiasis, formation of urinary calculi (urinary stones) occurs anywhere in the urinary system (Pearle et al., 2007). It comprises nephrolithiasis (the formation of kidney stones), ureterolithiasis (the formation of stones in the ureters), and cystolithiasis (the formation of bladder stones) (Mc Nutt., 1893). One of the common types of urolithiaisis is renal calculus also known as kidney stone which is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Kidney stones typically leave the body by passage in the urine stream. However, many stones are formed and passed without causing symptoms. If stones grow to a sufficient size (usually at least 3 millimeters, they can cause obstruction of the ureter and causes post renal azotemia and hydronephrosis (distension and dilation of the renal pelvis and calyces), as well as spasm of the ureter. This leads to pain, most commonly felt in the flank (the area between the ribs and hip), lower abdomen, and groin (a condition called renal colic). Renal colic is commonly associated with nausea, vomiting, fever, blood in the urine, pus in the urine, and painful urination. Renal colic typically comes in waves lasting 20 to 60 minutes, beginning in the flank or lower back and often radiating to the groin or genitals.
It has been reported that kidney or ureter stones occur in 1 in 20 people at some period in their life time (Figure-1). The development of the stones is usually related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate. The stones formed in the urine collecting area (the pelvis) of the kidney and may range in size from tiny to staghorn stones the size of the renal pelvis itself. The diagnosis of kidney stones is made on the basis of information obtained from the history, physical examination, urinalysis, and radiographic studies besides ultrasound examination.

**Figure 1. Stones in the kidney and ureter** (Kshetrimayum BS and Saitluangpuii S, 2013)

Since the last two decades, there has been huge effort and different studies have been undertaken from the scientific communities towards clarifying the etiology of the disease in different population and ethnic communities of the world in order to contain the disease. Safarinejad in 2007, determined the prevalence, incidence, and risk factors of adult urolithiasis in Iran in a cross sectional study. In this study, data on risk factors for urolithiasis including age, race, education, body mass index, hypertension, and current use of medication were also obtained by a self-administered questionnaire and reported that urinary stones were more in number among men and women who lived in south central and southwest counties, with odds increasing from west to east and from north to south. It was also reported that a positive association was found between urolithiasis and unemployment, consumption of tea,
consumption of cola and meat consumption. This study provides a quantitative estimate of the prevalence, incidence, and main risk factors for adult urolithiasis in the Iranian population. He further warranted for further studies in order to determine the incidence and prevalence of urolithiasis in different ethnic groups. In another study, a nationwide survey was conducted to investigate the prevalence of upper urinary calculi in Taiwan with the help of postal questionnaire and revealed that alcohol consumption and family history of kidney stone were significant risk factors for stone occurrence with reported overall prevalence being 9.6% (14.5% in males and 4.3% in females). The subtropical temperature and gradually higher socioeconomic standards of living may also contribute to the high prevalence in this region. Further, it has been reported that urinary stones from endemic patients had higher fluoride, oxalate and Ca levels than those from non-endemic patients. In vitro studies also suggested that fluoride did not influence the heterogenous mineralization of calcium oxalate and fluoride in vivo may behave as a mild promoter of urinary stone formation by (a) excretion of insoluble calcium fluoride, (b) increasing oxalate excretion and (c) mildly increasing the oxidative burden. In an interesting study, Bastian and Vahlensieck in 1975, determined the excretion of Mg, Ca, Zn and citric acid in the urine of 11 patients with urinary calculi and in 5 healthy subjects on a standard diet for a period of 5 days and found that there was a more or less distinct dependence of electrolytes and citric acid on the standard diet and the excretion of Mg and citric acid in the urine was reduced in patients with calcium oxalate stone, while that of calcium and zinc were increased. Moreover, Roswitha et al., in 2005, designed a study to evaluate the effect of dietary intervention on urinary risk factors for recurrence in calcium oxalate stone formers and suggested that nutrition is the major environmental risk factor in idiopathic calcium oxalate stone disease. In this study, the evaluation of urinary risk profiles of the patients on their usual dietary habits revealed a high risk for calcium oxalate stone formation. They also reported that a low fluid intake and an
increased intake of protein and alcohol were identified as the most important dietary risk factors and a shift to a nutritionally balanced diet according to the recommendations for calcium oxalate stone formers significantly reduced the stone forming potential. In addition to this, Fardellon et al., in 2001, administered a frequential type self-questionnaire enabling evaluation of the calcium content of the diet of an individual as well as of a given population on the basis of 20 different types of food (items) rich in calcium and/or frequently eaten in metropolitan France. The self-questionnaire with its 20 items thus offers a simple and rapid method for estimation of the daily calcium intake of a given individual to within an accuracy of 20% and provided a dietetic evaluation technique suitable for both clinical and epidemiological use. In order to determine whether kidney stone disease prevalence increased in the United States over a 20-year period and the influence of region, race/ethnicity, and gender on stone disease risk, Stamatelou et al., in 2003 measured the prevalence of kidney stone disease history from the United States National Health and Nutrition Examination Survey (II and III), population-based, cross-sectional studies, involving 15,364 adult United States residents in 1976 to 1980 and 16,115 adult United States residents in 1988 to 1994. The study reported that the prevalence of kidney stone disease history in the United States population increased between 1980 and 1994 and history of stone disease was strongly associated with race/ethnicity and region of residence.

Mithani S et al., in 2005, studied to identify difference in urinary excretion between stone formers and healthy volunteers as a metabolic factor and reported that urinary citrate excretion level among the stone patients is similar to normal volunteers and is not a predisposing factor for the lithogenesis. Boonla C et al in 2006 also investigated the composition of the urinary tract stones and prospectively identify the risk of urinary stone in Udon Thani province of Thailand and reported that high carbohydrate and low fat diet consumptions combined with low citrus fruit intake are chief dietary risks of stone
development in the population. Moreover, in an systematic study to explore the etiopathogenesis of disease in Kathmandu region of Nepal, Sanjiv R et al., in 2006, studied the qualitative composition of 47 renal stones collected from surgical patients admitted to NMCTH over a period of 13 months and suggests that calcium oxalate stones are predominant and the prevalence was very high among 20 yrs age group. Monthira et al., in 2005 also studied the epidemiology of urolithiasis in southern Thailand and revealed that oxalate and uric acid was found in all the renal calculi.

In another study, Naghii and Hedayati in 2010 studied the influence of sex hormones on the stone formation and suggested the association between serum gonadal steroids and urolithiasis in males received only limited attention and the recommendation for steroid investigation as a basic evaluation to rule out treatable systemic causes in urolithiasis patients is warranted. In addition to this, Iguchi M et al., in 1999, studied the effect of the female sex hormone on urinary stone formation by using ethylene glycol and vitamin –D induced rat urolithiasis model and suggested that the female sex hormones can inhibit renal crystal deposition in ethylene glycol treated rats by suppressing urinary oxalate excretion. Moreover, Paryani and Ather in 2002, assessed mean serum creatinine in order to confirmed whether definitive treatment of urolithiasis following relief of obstruction in patients with renal insufficiency reported in further improvement in renal function as determined by serum creatinine and reported that renal calculi and concurrent mild to moderate renal insufficiency warrants aggressive treatments and patients demonstrate significant improvement in renal function independent of relief of obstruction.

