Discussion
Fluorine is the most electronegative element, distributed ubiquitously as fluorides in nature. Water is the major medium of fluoride intake by humans (WHO, 1984). Fluoride can rapidly cross the cell membrane and is distributed in skeletal and cardiac muscle, liver, skin and erythrocytes (Carlson et al, 1960 and Jacyszyn and Marut, 1986). Fluorosis is a major public health problem resulting from long-term consumption of water with high fluoride levels. It is characterized by dental mottling and skeletal manifestations such as crippling deformities, osteoporosis, and osteosclerosis. In India, as many as 15 states are affected by endemic fluorosis, and an extensive belt of high fluoride in water and soil is reported in South India (Siddiqui, 1955; Krishnamachari, 1976). Particularly Andhra Pradesh become as a more endemic area in this regard. Next to Nalgonda, the southern part of Andhra Pradesh (Nellore) is also getting polluted by fluoride water (Brindha et al., 2010; Jaganmohan et al., 2010a).

Water and water-based beverages are the largest contributors to an individual’s total exposure to fluoride in India and Pakistan (Azizullah et al., 2010). For an average person, depending on age, drinking water accounts for 57% to 90% of total fluoride exposure at concentrations of 2 mg/L and accounts for 72% to 94% of total fluoride exposure at concentrations of 4 mg/L. Non-beverage food sources containing various concentrations of fluoride are the second largest contributor to
fluoride exposure. The greatest sources of nondietary fluoride are dental products, primarily toothpastes. The public is also exposed to fluoride from background air concentrations and from some pesticide residues. Other sources include some pharmaceuticals and consumer products. Exposure to fluoride can cause a condition known as enamel fluorosis (Rao, 2009). Depending on the amount of fluoride exposure (the dose) and the period of tooth development at which the exposure occurs, the effects of enamel fluorosis can range from mild discoloration of the tooth surface to severe staining, enamel loss, and pitting (Rao and Rao, 2009). The condition is permanent after it develops in children during tooth formation (from birth until about the age of 8). Severe enamel fluorosis occurs at an appreciable frequency, approximately 10% on average, among children in U.S. communities with water fluoride concentrations at or near the current allowable concentration of 4 mg/L (WHO, 1994). The prevalence of severe enamel fluorosis is very low below about 2 mg/L of fluoride in drinking water. The biggest debate concerning enamel fluorosis, particularly the moderate to severe forms, is whether to consider it as an adverse health effect or a cosmetic effect (Chandrajith et al., 2011). Previous assessments considered all forms of enamel fluorosis to be aesthetically displeasing, but not adverse to health. This view has been based largely on the lack of direct evidence that severe enamel fluorosis results in tooth loss, loss of tooth function, or psychological, behavioral, or social problems (Dhar and Bhatnagar,
Fluoride is readily incorporated into the crystalline structure of bone, and will accumulate over time. Concerns about fluoride’s effects on the musculoskeletal system are focused on a condition called skeletal fluorosis and also on increased risks of bone fracture. Models that estimate the accumulation of fluoride into bone (pharmacokinetic models) have been developed that are useful in understanding fluoride’s effect on bone (Nayak et al., 2009). Skeletal fluorosis is a bone and joint condition associated with prolonged exposure to high concentrations of fluoride. Fluoride increases bone density and causes changes in the bone that lead to joint stiffness and pain (Hrishikesh Kumar et al., 2009). The condition is categorized into a preclinical stage and stage I, II, and III, the last of which is sometimes referred to as the “crippling” stage because mobility is affected. At stage II, mobility is not significantly affected, but it is characterized by sporadic pain, stiffness of joints, and osteosclerosis (bone thickening) of the pelvis and spine. The committee concluded that both stage II and stage III skeletal fluorosis should be considered adverse. There are very few known clinical cases of skeletal fluorosis in the United States (WHO, 1994). Pharmacokinetic models show that bone fluoride concentrations resulting from lifetime exposure to fluoride in drinking water at 2 mg/L or 4 mg/L fall within or exceed the ranges historically associated with stage II and stage III skeletal fluorosis (Singh and Jolly, 1970). However, this evidence is not conclusive because the levels at which skeletal fluorosis occurs vary
widely, and because it appears to be rare in the United States. There were few studies to assess risks of bone fracture in populations exposed to fluoride at 2 mg/L in drinking water. The best available study suggested an increased rate of hip fracture in populations exposed to fluoride at concentrations above 1.5 mg/L. However, this study alone is not sufficient to judge fracture risk for people exposed to fluoride at 2 mg/L. Thus, no conclusions could be drawn about fracture risks at 2 mg/L. Whether fluoride might be associated with bone cancer has been a subject of debate. Animal studies have suggested the possibility of increased risk of osteosarcoma (a bone cancer) in male rats, but no new animal bioassays have been performed to evaluate this further. Several new population studies investigating cancer in relation to fluoride exposure are now available. Some of those studies had significant methodological limitations that make it difficult to draw conclusions (Teotia et al., 1998). Overall, the results were mixed, with some studies reporting a positive association and others no association. The committee concluded that the evidence to date is tentative and mixed as to whether fluoride has the potential to initiate or promote cancers, particularly of the bone (WHO, 1994).