In India, urolithiasis constituted one of the commonest afflictions requiring surgical intervention and there are about 5-7 million patients suffering from urinary calculus disease. It is for these reasons that the Indian Council of Medical Research (ICMR) has classified this disease as one of the refractory diseases and stressed that efficient effort should be made to
find out the causes of the disease and to search for suitable drugs for its cure (Satyawati GV, 1982). Pushpa D et al., in 2010, analyze qualitatively the uroliths obtained by surgical intervention at Krishna hospital Karad, a South West region in Maharashtra (India), to evaluate the predominant constituent present in them reported that urolithiasis was more suffered by individuals between the age group of 30 to 60 years with more predominance in males than females. In this study, the chemical analysis of uroliths showed that all the assessed stones were of mixed heterogeneous type with magnesium ammonium phosphate (71.2%) was predominant constituent followed by calcium oxalate (68.8%), calcium carbonate (64.0%), urate (44.8%), cystine (12.8%), xanthine (2.4%) and fibrin (1.6%). The study provides simple qualitative laboratory based method for assessing chemical composition of various uroliths and a reliable diagnosis of stone contents whose data may be useful in advising the people of this region for taking preventive measures for reducing the risk of prevalence and recurrence of urolithiasis in them. Rao TVRK et al., in 2006, carried out epidemiology of the urolithiasis in the Purnia division of Bihar and analyzed the chemical composition of the stone and suggested that there is gradual increase in the urolithiasis during the past ten years and most of the stones are found to be mixed crystalloid composition containing calcium oxalate, phosphates, magnesium ammonium phosphate and ammonium urate etc. Moreover, Girija EK et al., in 2007 also analyzed the urinary calculi of population of southern Indian States and found that majority of the stones are the pure or calcium phosphate mixed calcium oxalate stones.

Jawalekar SL et al., 2010 in order to find out risk factors for urolithiasis analyzed the urinary constituents and serum parameters in the patients of urolithiasis in Maharastra and revealed the positive association between parathyroid hormone and urolithiasis. Rathee N et al., in 2004 measured fluoride content in 100 urinary stones retrieved by open surgery of stone formers admitted at PGIMS Rohtak and their respective urine and serum and compared
with those of healthy individuals. The concentration of fluoride was also measured in the sources of drinking water of these stone formers and reported that the concentration of fluoride was probably significantly higher in drinking water of these stone formers than the normal ones. This study further reported that there is a positive correlation between the content of fluoride of urinary stones and urine of stone patients, stone and serum, drinking water and stone and their urine and serum, urine and drinking water and serum and water.

On the contrary, Rajkiran et al., in 1996, studied the nutrient intake of 69 stone formers (SFs) from three subsets of the local population (urban 22, rural tribal 22 and rural nontribal 25) and 69 age, sex, weight and socioeconomically matched control subjects (NSs) (urban 20, rural tribal 22 and rural nontribal 27). The influence of dietary intake of protein, carbohydrate, fat, fiber, calcium and oxalic acid on urinary excretion of Ca, oxalic acid, uric acid, inorganic phosphorus, Mg and citric acid was examined using the chi-square test. The study reported that low nutrient intake did not influence the lithogenic process since there was no association between them. Pendse and Singh in 1996, studied fifty-two (52) cases of urinary tract calculus disease for dietary habits, routine chemical and microscopic urinalysis, bacterial culture, quantitative analysis of 24h urine sample and qualitative analysis of the stones. The results of the study strongly suggested the multifactorial etiology of stone disease in this region and reported that imbalanced nutrition and urinary tract infection were the principal risk factors for urolithiasis in this study.

1.2. TYPES AND NATURE OF STONES IN UROLITHIASIS

Urinary stones can be classified according to size, location, X-ray characteristics, aetiology of formation, composition, and risk of recurrence (Leusmann DB, 1990; Leusmann DB, 2000; Kim SC et al., 2007; Hesse A et al., 2003). Accordingly, the following types were recorded:
(i) **Stone size:** Stone size is usually given in one or two dimensions, and stratified into those measuring up to 5 mm, 5-10 mm, 10-20 mm and > 20 mm in largest diameter.

(ii) **Stone location:** Stones can be classified according to anatomical position: upper, middle or lower calyx; renal pelvis; upper, middle or distal ureter; and urinary bladder.

(iii) **X-ray characteristics:** Stones can be classified according to plain X-ray appearance which varies according to mineral composition (Kim SC *et al.*, 2007). Non-Contrast-Enhanced Computer Tomography (NCCT) can be used to classify stones according to density, inner structure and composition. Accordingly, stones were classified as Radiopaque (Calcium oxalate dehydrate, Calcium oxalate monohydrate, Calcium phosphates), Poor radiopacity (Magnesium ammonium phosphate, Apatite, Cystine) and Radiolucent(Uric acid, Ammonium urate, Xanthine and 2,8-dihydroxyadenine (Leusmann DB, 2000; Kim SC *et al.*, 2007).

(iv) **Aetiology of stone formation:** Stones can also be classified into those caused by: infection, or non-infectious causes (infection and non-infection stones); genetic defects or adverse drug effects (drug stones). Non-infection stones are calcium oxalate, Calcium phosphate (including brushite and carbonate apatite), and Uric acid while infection stones are Magnesium ammonium phosphate, Carbonate apatite and Ammonium urate. Genetic causes/Drug stones comprises Cystine, Xanthine and 2,8-dihydroxyadenine (Yasui T *et al.*, 2013).

(v) **Stone composition** - Metabolic aspects are important in stone formation, and metabolic evaluation is required to rule out any disorders and thus Analysis in relation to metabolic disorders is the basis for further diagnostic and management decisions. Stones are often formed from a mixture of substances. The clinically most relevant substances and their mineral components are calcium oxalate monohydrate (CaC$_2$O$_4$.H$_2$O), Calcium oxalate dehydrate(CaC$_2$O$_4$.2H$_2$O), Basic calcium phosphate(Ca$_{10}$(PO$_4$)$_6$.6(OH)$_2$), b-tricalcium phosphate(Ca$_3$(PO$_4$)$_2$), Carbonate apatite phosphate(Ca$_5$(PO$_4$)$_3$OH), Calcium hydrogen
phosphate (CaHPO$_4$.2H$_2$O), Calcium carbonate(CaCO$_3$), Octacalcium phosphate(Ca$_8$H$_2$(PO$_4$)$_6$.5H$_2$O), Uric acid dehydrate(C$_5$H$_4$N$_4$O$_3$), Ammonium urate(NH$_4$C$_5$H$_3$N$_4$O$_3$), Sodium acid urate monohydrate(NaC$_5$H$_3$N$_4$O$_3$. H$_2$O), Magnesium ammonium phosphate (MgNH$_4$PO$_4$.6H$_2$O), Magnesium acid phosphate trihydrate (MgHPO$_4$.3H$_2$O), Magnesium ammonium phosphate, Monohydrate (MgNH$_4$ (PO$_4$).1H$_2$O), Cystine ([SCH$_2$CH (NH$_2$) COOH]$_2$), Gypsum, 2,8-dihydroxyadenine, Proteins, Cholesterol, Calcite, Potassium urate, Trimagnesium phosphate, Melamine, Matrix, Drug stones.

(vi). Stone classification based on chemical composition: Urinary stones are composed of a combination of crystals (both inorganic and organic) and proteins. Calcium-based stones, which include calcium oxalate monohydrate, calcium oxalate dihydrate, and calcium phosphate stones, account for 70%–80% of upper urinary tract stones. Struvite stones account for 5%–15% of stones and are composed of magnesium ammonium phosphate. In contrast, uric acid stones are unique in that they can often be dissolved with urinary alkalinization; they account for 5%–10% of stones and occur in acidic urine (pH <5.8). Other stones, including cystine, xanthine, and protein matrix stones, as well as drug (eg, triamterene, indinavir)–induced calculi, account for less than 5% of stones (Moe OW, 2006; Sandhu C et al., 2003). Major types of stones according to the chemical components are calcium stones (70-80%), Uric Acid stone (5-10%), Cysteine stone (1%), Struvite (1%), xanthine stone (1%), Mixed stones (50-60%) while major subtypes are calcium oxalate monohydrate (40-60%), calcium oxalate dehydrate (40-60%), calcium hydrogen phosphate( brushite) (2-4%), calcium orthophosphate (<1%), mixed calcium oxalate-phosphate (35-40%) and mixed uric acid- calcium oxalate (5%).( Barnela et al., 2012).
1.3. **CAUSATIVE FACTORS OF UROLITHIASIS**

Urolithiasis often has no definite, single cause, although several factors may increase the risk. It may be linked to environmental, genetical and dietary factors etc. Kidney stones form when urine contains more crystal-forming substances — such as calcium, oxalate and uric acid — than the fluid in urine can dilute. At the same time, urine may lack substances that prevent crystals from sticking together, creating an ideal environment for kidney stones to form. Factors that increase risk of developing kidney stones include:

(i). **Genetic factors and candidate genes for Urolithiasis**: Genetic factors are known to play a major role in urolithiasis. Studies have tried to identify genes related to ureter calculi in an effort to clarify the cause of urolithiasis and to advance the diagnosis and treatment of urolithiasis (Danpure CJ., 2000; McGeown MG., 1968). It has been reported that the use of single-nucleotide polymorphisms (SNPs) associated with genetic diseases has been fruitful in identifying candidate disease genes. Recent genetic advances in urolithiasis indicate the potential of a new approach towards the gene polymorphism (Resnick M et al., 1968; Goodman HO et al., 1995; Chen WC et al., 2001a; Chen WC et al., 2001b) Moreover, polymorphism in manganese superoxide dismutase gene (Mn-SOD) is a new approach to identify its probable association with urolithiasis through oxidative stress. MnSOD is one of the primary enzymes that directly scavenge poten-tial harmful oxidizing species. It has been reported that A valine (Val) to alanine (Ala) substitution at amino acid 16, occurring in the mito-chondrial targeting sequence of the MnSOD gene, has been associated with an increase in urolithiasis risk (Tugcu et al., 2007). Moreover, number of studies has been carried out by many scientists in many parts of the world to identify the probable candidate genes responsible for urolithiasis. Some of the results as reported from the studies done by scientists are shown below:
<table>
<thead>
<tr>
<th>Candidate Genes</th>
<th>Type of patients</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>TaqI and ApaI gene polymorphism</td>
<td>Calcium stone patients</td>
<td>Nishijima et al. 2002</td>
</tr>
<tr>
<td>BsmI endonuclease polymorphism</td>
<td>Calcium oxalate stone patients</td>
<td>Wen-Chi Chen et al. 2001</td>
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<tr>
<td>VEGF gene BstUI polymorphism</td>
<td>Calcium oxalate stone patients</td>
<td>Chen et al. 2001a</td>
</tr>
<tr>
<td>FokI and TaqI VDR genes polymorphism</td>
<td>Calcium oxalate stone patients</td>
<td>Mittal et al. 2010</td>
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Numerous studies have been dedicated to interpreting the possible association between the polymorphisms of genes and urolithiasis susceptibility. However, the results were remained inconclusive. The controversial results across many of these studies could possibly be related to the small sample size from an individual study, ethnic difference or the biological genetic model applied for the analysis. Therefore, it was necessary to quantify the potential between-study heterogeneity and summarize results from all eligible studies with rigorous methods. In view of this, very recently, Yiwei Lin et al. 2011 carried out a meta-analysis with the most updated data in order to revisit the association between VDR (vitamin-D receptor) variants (i.e., ApaI, BsmI, FokI and TaqI) and urolithiasis risk and reported that some VDR gene polymorphisms are associated with an increase in the probability of urolithiasis with certain populations under an indicated genetic model. Considering the
predictive value, this meta-analysis study also warrants further investigation in this field to
to better clarify these SNP-urolithiasis associations and to reinforce their findings.

(ii). **Family or personal history:** There is a higher risk of developing kidney stones if a
member of the family already has it.

(iii) **Dehydration:** Not drinking enough water each day can increase risk of kidney stones.
And it has been reported that people who live in warm climates and those who sweat a lot
may be at higher risk than others.

(iv) **Certain diets:** Eating a diet that's high in protein, sodium and sugar content may
increase risk of some types of kidney stones. This is especially true with a high-sodium diet.
Too much sodium in the diet increases the amount of calcium in the kidneys significantly
increases risk of kidney stones.

(v) **Being obese:** It has been reported that High body mass index (BMI), large waist size and
weight gain have been linked to an increased risk of kidney stones.

(vi) **Digestive diseases and surgery:** Gastric bypass surgery, inflammatory bowel disease
or chronic diarrhea can cause changes in the digestive process that affect absorption of
calcium and water and thus increasing the levels of stone-forming substances in urine.

(vii) **Other medical conditions.** Diseases and conditions that may increase risk of kidney
stones include renal tubular acidosis, cystinuria, hyperparathyroidism, certain medications
and some urinary tract infections.

1.4. **INFLUENCE/ROLE OF DIET IN UROLITHIASIS**

Urolithiasis as a multifactorial recurrent disease has various etiological and risk
factors. It includes both intrinsic factors such as demographic, anatomic and genetic
disposition, and extrinsic factors such as geographic predilection, climatic condition, lifestyle
predilection as well as dietary habits. Understanding how the stone forms *in vivo* will herald
means to prevent its development. Dietary factors play an important role in kidney stone
formation, and dietary modification can reduce the risk of stone recurrence. Super saturation of urinary lithogenic promoters such as calcium, oxalate, phosphate and uric acid are considered as the risk factors of renal stone formation. On the other hand, a marked decrease in the urinary concentration of stone inhibitors such as citrate, potassium and magnesium is also a critical risk. And the urinary levels of these stone modulators are greatly influenced by diet (Siener et al., 2003; Siener et al., 2005).

Though only 10% to 20% of urinary oxalates come from dietary sources (Morton, Iliescu & Wilson., 2002), dietary reduction is commonly advised for calcium oxalate stone formers. It has been suggested that because there is much less oxalate in the urine than calcium in the urine, urinary oxalate concentration is much more critical to the formation of calcium oxalate crystals than is the urinary calcium concentration; reducing urine oxalates may have a more powerful effect on stone formation than can reduction of urine calcium (Morton et al., 2002). Patients with calcium oxalate stones, particularly those with documented hyperoxaluria, should avoid foods high in oxalates. Vitamin C is a precursor to endogenous production of oxalates, so some clinicians recommend avoiding mega-doses of vitamin C. The rare genetic condition of primary hyperoxaluria is only slightly impacted by dietary reduction, and causes serious medical problems besides kidney stones. The effect of excess animal protein (purine) is also most obvious for the uric acid stone former. Uric acid, a byproduct of purine metabolism, is excreted in large quantities in the urine. Excess protein consumption creates urine leading potentially super saturation of uric acid, and a low pH, a necessary condition for formation of uric acid stones. There is no inhibitor of uric acid crystal formation (Menon & Resnick., 2002), so dietary measures focus on reducing uric acid and increasing urine volume. In this regard, reduction of animal protein to 350 gram per day for adults is recommended. This is enough to meet the dietary needs of most population in India, many of whom typically consume several more grams of animal protein daily than the
recommended level. Protein from plant sources (beans, legumes, etc.) can be substituted as a dietary alternative without negative consequences however calcium oxalate stone formers reducing their animal protein should also take into consideration the oxalate content of substitute proteins. The role of excess protein in promoting calcium stone formation is equally important as high dietary protein is associated with increased urinary calcium. Thus, there is a link between meat consumption and both uric acid and calcium stone formation. In fact, vegetarians form stones at one-third the rate of those eating a mixed diet (Lemann J., 2002). Clearly, the benefits of protein restriction for stone formers are many. The eating habits of people can make them prone to suffer from higher risks of having cases of Urolithiasis. Studies have been done on many places where it is found that in places where the rate of Urolithiasis is high, the diet of the people are usually to be blamed.

Epidemiological studies and metabolic investigations of the chemical composition of urine have suggested that a number of nutrients may influence the formation of stones in the upper urinary tract. These stones, which are predominantly calcium oxalate in composition, are more common in affluent countries where there is relatively high consumption of protein and fat and low consumption of carbohydrate (Anderson DA., 1972). The availability of calcium for stone formation depends ultimately on the dietary intake, intestinal absorption, excretion in the faeces, transport across cell membranes from the extracellular to the intracellular components of the body fluids and the renal tubular reabsorption of calcium.

With regard to Urolithiasis, the lithogenic potential of dietary protein is believed to be through different mechanisms. It results from a combination of a higher renal load of lithogenic substances, and a tendency towards their increased precipitation in the kidneys. Animal proteins are rich in sulfur-containing amino acids such as cystine and methionine. Oxidation of sulfur to sulfate generates acid load that aggravates calcium mobilization from bones (Arnett TR., 2008). Further, calcium forms soluble complex with sulfate generated
from the oxidation of sulfur in proteins. The acid load increases calcium mobilization from
the bones, and causes hypercalciuria and low urine citrate levels, which is considered the
strongest complexing agent for calcium in urine (Sharma AP et al., 2007). The acid load also
implemented decreases renal tubular reabsorption and imposes an additional risk for negative
calcium balance and osteopenia, since urinary calcium excretion raises further (Robertson
WG et al., 1982; Goldfarb S, 1988). In addition, chronic over consumption of animal protein
may increase renal mass and thereby up-regulate calcitriol production (Hess B et al., 1995).
This contributes to hypercalciuria by down-regulating parathyroid hormone secretion with
subsequent reduction in renal calcium reabsorption (Hess B et al., 1995).

It has been also reported dietary sodium increases the risk of urolithiasis. Salt intake
expands intravascular volume, which can increase urinary calcium level, likely by decreasing
renal tubular calcium reabsorption. Increased in salt intake can also induced mild systemic
metabolic acidosis, this can lower urinary citrate levels, and increases the risk of calcium
precipitation in kidneys (Sakhaee K et al., 1993). Further, sodium intake is another
significant dietary risk factor for kidney stone disease and hypercalciuria because urinary
sodium excretion is directly correlated with urinary calcium excretion, such that increasing
the excretion of one leads to an increase in excretion of the other. High sodium intake
contributes to stone formation in several ways; first it increases the urinary calcium level by
reducing renal tubular reabsorption of calcium. Second, high sodium intake can cause a mild
reduction in urinary citrate level by provoking mild bicarbonaturia and metabolic acidosis.
Third it can increase urinary saturation of monosodium urate, causing urate induced calcium
oxalate crystallization (Breslau NA et al., 1982).

On the other hand, potassium-rich foods decreases stone formation through a decrease
in urinary calcium excretion & high urinary potassium is believed to increase renal tubular
phosphate absorption and consequently inhibit 1, 25- dihydroxyvitamin synthesis which
slows intestinal calcium absorption (Osorio AV et al., 1997). Potassium rich foods offer the additional advantage of high citrate content thus decreasing the precipitation of urinary calcium (Srivastava T et al., 2005). There are studies which reported that urine specific gravity, which measures urine density function, may be a better indicator of urinary dilution and a stronger predictor of stone formation and linked to diet one’s consumed (Chen Y et al., 2001). It was also suggested that maintaining urine specific gravity below a certain level might reduces the occurrence of urinary stones, along with appropriate fluid intake. A diet high in animal proteins results in higher acid excretion and lower urinary pH compared with a vegetarian diet and the formation of calcium phosphate crystals is highly dependent on the urinary pH (Robertson WG et al., 1975), where as the formation of calcium oxalate in a solution seems to be independent of variations in pH within the physiological range.

Correlation of the dietary pattern with the incidence of kidney stone disease in the Indian subcontinent had revealed that kidney stone occurred more frequently in the areas where the staple diet has been wheat than among the rice eaters (Teotia M et al., 1976). Whole-wheat flour when consumed as a staple food leads to the production of urine supersaturated with uric acid, which appears to be an essential pre-requisite for the formation of primary stone. In addition to this increased animal-protein intake increases the excretion of uric acid and calcium and lowers urinary citrate excretion, all of which predispose a person to the formation of calcium stones (Gary C Curhan et al., 1993). Increased uric acid saturation may favour the nucleation and growth of calcium oxalate and / or ammonium acid urate by blocking the action of acid mucopolysaccharide inhibitors. Another possibility could be that whole wheat flour when eaten as a staple food for a long period may lead to the production of some unknown chemical factor that inhibits the reabsorption of uric acid in the proximal renal tubule and thus causes increased urinary concentration of uric acid. Whether or not such a mechanism is genetically determined requires further studies (Teotia M et al., 1976).
Dietary protein intake also increases net fixed acid production and acid excretion, thus inhibiting renal tubular reabsorption of calcium and imposes an additional risk for negative calcium balance and osteopenia, since urinary calcium excretion rises further (Lemann J *et al.*, 1986).  

It has also been reported that vegetarian diets have been associated with increase excretion of calcium, oxalate and uric acid and a significant increase in oxalate excretion. Dietary intake of oxalate also varies among individuals based on food choices and it is especially high in vegetarians because green leafy vegetables contain large amounts of oxalate. On the other hand Ca restriction increases the absorption of oxalate in the gastrointestinal tract leading to an increase of urinary oxalate excretion suggesting that the inverse relation between dietary calcium and kidney stones may be due to increased binding of oxalate by calcium in the gastrointestinal tract. In this case, urinary oxalate may be more important than urinary calcium for stone formation, because calcium oxalate saturation of urine increases rapidly with small increases in the oxalate concentration. In this aspect, calcium restriction could actually be harmful in that it may lead to increased urinary oxalate excretion (Borsatti A, 1991).

Increase intake of Mg is favorable as it decreases calcium absorption and increases magnesium absorption which as an inhibitor reduces risk factors of the disease. Mg is a divalent cation is a complexing agent for oxalate. Magnesium inhibits oxalate absorption and excretion thus prevents its supersaturation. Normally magnesium is complexed with calcium as well as oxalate and decreases its excretion. Thus decreased magnesium in nephrolithiasis results in increased urinary oxalate level, as sufficient magnesium is not available to form the magnesium oxalate complex (Schwille PO *et al.*, 1999). Intake of increased phosphorus has an effect it decreases calcitriol production and enhance urinary excretion of a natural inhibitor of oxalate precipitation, pyrophosphate. Phosphorus has also been shown to partially protect
the kidney against calcium-induced damage. Phosphorus acts as a urinary acidifier and helps prevent stones from forming in the kidney (Tieder M et al., 1985).

Majority of urinary stones contain oxalate and it seems logical to restrict dietary oxalate to lessen the stone risk. Dietary oxalate contributes to 10-20% (more recent studies suggest up to 80%) of urinary oxalate and the rest comes from body metabolism. Reduction in dietary oxalate is a standard recommendation to individuals with calcium oxalate stone disease. Diets rich in oxalate are spinach, rhubarb, beetroot, beet, cocoa, chocolates, coffee and tea, nuts (pea nut, almond, and cashew), asparagus, cumin seed, cranberry, raspberry, dried beans, bran flakes, wheat bran, and strawberries. Other high-oxalate foods are grits, bran cereals, berries, figs, citrus peels, kiwis, tangerines, green leafy vegetables, okra, olives, beans, parsley, zucchini, potatoes and sweet potatoes, peppers, eggplant, black pepper, marmalade and soy sauce. Beverages with oxalate include coffee, chocolate milk and hot chocolate, dark beers, black tea, soy drinks and juices made out of high-oxalate fruits.

There was also report of some dissolved chemicals present in the drinking water could be an etiological factor for this disease. Siener in 2006 reported that for a majority of male kidney stone patients (88.46 per cent), the source of water was hand pump (groundwater). Only 2.56 per cent and 8.98 per cent of males were using water from tap (surface water) and babdi (stagnant water), respectively. In case of females kidney stone patients, 61.54 per cent were using water from hand pump while 28.85 per cent were using tap water. 9.61 per cent were still dependent on babdi for drinking water. Different rocks contain different minerals and groundwater is always in contact with these rocks, for example, sandstone, limestone and basalt and minerals. As a result, groundwater often contains more dissolved minerals than surface water. Consumption of groundwater may be one of the reasons of stone formation in patients (Siener R., 2006).
1.5. **OXIDATIVE STRESS AND UROLITHIASIS**

Oxidative stress is defined as a “state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them”. In other words, oxidative stress is the general phenomenon of oxidant exposure and antioxidant depletion or oxidant-antioxidant balance. It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. During normal oxidation process in the body, oxygen combines with reduced molecules, such as carbohydrates or fats, and provides energy and when there is decreased oxidation or decreased energy production, the cells can no longer function efficiently and disease results. However, this normal process propagates short-lived intermediates known as free radicals, and some free radicals escape and initiate further oxidation setting up a chain reaction. So, potentially harmful reactive oxygen species are produced as a consequence of biological metabolism, and by exposure to environmental factors. Free radicals are then usually removed or inactivated by a team of natural antioxidants which prevent these reactive species from causing excessive cellular damage.