Even though the knowledge of science has been improved rapidly, the studies related to the fluoride toxicity and development of renal failure is not much focused area. Most of the fluoride toxicity
related to tooth and bone has been exploited (Whitford and Pashley, 1982). But studies related to this area are scanty. To fill this scientific gap present study has been developed and conducted in the Nellore district which was recently noted by the government as well as newspapers as most threaten area. Nellore is the geographically south part of the country very near to Bay of Bengal. Particularly the sub areas in this district like Udayagiri mandal become more susceptible to fluoride poisoning (Local Medical/Hospital data). Especially people belonging to this area are getting frequent renal problems and admitting to the hospitals and are even deaths also noted some times. This information initiated us to conduct a research work on the fluoride mediated renal damage and its involvement with several biological parameters. The study was started with a sum of total ten fluoride affected villages which were find out with the help of water control department and the water samples has been taken for the analysis of water fluoride content. According to the WHO the control limit fluoride content in water should be 1 ppm (WHO, 1984). Water samples from different bore wells of ten villages showed a maximum range of 2.37 to 6.74 ppm by SPADNS method (Table-3). Similar type analysis in the drinking water fluoridation has been earlier reported by several workers (Shivashankara et al., 2000; Ayoob and Gupta, 2006; Karunakaran et al., 2009; Kirti Avishek et al., 2010; Arveti et al., 2011; Radha Goutam et al., 2011).
Among the selected ten villages three are showing high levels of fluoride content in their drinking water (ranges 4-7 ppm). Particularly Varikunta padu showing a maximum fluoride content of 6.74 ppm. Next to fluoride remaining all other water parameters are also important due to their reactive nature and purity for consumption (Dhar and Bhatnagar, 2009). For that total water analysis was made in the water samples from the ten villages. This analysis includes the estimation of pH, alkalinity, hardness, Ammonia, Calcium and magnesium in addition to fluoride content (Table-4). All these parameters showed abnormality in all the selected areas. Out of ten villages selected only three villages with high fluoride content namely, Varikunta padu (6.74 ppm), Kolangadi palli (5.12 ppm) and Gangireddy palli (4.43 ppm) were taken for the further entire study. For that we have gathered the information like age, sex, duration of stay in the specified area, drinking water source, any complications like diabetes and renal failures and hypertension etc.

Present study was confined to the fluoride mediated renal problems. Hence we eliminated the samples which are shown to be diabetic and hypertensive. Because diabetes or hypertension can develop the renal failures due to their high frequency as well as extreme blood flow (Marshall, 2004). From the collected information we have selected people who have never suffered from hypertension and diabetes. Around 90 villagers were selected for the entire analysis.
Blood and urine samples were collected. Analysis of the samples showed the fluoride content in abnormal range both in urine and serum (Table-5). The generally accepted average normal serum fluoride value is 8 µM (0.15 ppm.) as found by Singer et al. (1969). Incase of urine fluoride acceptable point is 1 mg per liter. But in the case of the selected objects it seems to be more when compared to the normal value. Particularly Kolangadi palli people showed a maximum of 2.27 ppm of serum fluoride and in case of urine fluoride Varikuntapadu people have shown a maximum range of 4.00 mg (Jaganmohan et al., 2010). The concentration of fluoride ions in serum is directly related to the fluoride content of the drinking water. This close relationship has been clearly demonstrated by several authors (Guy et al., 1976; Singer and Ophaug, 1979). Saralakumari and Ramakrishna Rao (1993) observed a correlation between fluoride toxicity and duration of stay in the village and same was observed in rabbits (Naresh kumar et al., 2010) nutritional and socio-economic status of the individuals. Ekstrand (1978) showed that plasma fluoride increased with age between 10 and 38 years. This difference in serum fluoride levels could be attributed to a difference in fluoride uptake by the skeleton. The young, growing skeleton, being low in fluoride, has a greater capacity for taking it up. In older people, the bone fluoride is higher and the plasma approaches equilibrium with it, hence there occurs a rise in plasma fluoride with advancing age (Murray et al., 1991). Experiments on dogs and puppies
also revealed age-dependant increase in blood fluoride levels (Whitford and Pashley, 1982). Thus there is a direct correlation between the age of the individual and fluoride retention.

The fluoride contents of urine, serum and drinking water were significantly higher in selected villagers as compared to normal. These observations are in agreement with the earlier report from Jodhpur (Verma et al., 1990), which showed significant increase in fluoride content of urine and serum of threaten people compared to healthy control, but in disagreement with earlier report which showed normal plasma and urinary excretion of fluoride in children having endemic vesicle stones (Teotia et al., 1991). Even though the selection was done specifically remove the hypertension and diabetic people, again a cross check has been made to know the random blood glucose levels as well as blood pressure of the selected 90 fluoride threaten individuals (Table-6). Blood pressure was measured with the help of a local rural medical practitioner. Random blood glucose level was assayed with the help of one pick glucometer. These results showed that the values are not significant (p<0.001) and there was not much change when compared to that of control value. Mean value of RBS showed to be 175 mg/dl, where as control mean value is 173 mg/dl (Table-6).
The best indicator of kidney function is considered to be the rate that blood is filtered at the glomerulus, or glomerular filtration rate (GFR). Chronic kidney disease can then be quantitatively defined as a GFR <60 mL/min/1.73m$^2$ for three months or more, irrespective of the cause (National Kidney Foundation, 2002). While the GFR can be measured directly by clearance studies of exogenous markers, such as inulin, iohexol, iothalamate, and Cr$^{51}$-EDTA. These procedures are costly and time consuming and are not suited to the routine detection of kidney disease. Even to measure the clearance of endogenous substances, such as urea and creatinine, requires both serum and an accurately timed urine collection, so efforts have been directed at more convenient “urine-free” estimates (Cockcroft and Gault, 1976).

Creatinine is a non-protein waste product of creatine phosphate metabolism by skeletal muscle tissue. Creatinine production is continuous and is proportional to muscle mass. Creatinine is freely filtered and therefore the serum creatinine level depends on the Glomerular Filtration Rate (GFR). Renal dysfunction diminishes the ability to filter creatinine and the serum creatinine rises. Hence creatinine was measured in the test and control samples. If the serum creatinine level doubles, the GFR is considered to have been halved. A three fold increase is considered to reflect a 75% loss of kidney function. Present study reveals that there was a drastic increase, almost
doubled with the control value indicates the loss of renal function and symptoms of renal failure (Table-6). Control subjects showed the creatinine content of 1.43 mg/dl, where as the disordered subjects showed a value of 2.78 mg/dl, which shows a drastic increase in the serum creatinine value and the loss of renal function. From the results we can observe a significant (p<0.001) increase in the serum creatinine content. From the literature it is clear that increase serum creatinine levels are seen in impaired renal function, chronic nephritis, and urinary tract obstruction, muscle diseases such as gigantism, acromegaly, and myasthenia gravis, congestive heart failure or even with stroke.

After knowing the serum creatinine and confirmation of the selected subjects were exempted from diabetic and hypertension people, the studies were further extended to know the alterations at different levels. Complete blood picture can provide a clear picture of the cellular as well as chemical components of the living system. Hence, studies were conducted in the control as well fluoride affected people. Selected objects blood samples were used for the analysis. Table 7 shows some of the important haematological parameters like WBC, RBC, Hb percentage, HCT, MCV and MCH. There are only a few reports in the literature of anemia in fluorotic individuals. Haemoglobin levels in the endemic villages were low, compared to those in the control population. Same results were observed by Susheela (2010) in
women suffering from fluoride toxicity. Though the difference between mean values was not significant, individual values in the endemic population showed great fluctuation. Though fluoride is capable of causing anemia, the haemoglobin level is also governed by the individual's nutritional status. Decreases in haemoglobin and erythrocyte count were also reported in camels near a super-phosphate factory (Karram and Ibrahim, 1992). Results showed that there was a slight increase in WBC content, shows a significant (p<0.001) increase. But in the case of other parameters there was a different response. Except WBC remaining all mentioned parameters showed to be significantly (P<0.001) decreased. In the case of HCT and MCV there was a much difference when compared to that of controls. In case of controls HCT and MCV showed 41.92 and 92.16 respectively, but in affected the values decreased to 38.46 and 81.97 respectively (Table-7). Table 8 shows the continuation of haematological parameters like MCHC, PLT, RDW-SD, RDW-CV, PPW and MPV. Here also there was significant (p<0.001) increase in the case of MCHC and PLT i.e. 28.86 and 151.47 were observed in controls, where treated subjects showed 30.85 and 198.22 respectively. Remaining all parameters was shown to be decreased (Jaganmohan et al., 2010b).