Although reactive oxygen/nitrogen species (ROS/RNS) play an important role in immunemediated defense against invading microorganisms and serve as cell-signalling molecules, a high concentrations of ROS/RNS are capable of damaging host tissues and can modify or damage DNA, lipids, and proteins. In human body, ROS/RNS levels are controlled through an intricate network of endogenous and exogenous antioxidant molecules that are responsible for scavenging and consumption of specific reactive species. In this regard, intake of dietary antioxidants has received much attention, with the concept being that these molecules can affect disease by modulating the biological reactivity of free radicals.

In humans, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms (Peter H Proctor., 1989; Peter Proctor et al.,
These include cancer (Halliwell B., 2007), Parkinson's disease, Alzheimer's disease (Valko M et al., 2007), atherosclerosis, heart failure (Singh N et al., 1995), myocardial infarction (Ramond A et al., 2011), Schizophrenia (Proctor P, 1972; Boskovic M et al., 2011) Bipolar disorder (Rodrigo MV et al., 2008; Dean OM et al., 2011), fragile X syndrome (Diego OY et al., 2009), Sickle Cell Disease (Amer J et al., 2006), lichen planus (Aly DG et al., 2010), vitiligo (Arican O et al., 2008), autism (James SJ et al., 2004), and chronic fatigue syndrome (Gwen Kennedy et al., 2005).

From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs. In addition to this, oxidative stress has not only a cytotoxic effect, but also plays an important role in the modulation of messengers that regulate essential cell membrane functions, which are vital for survival. It affects the intracellular redox status, leading to the activation of protein kinases, including a series of receptor and non-receptor tyrosine kinases, protein kinase C, and the MAP kinase cascade, and hence induces various cellular responses. These protein kinases play an important role in cellular responses such as activation, proliferation, and differentiation, as well as various other functions. Accordingly, the protein kinases have attracted the most attention in the investigation of the association between oxidative stress and disease. In addition to this, oxidative stress may also be involved in the development of stone formation in the renal system. Manganese superoxide dismutase (MnSOD) is one of the primary enzymes that directly scavenge potentially harmful oxidizing reactive oxygen species. It has been reported that a valine (Val) to alanine (Ala) substitution at the site of amino acid 16, in the mitochondrial targeting sequence of the MnSOD gene, has been associated with an increase in Urolithiasis risk (Tugcu et al., 2007). For most human diseases, increased formation of reactive oxygen species is a secondary to primary disease process.
Similarly association of Urolithiasis and free radicals has been reported (Selvam and Kalaiselvi, 2001). Experiments performed on animals (Muthukumar and Selvam., 1997), cultures (Thamilselvan et al., 2003) and human sera (Singh and Barjatia, 2002) have revealed that there is an enhanced oxidative stress in stone forming conditions. Oxalate is known to induce lipid peroxidation by unknown mechanism which causes disruption of the structural integrity of the membranes (Thamilselvan et al., 1997). The levels of serum malondialdehyde, nitrite, α-tocopherol, plasma ascorbate and erythrocyte superoxide dismutase are biomarkers for the pathogenesis of urolithiasis (Pillai and Pillai, 2002).

For most human diseases, oxidative stress characterized by increased formation of reactive oxygen species and a state of damage caused by reactive oxygen species (ROS) is considered secondary to primary disease process (Halliwell B., 1991). Similarly, an association of enhance oxidative stress and stone forming conditions have been reported both in animals (Selvam and Kalaisel., 2001) and human studies (Singh PP and Barjatia., 2002). One of the consequences of this is manifested in the formation of lipid peroxides (LPO) in cell membranes, resulting in dysfunction of the same. There are studies in experimental animals which reported induction of lipid peroxidation and acute phase proinflammatory cytokines (IL-1, IL-6, and TNF-α) by oxalate and oxalate load which causes disruption of the structural integrity of the membranes (Thamilselvan S and Khan SR., 1997) and of which free radical plays a prominent role. Given the higher reactivity of the ROS, living things have been developed several efficient mechanisms that enable stabilization and disposal of them to mitigate their harmful effects. Among them are inbuilt antioxidant scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and small molecular antioxidants such as reduced glutathione (GSH).
1.6. FREE RADICALS

The role of free radical is gaining worldwide attention since so many physiological and pathophysiological phenomenons are related to redox status of the cell. A free radical is any atom or molecule that contains one or more unpaired electrons. The unpaired electron alters the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding nonradical (Halliwell and Gutteridge, 1989). Despite being essential for most form of life, the high content of O₂ in atmosphere means that oxidation reactions are common place in our environment. Although, our body uses O₂ and oxidation reactions to good effect for generating energy and killing invaders, unwanted side reaction are unavoidable. Therefore to support the aerobic metabolism, mechanisms had to evolve for the biological control of O₂. One such mechanism involves its complete reduction to water which produces free radical O²⁻ by 1 electron reduction of molecular oxygen as the first intermediate in this pathway (Darley-Usmar et al., 1995). Free radicals are also molecules that contained an unpaired electron in its outer orbit and that can exist independently. Molecular oxygen is a diradical, containing 2 unpaired electrons with parallel spin configurations. Because electrons must have opposite spin to occupy the same orbit, electron added to molecular oxygen must be transferred one at a time during its reduction (Sen, 1995; Yu, 1994) resulting several highly reactive intermediates (Yu, 1994). The complete reduction of oxygen to H₂O requires 4 steps and the generation of several free radicals and H₂O₂. H₂O₂ is not free radical in itself because it contains no unpaired electrons. H₂O₂ is however considered as reactive oxygen species because of its ability to generate highly reactive hydroxyl free radicals through interactions with reactive
transition metals. The complete reduction of oxygen is summarized in the following equations:

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^- \text{ Superoxide radical} \ldots \ldots \ldots (i) \]

\[ \text{O}_2^- + \text{H}_2\text{O} \rightarrow \text{HO}_2^- + \text{OH}^- \text{ Hydroperoxyl radical} \ldots \ldots \ldots (ii) \]

\[ \text{HO}_2^- + e^- + \text{H} \rightarrow \text{H}_2\text{O}_2 \text{ hydrogen peroxide} \ldots \ldots \ldots (iii) \]

\[ \text{H}_2\text{O}_2 + e^- \rightarrow \text{OH} + \text{OH}^- \text{ hydroxyl radical} \ldots \ldots \ldots (iv) \]

Each of these oxygen derived intermediates is considered highly reactive because its unstable electron configurations allow for the attraction of electrons from other molecules resulting another free radicals that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to the lipid peroxidation (Hochstein and Ernsster, 1963), DNA damage (Kasai et al., 1986) and protein degradation (Griffith et al., 1988) during oxidatively stressful events. Although all the intermediates are potentially reactive, the intermediates vary in their biological importance. The superoxide radical (\( \text{O}_2^- \)) is the most well known oxygen derive free radicals and unlike other free oxygen radicals can lead to the formation of other additional species (Harris, 1992). Hydrogen peroxide although not a free radicals by definitions is biologically important oxidant because of its ability to generate the hydroxyl radicals, which are extremely potent radicals (Aruoma and Halliwell, 1987). Further because of its nonionized and low charge state, \( \text{H}_2\text{O}_2 \) is able to diffuse through hydrophobic membranes as seen with leakage of \( \text{H}_2\text{O}_2 \) from mitochondria (Yu, 1994). The hydroxyl radicals are formed not only by the reduction of hydrogen peroxide but also through the interaction of superoxide with hydrogen peroxide and the reduced form of the metal ions i.e copper and zinc (Ross and Moldeus, 1991). The ability of hydroxyl radical to
remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems.

Free radicals are very unstable due to their high reactivity (Paolisso et al., 1996; Halliwell and Gutteridge, 1989). Because of their nature, they have short life time and are difficult to measure and accurately determined in vivo as well as in biological materials such as plasma or other fluids (Granic, 2001; Sano et al., 1998; Borcea et al., 1999). In clinical states, their existence is determined by their influence on other molecules or antioxidant mechanisms which they cause (Borcea et al., 1999). It is more reliable to measure the consequence of their action, mostly decreased level of antioxidant enzyme activity (Bambolkar and Sainani, 1995).