Table-9 shows the continuation of parameters of haematology like P-LCR, LYM percentage, MXD percentage, neutrophils, lymphocytes
number, MXD number and NEUT number. Here also we can find increase in some of the parameters like LYM percentage, MXD percentage, lymphocytes number, MXD number and NEUT number. Particularly drastic increase was observed in MXD percentage i.e. 2.57 in control where as fluoride affected people showed 8.58, which shows a significant (P<0.001) increase. Next to this a significant change (P<0.001) was noted in case of LYM percentage. Control value shows 38.62 whereas fluoride threatens subjects showed 44.26 (Table-9).

The various biochemical parameters investigated in this study are useful indices of evaluating the toxicity of fluoride in human beings (Guy et al., 1976; Saralakumari and Ramakrishna Rao, 1993). Assessment of haematological parameters can be used to determine the extent of deleterious effect of fluoride on the blood of an animal (Ekstrand, 1978; Ashafa et al., 2009). It can also be used to explain blood relating functions of a metal or its chemical products (Guy et al., 1976). Such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for lower animals or human toxicity, when the data are translated from animal or human studies (Olson et al., 2000). The non-significant effect of the fluoride on the RBC may be an indication that the balance between the rate of production and destruction of the blood corpuscles (Erythropoiesis) was not altered. MCHC, MCH and MCV relates to individual red blood cells while Hb,
RBC, PCV, LUC and RCDW are associated with the total population of red blood cells. Therefore, the absence of significant effect of fluoride on RBC, Hb, MCH, MCHC and RDW could mean that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered (Adebayo et al., 2010). The decreased MCV and the elevated levels of PLT and MXD percentage in the fluoride affected people further suggest selective toxicity of the fluoride and its components. The significant increase in the lymphocytes and MXD could possibly suggest enhancement in the ability of the blood component to phagocytose. Lymphocytes are the main effector cells of the immune system (McKnight et al, 1999; Adebayo et al., 2010). The enhancement in the lymphocytes in this study may affect the effector cells of the immune system. All these alterations suggest selective toxicity of the drinking water fluoride on the haematological parameters investigated in this study.