Free radicals formation occurs continuously in cells as a consequence of the both enzymatic and non enzymatic reactions. Enzymatic reactions which serve as sources of free radicals include those involved in the respiratory chain, phagocytosis, prostaglandin synthesis and in cytochrome P-450 system. Free radicals also arise in non enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. Some internally generated sources of free radicals are mitochondria, phagocytes, xanthene oxidase reaction involving iron and other transition metals, arachidonate pathways, exercise, inflammation. Some externally generated sources of free radicals are cigarette smoke, environmental pollutants, radiation, ultraviolet light, certain drugs, pesticides, aneaaesthetic and industrial solvents and ozone (Langseth, 1996).

1.7. LIPID PEROXIDATION

Lipid peroxidation (LPO) is a complex process whereby polyunsaturated fatty acids (PUFAs) in the phospholipids of cellular membranes undergo reaction with oxygen to yield lipid hydroperoxides (LOOH). The reaction occurs through a free radical chain mechanism
initiated by the abstraction of a hydrogen atom from a PUFA by a reactive free radical, followed by a complex sequence of propagative reactions. The LOOH and conjugated dienes that are formed can decompose to form numerous other products including alkanals, alkenals, hydroxyalkenals, malondialdehyde (MDA) and volatile hydrocarbons (Halliwell B et al., 1989). LPO is often the first parameter to confirm the involvement of free radicals in cell damage as LPO is an extremely likely consequence if a reactive free radical is formed in a biological tissue where PUFAs are generally abundant and extremely demaging to cells. Moreover, a vast array of analytical techniques has been developed to measure lipid peroxidation, though not all of them are applicable to the situation in vivo (Cheeseman K, 1989).

For all assays it’s important that artifactual changes in lipid peroxidation products are minimized both during and after sampling. Radicals scavenging antioxidants and metal-chelating agents are added to prevent the further formation of lipid hydroperoxides and the breakdown of existing lipid hydroperoxides. Enzymic reactions that may affect levels of products are inhibited by mixing the sample with acid or organic solvents. It is generally advisable to assay samples as quickly as possible after taking them, since a tendency to increased lipid peroxidation on storage has been reported (Young IS et al., 1991; Duthie GG et al., 1992). Conversely, lipid hydroperoxides can deteriorate on storage (Holley A et al., 1991).

The lipid peroxidation’s reaction in biological membranes causes impairment of membrane functioning (Slater TF et al., 1971; Comporti M, 1987), decreases fluibility, inactivation of membrane-bound receptors and enzymes and increases non-specific permeability to ions such as Ca$^{2+}$. Additionally, lipid hydroperoxides decompose upon exposure to iron or copper ions, simple chelates of these metal ions (e.g. with phosphate esters), haem, and some iron proteins, including haemoglobin and myoglobin. Products of
these complex decomposition reactions include hydrocarbon gases (such as ethane and pentane), radicals that can abstract further hydrogen atoms from fatty acid side chains and cytotoxic carbonyl molecules, of which the most harmful are the unsaturated aldehydes such as 4-hydroxy-2-trans-nonenal. Indeed, a major contributor to extracellular antioxidant defence in mammals is the existence in body fluids of proteins that bind copper ions (caeruloplasmin and albumin), iron ions (transferrin), haem (haemopexin) or haem proteins (haptoglobins) and stop them from accelerating lipid peroxidation and other free radical reactions (Kappus H, 1987; Gutteridge JMC et al., 1988).

The measurement of putative “elevated end products of lipid peroxidation” in human samples is probably the evidence most frequently quoted in support of the involvement of free radical reactions in tissue damage by disease or toxins. Studies beginning in the 1950s provided good evidence that several halogenated hydrocarbons exert some, or all, of their toxic effects by stimulating lipid peroxidation in vivo. This early choice of halogenated hydrocarbons for study was both casual (in that it gave early emphasis to the important biological role of free radical reactions) but also unfortunate, since later studies have shown that most toxins stimulating oxidative damage to cells do not appear to act by accelerating the bulk peroxidation of cell membrane lipids (Gutteridge JMC et al., 1988):

\[ \text{toxin} \rightarrow \text{lipid peroxidation} \rightarrow \text{cell damage} \]

In the process of LPO, oxidation of lipids can be measured at different stages, including:

a. Losses of unsaturated fatty acids;

b. Measurement of primary peroxidation products and


Between phases a, b and c it is possible detect carbon-and oxygen-centered radicals and identify these radicals by their ESR spectra (Halliwell B, 1989).
LPO, however, is often a late event, accompanying rather than causing final cell death. Indeed, cell and tissue destruction whether mediated by radicals or otherwise can often lead to more lipid peroxidation because antioxidants are diluted out and transition metal ions that can stimulate the peroxidation process are released from disrupted cells (Halliwell B, 1987). This stimulation of lipid peroxidation as a consequence of tissue injury can sometimes make a relevant contribution to worsening the injury. For example, in atherosclerosis there is good evidence that lipid peroxidation occurs within the atherosclerotic lesion and leads to foam cell generation and hence lesion growth (Halliwell B et al., 1984). In traumatic injury to the brain and spinal cord, good evidence again exists that iron ion release into the surrounding area, and consequent iron-stimulated free radical reactions, worsen the injury (Steinberg D et al., 1989). It is equally likely that in some other diseases, the increased rates of free radical reactions induced as a result of tissue injury make no significant contribution to the disease pathology. Each proposal that free radicals in general or lipid peroxidation in particular, are important contributors to the pathology of a given disease must be carefully evaluated on its merits. This obviously requires accurate methodology for measuring these processes in cells, tissues and whole organisms. It should be noted that the chemical composition of the end products of peroxidation will depend on the fatty acid composition of the lipid substrate used and upon what metal ions (if any) are present.

1.8. ANTIOXIDANT ENZYMES (SUPEROXIDE DISMUTASE AND CATALASE)

During the evolution, aerobic organisms have developed protection and defense mechanisms against oxidants and free radicals. The antioxidant systems include numerous enzymes and non enzyme type antioxidant groups that are located in the cell and extracellular fluid. It has been speculated that the susceptibility of an organisms to oxidative damage is influenced by the antioxidant defense system’s
ability to cope with stress, which in turn can be influenced by the nutritional intervention with antioxidants (Chow, 1988). Inherent antioxidant defense system consisting of enzymes such as catalases and superoxide dismutase etc. and nutrients may participate in coping oxidative stress (Wills, 1985; Machlin and Bendich, 1987; Leibovitz et al., 1990). As antioxidant enzymes have important role in protection against free radical damages, decrease in the activities or expression of these enzymes may predispose tissues to free radical damage (Hwang and FWu, 1993; L’Abbe et al., 1991).

Antioxidant enzymes dependent defences play an important role in scavenging free radicals produced under oxidative stress (Harris, 1992). In mammalian cells, there are several mechanisms in which organisms defend themselves against oxidative stress. Among them, there are small molecular antioxidants such as reduced glutathione (GSH) and antioxidant scavenging enzymes such as cellular Cu, Zn-superoxide dismutase (SOD), catalase (CAT), cellular glutathione peroixdase (GPx) and Glutathione-s-transferase (Kasapoglu and Ozen, 2001; Touyz, 2000). Sato et al., (2002) postulated that SOD is the key enzyme in protecting the vessel wall against oxidative stress. Organisms, depending on the oxidative metabolism, have evolved number of enzymes to reduce O$_2^-$ which is formed as an intermediate. SOD is one such enzyme which catalyzes the reaction of O$_2^-$ with an electron and 2 protons to form H$_2$O$_2$. Three mammalian SODs have so far been identified: copper- zinc SOD (SOD-1), MnSOD (SOD-2) and extracellular SOD (SOD-3). The three SODs gene have been characterized and cloned (Groner et al., 1985; Wan et al., 1994; Folz and Crapo, 1994). SOD is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. SOD catalyzes the destruction of the O$_2^-$ free radical.
\[ 2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \]

It protects oxygen-metabolizing cells against harmful effects of superoxide free-radicals (Petkau et al. 1975; Fridovich 1972, 1973; Lavelle et al. 1973; Paschen and Weser 1973). McCord in the year 1974 reported that SOD protects hyaluronate against depolymerization by free-radicals and indicated that exogenous SOD might have an anti-inflammatory effect (Salin and McCord 1975). The O₂⁻ ion, which has been considered important in aging, lipid peroxidation and the peroxidative hemolysis of red blood cells is formed by the univalent reduction of O₂ during various enzymatic reactions or by ionizing radiation (Fee and Teitelbaum 1972, Fee et al. 1975). In addition to this, there is also superoxide radical formation during leukocyte phagocytosis indicating that SOD deficiency might lead to Heinz body hemolytic anemia (Allen et al., 1974; DeChatelet et al., 1974; Dionisi et al., 1975; Winterbourn et al., 1975).

SOD is also reported to be found in both the dermis and the epidermis and is the key to the production of healthy fibroblasts. Studies have shown that SOD acts as both an antioxidant and anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes. Researchers are currently studying the potential of SOD as an anti-aging treatment, since it is now known that SOD levels drop while free radical levels increase as we aged. There are two types of SOD: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. Cu/Zn SOD protects the cytoplasm of the cell, and Mn SOD protects their mitochondria from free radical damage. It has been reported that abnormalities in the Cu-Zn SOD gene may contribute to the development of Amyotrophic Lateral Sclerosis (ALS), or Lou Gehrig’s disease, in some people. ALS is a fatal disease that causes deterioration of motor nerve cells in the brain and spinal cord. It has been theorized that low levels of SOD in those with ALS leaves nerve cells unprotected from the free radicals that can kill them, so researchers have
been studying the effect of vitamin E and other antioxidant supplements on the progression of this disease. It was hoped that regular doses of antioxidants could make up for the lack of SOD and help neutralize free radicals. Initial studies were promising, and indicated that vitamin-E supplementation could potentially slow the progression of ALS, with some researchers claiming that the risk of death from ALS was as much as 62 percent lower in regular vitamin E users compared to nonusers.

CAT on other hand is important in antioxidant defense against hydrogen peroxide (Robertson et al., 2003; Hansen et al., 1999). The tretameric peroximal catalase converts H$_2$O$_2$ to water and molecular oxygen and in the presence of H$^+$ donors it facilitates the reduction of organic hydroperoxides. CAT gene expression is regulated by H$_2$O$_2$. In mammals H$_2$O$_2$ is detoxified by CAT and GPx. CAT protects the cells from H$_2$O$_2$ generated within them and plays an important role in inactivation of ROS and in adaptation to oxidative stress. The mechanism responsible for the down regulation of CAT in disease animals is uncertain. However, it may be the consequence of decreased Cu -Zn SOD and the reduction of H$_2$O$_2$ production which regulates CAT expression (Mates et al., 1999).

Catalase (CAT) is considered a common enzyme found in nearly all living organisms exposed to oxygen and catalyzed the decomposition of hydrogen peroxide (H$_2$O$_2$) to water and oxygen (Chelikani P et al., 2004).

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

While the complete mechanism of CAT is not currently known, the reaction is believed to occur in two stages:

\[ \text{H}_2\text{O}_2 + \text{Fe (III)-E} \rightarrow \text{H}_2\text{O} + \text{O=Fe (IV)-E (. +)} \]

\[ \text{H}_2\text{O}_2 + \text{O=Fe (IV)-E (. +)} \rightarrow \text{H}_2\text{O} + \text{Fe (III)-E + O}_2 \] (Boon EM et al., 2007)
Here Fe ()-E represents the iron center of the heme group attached to the enzyme. Fe (IV)-E (+) is a mesomeric form of Fe(V)-E, meaning the iron is not completely oxidized to +V, but receives some "supporting electrons" from the heme ligand. This heme has to be drawn then as a radical cation (+).

As hydrogen peroxide enters the active site, it interacts with the amino acids Asn147 (asparagine at position 147) and His74, causing a proton (hydrogen ion) to transfer between the oxygen atoms. The free oxygen atom coordinates, freeing the newly formed water molecule and Fe(IV)=O. Fe(IV)=O reacts with a second hydrogen peroxide molecule to reform Fe(III)-E and produce water and oxygen. The reactivity of the iron center may be improved by the presence of the phenolate ligand of Tyr357 in the fifth iron ligand, which can assist in the oxidation of the Fe (III) to Fe (IV). The efficiency of the reaction may also be improved by the interactions of His74 and Asn147 with reaction intermediates and the rate of the reaction can be determined by the Michaelis-Menten equation (Maass E, 1995).

CAT is also a very important enzyme in reproductive reactions as it has highest turnover numbers of all enzymes and can convert millions of molecules of H₂O₂ to water and oxygen each second (Goodsell DS, 2004). CAT is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon EM et al., 2007). It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human CAT is approximately 7 and has a fairly broad maximum pH in the ranged between 6.8 and 7.5. (Maehly A et al., 1954; Aebi H, 1984) however, the pH optimum for other CAT varies between 4 and 11 depending on the species (Toner K et al., 2000). CAT can also catalyze the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It does so according to the following reaction:

\[ \text{H}_2\text{O}_2 + \text{H}_2\text{R} \rightarrow 2\text{H}_2\text{O} + \text{R} \]
Any heavy metal ion (such as copper cations in copper (II) sulfate) can act as a non-competitive inhibitor of catalase. Also, the poison cyanide is a competitive inhibitor of CAT, strongly binding to the heme of catalase and stopping the enzyme's action. The true biological significance of CAT is not always straightforward to assess: Mice genetically engineered to lack catalase are phenotypically normal, indicating this enzyme is dispensable in animals under some conditions (Ho YS et al., 2004). A catalase deficiency may increase the likelihood of developing type 2 diabetes (Laszlo Goth et al., 2001; Laszlo Goth, 2008). Some humans have very low levels of catalase (acatalasia), yet show few ill effects.

1.9. ANTIOXIDANTS VITAMINS (VITAMIN-C AND VITAMIN-E)

Antioxidant is a substance that protects the biological tissue from damage by the free radicals and can be recycled or regenerated by biological reducers (Rosen et al., 2001). Vitamin C or L-ascorbic acid/or ascorbate is an essential antioxidant for humans and certain other animal species. Vitamin C refers to a number of vitamers that have vitamin C activity in animals, including ascorbic acid and its salts, and some oxidized forms of the molecule like dehydroascorbic acid. Ascorbate and ascorbic acid are both naturally present in the body when either of these is introduced into cells, since the forms interconvert according to pH. Vitamin C is a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy (FSA, 2007). In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries. Ascorbate may also act as an antioxidant against oxidative stress (Padayatty SJ et al., 2003). However, the fact that the enantiomer D-ascorbate has identical antioxidant activity to L-ascorbate, yet far less vitamin activity underscores the fact that most of the function of L-ascorbate as a vitamin relies not on its antioxidant properties, but upon enzymic reactions that are stereospecific (Aboul-Enein HY et al., 2003).
The biological role of ascorbate is to act as a reducing agent, donating electrons to various enzymatic and a few non-enzymatic reactions. The one- and two-electron oxidized forms of vitamin C, semidehydroascorbic acid and dehydroascorbic acid, respectively, can be reduced in the body by glutathione and NADPH-dependent enzymatic mechanisms (Meister A, 1994; Michels A et al., 2012). The presence of glutathione in cells and extracellular fluids helps maintain ascorbate in a reduced state (Gropper SS et al., 2005). The vast majority of animals and plants are able to synthesize vitamin C, through a sequence of enzyme-driven steps, which convert monosaccharides to vitamin C. In case of plants, this is accomplished through the conversion of mannose orgalactose to ascorbic acid while in case of some animals, glucose needed to produce ascorbate in the liver (in mammals and perching birds) is extracted from glycogen; ascorbate synthesis is a glycogenolysis-dependent process (Wheeler GL et al., 1998 Banhegyi G et al., 2001).

Vitamin-C is absorbed in the body by both active transport and simple diffusion. Na-dependent active transport—Na-Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs)—are the two transporters required for absorption. SVCT1 and SVCT2 import the reduced form of ascorbate across plasma membrane (Savini I et al., 2008). GLUT1 and GLUT3 are the two glucose transporters, and transfer only dehydroascorbic acid form of Vitamin C (Rumsey SC et al., 1997). Although dehydroascorbic acid is absorbed in higher rate than ascorbate, the amount of dehydroascorbic acid found in plasma and tissues under normal conditions is low, as cells rapidly reduce dehydroascorbic acid to ascorbate (May JM et al., 2003; Packer L, 1997). Thus, SVCTs appear to be the predominant system for vitamin C transport in the body. SVCT2 is involved in vitamin C transport in almost every tissue, the notable exception being red blood cells, which lose SVCT proteins during maturation (May JM et al., 2007). "SVCT2 knockout" animals genetically engineered to lack this functional gene, die
shortly after birth suggesting that SVCT2-mediated vitamin C transport is necessary for life (Sotiriou S et al., 2002).

Although the body's maximal store of vitamin C is largely determined by the renal threshold for blood, there are many tissues that maintain vitamin C concentrations far higher than in blood. Biological tissues that accumulate over 100 times the level in blood plasma of vitamin C are the adrenal glands, pituitary, thymus, corpus luteum, and retina (Hedige MA, 2002). Those with 10 to 50 times the concentration present in blood plasma include the brain, spleen, lung, testicle, lymph nodes, liver, thyroid, small intestinal mucosa, leukocytes, pancreas, kidney and salivary glands. Ascorbic acid can be oxidized (broken down) in the human body by the enzyme L-ascorbate oxidase. Ascorbate that is not directly excreted in the urine as a result of body saturation or destroyed in other body metabolism is oxidized by this enzyme and removed.

On the other hand vitamin- E consists of two families of compounds namely the tocopherols and tocotrienols, characterised by a 6-chromanol ring and an isoprenoid side chain. The members of each family are designated alpha (α)-, beta (β)-, gamma (γ)-, or delta (δ)- according to the position of methyl groups attached to the chroman nucleus. Therefore, 8 stereoisomers of the large vitamin E family are possible but only the RRR-form occurs naturally. Tocopherols and tocotrienols are differentiated by their phenyl “tails” as these are saturated in the tocopherols but unsaturated in the tocotrienols (Combs, 1992). Unlike other antioxidant vitamins, a specific role for vitamin E in a required metabolic function has not been found. Major functions of vitamin-E appear to be as a non-specific chain-breaking antioxidant that prevents the propagation of free-radical reactions and especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and in plasma lipoproteins. It has been indicated that vitamin E functions primarily as an antioxidant in biological systems by trapping peroxyl free radicals (Combs, 1992; IOM, 2000). In this
regard, vitamin E is found in cellular membranes associated with PUFA in phospholipids. In the case of vitamin-E deficiency, the oxidation of PUFA is more readily propagated along the membrane, leading to cell damage and eventually symptoms, mainly neurological.

Vitamin-E is known to transport in the blood by the plasma lipoproteins and erythrocytes. It is absorbed with the fat component of food, “piggy-rides” on chylomicrons (formed in intestinal mucosal cells) through the lymphatic system and are finally released into the blood stream, though the efficiency of vitamin -E absorption is low in humans (IOM, 2000). In human, the primary human vitamin-E deficiency symptom is a peripheral neuropathy characterized by the degeneration of the large-caliber axons in the sensory neurons. Other symptoms observed in humans include spinocerebellar ataxia, skeletal myopathy, and pigmented retinopathy. Vitamin- E requirements have thus been reported to increase when intakes of polyunsaturated fatty acids (PUFAs) are increased. It has been suggested that a ratio of at least 0.4 mg (1 μmol) α-tocopherol per gram of PUFA should be consumed by adults. However, the method of determining the vitamin E requirement generated by PUFA intakes is not universally accepted. There are also data to suggest that low-density lipoprotein (LDL) oxidation susceptibility in vitro is dependent upon its PUFA content. Although it is clear that the relationship between dietary PUFA and vitamin E needs is not simple, high PUFA intakes should certainly be accompanied by increased vitamin E intakes.

Along inbuilt antioxidant enzymes, the synergistic effect of α-tocopherol and ascorbic acid has proved to be an efficient protector to the membrane integrity in response to the damaging peroxidative effect (Pillai CK and Pillai KS, 2002). A combined study relating peroxidative stress and antioxidant capacity in stone forming conditions in humans has not been cited yet. Therefore in light of the above concepts the present study was planned to quantitate the levels of serum malondialdehyde, nitrite, α-tocopherol, plasma ascorbate and
erythrocyte superoxide dismutase and also to investigate their possible bearings in pathogenesis of urolithiasis is very important. While oxidative stress is a well-known mechanism of action in the genesis of cell injury in different pathologies in the context of urolithiasis, most of the studies supporting this hypothesis are experimental, with few clinical data available (Broche F et al 1997).

1.10. STATUS OF UROLITHIASIS IN NORTHEAST INDIA:

The northeastern states of India, which border Burma (Myanmar) on one side and can be said to fall in the broad belt area of stone disease covering south-east, middle-east, north-east Asia and facing an acute problem of stone diseases. Due to lack of research facilities, the remote-ness, difficult geographical situations, the prevalence of urolithiasis is virtually unknown outside of these states. A preliminary survey from the laboratory highlighted the fact that urolithiasis is a major problem in these regions and required urgent attention. The incidence of urolithiasis is very high among the natives of these regions who are different in food habits, and also socially, culturally and ethnically from the rest part of India. (Singh PP, 1978). Most of the living population in these states has different food habits like rice as staple diet, high consumption of fermented fishes, soybeans, bam-boos and other types of indigenous food stuffs. Non-vegetarian foods are one of the major recipes in the daily menu of the most of the people living in these regions. But study of literatures revealed that there have been non-existent of data on the studies of etiologic chemical factors of urolithiasis found in the in the different vegetables and meat foodstuffs commonly available and consumed by the natives of these regions. Moreover, no publish literature have been reported on clinical studies from the patients of urolithiasis from these regions of India. In fact, the following key questions are still needed to consider as far as the prevalence of urolithiasis in the northeastern states of India is concern:
(a) Why the prevalence of urolithiasis is very high in the living population of north eastern states of India as compared to other parts of India and World? And is the epidemiology of urolithiasis endemic to these regions only?

(b) Is the different dietary habit of the natives of these regions playing any role for the high prevalence of urolithiasis?

(c) Is genetic factor playing a role in the pathogenesis of urolithiasis in the natives of these regions?

(d) Is different climatic condition from other parts of the country play a major role for the epidemiology of urolithiasis in these regions?

(e) What are the remedies and preventive measures which can be look out by the scientist and health professionals to contain this disease?

Among the North Eastern States of India, Mizoram State is facing an acute problem of stone diseases. It is commonly believed that every family has a member afflicted with this disease. But literature reports revealed that there have been non-existent of data or limited studies on the role elements in the genesis of urolithiasis and their determination in the various vegetables and meat foodstuffs commonly available and consumed by the natives of this region using sophisticated techniques. Moreover, no published data has been reported on the role of oxidative stress in the pathogenesis of urolithiasis in the living population of this region of India. The study of literature reveals that etiology of urolithiasis is multilateral and multifactorial resulting primarily from diet and imbalances free radicals and antioxidants or antioxidant enzymes activities. If such factors was identified properly and underlying physiological mechanism for the causes of urolithiasis in the living population is established, the increasing prevalence of urolithiasis can be checked. Therefore, the scope of the present study is evaluation of the etiologic factors and oxidative stress status related to high prevalence of urolithiasis in the urban natives of Mizoram taking into account both the
physical and biological parameters and their correlation with this ailment. In view of the conflicting data reported from other parts of the world on the role of diet in the pathogenesis of urolithiasis and no clinical data to date from this region prompted us to investigate further in-depth studies in this field. Accordingly the present research proposal entitled “Evaluation of etiologic chemical factors and oxidative stress status associated with prevalence of urolithiasis in the urban areas of Mizoram” has been selected as a research thesis for the Ph.D. degree of the Mizoram University, Aizawl. The results of the study are given in the following pages.