After knowing changes in the complete blood picture, studies were conducted to know the status of next important parameters i.e. lipid profile. Any change in the body will reflect immediately in lipid content of the biological system. Lipid profile, also known as coronary risk panel or lipid panel, is the collective term given to the estimation of, typically, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, and triglycerides, used to assess risk of
coronary heart disease. An extended lipid profile may include very low
density lipoprotein cholesterol and non-HDL-C. Particularly alterations in
any metabolic activity reflect in the altered lipoprotein content as well as
the cholesterol. Here in the case of fluoride toxicity also we have
conducted experiment to know the lipid profile of the fluoride toxic
subjects. From the results it is clear that there was a drastic
enhancement was noticed in all lipid parameters except in HDL (Table-
10). In case of triglycerides control subjects showed 122.72 mg/dL,
where as the fluoride altered patients showed 146.96 mg/dL which
shows a drastic increase. That shows a direct relation between the
increased fluoride concentrations increases the lipid metabolism and
accumulation of fat content in the blood stream. For cholesterol control
people shows 147.4 mg/dL, where as test subjects showed an increase
to 164.09 mg/dL. In case of VLDL and LDL also there was an increase
when compared to that of controls (Table-10). This gives an idea that
fluoride is not much involved with lipid metabolism comparatively
(Jaganmohan et al., 2011c). Similar type of studies in albino rabbits
was observed by Seema Choudhury (2010). The well established high
incidence of hypertriglyceridemia (Bagdade et al., 1968; Attman et al.,
1984; Seema Choudhury et al, 2010) in fluoride affected people with
renal problem has proved in this study. Triglyceride levels were well
correlated with the levels of VLDL-cholesterol and, among
apolipoproteins. Similarly VLDL, LDL and cholesterol showed increased
values when compared to that of controls. This may be due to increase blood glucose levels by the fluoride leads to the altered lipoprotein activity. Overall fluoride is not having much involvement on the lipoproteins. But LDL, value seems to be high which intern able to develop coronary heart diseases. Even cholesterol is also at border range, even exceeds it also risk factor for the development of cardiac problems. Conflicting reports have been published regarding fluoride toxicity and lipid metabolism. Saralakumari et al. (1988) reported a decrease in plasma free fatty acids as well as total lipids, and an increase in serum cholesterol in rats supplemented with fluoride in drinking water for sixty days (Saralakumari et al., 1988), but Chinoy and Sequeira (1989) showed no changes in serum cholesterol in various reproductive tissues of rats (Chinoy, 1991; Chinoy et al., 1993; 1994) and mice (Chinoy and Sequeira, 1989) exposed to NaF for 30 days. The results of the present investigation also revealed normal levels of serum cholesterol, thus ruling out the occurrence of hypo / hypercholesterolemnia among fluorotic individuals in the early stages of the disease. The circulating levels of testosterone in fluorotic individuals were also not altered significantly in males. Chronic fluoride treatment of rats also resulted in decreased testicular cholesterol levels (Narayana and Chinoy, 1994). Chronic cases of fluorosis need to be investigated in detail since the chances of atherosclerosis cannot be ruled out.
After the lipid analysis, studies were made to assess the important and vital organ of the body, the liver. Liver function tests (LFTs or LFs), which include liver enzymes, are group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient’s liver. Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. Some tests are associated with functionality (eg. albumin); some with cellular integrity (eg. transaminase) and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to (1) detect the presence of liver disease, (2) distinguish among different types of liver disorders, (3) gauge the extent of known liver damage, and (4) follow the response to treatment. Table-11 explains about the detailed analysis of LFTs. Here we can find a minute enhancement with the control values. But there was no one parameter showing drastic change. Almost all parameters are in normal range of reference values. SGOT and SGPT enzyme activities were found to be slight decreased when compared to control values. These studies are comparable with the previous studies (Agarwal Shashi and Monika Bhardwaj, 2011). Serum bilirubin is also in normal range both in control and treated subjects. Protein contents like albumin and globulin seem to
be slightly increased (Table-11). Fluoride is known to inhibit protein synthesis, mainly due to impairment of peptide chain initiation (Hoerz and McCarty, 1971) and by interfering with peptide chains on ribosome (Ravel et al, 1966). That indicates fluoride is not showing much toxicity in the liver and even enzymatic activities were also seems to be not much elevated. Thus LFT results indicated that there was no much significant (P<0.001) alteration in the fluoride affected people.

The fluorotic group showed marked alteration in their serum electrolyte levels. As fluorine is the most electronegative element, distributed ubiquitously as fluoride in nature. On its interaction with biological system through water it definitely alters the electrolytes in the biological system, where sodium and potassium are the two important electrolytes. They maintain the homeostasis, acid base balance and also different biological functions in the membranes. Hence further studies were made to know the level of two important electrolytes (sodium and potassium) in the serum of the controls as well as the fluorides threaten people. Table-12 shows the analysis of serum sodium and potassium levels of the selected subjects. Sodium levels shown to be decreased in the selected subjects. Control value of serum sodium was 134.81ku/l and in case of the treated persons showing 139.37ku/L, that indicates fluoride increases significantly (P<0.001) the sodium concentration in the serum (Table-12). In case of potassium it was
showing similar to that of sodium. The control value is 4.04 mmol/L for the serum potassium level whereas fluoride affected peoples showing a slight significant (P<0.001) increase of 4.64 mmol/L. This clearly suggests that fluorine is involving with the electrolytes and altering the sodium and potassium levels. Potassium and sodium levels increased significantly, compared to controls. Differential distribution of these two cat ions is essential for normal membrane function and integrity. Similar augmentation in electrolyte levels was demonstrated in rats fed with sodium fluoride (Chinoy et al., 1993). But Suketa and Terui (1980) observed a reduction in the serum sodium level in rats after fluoride administration, while potassium levels, on the other hand, significantly increased. They attributed these changes to alteration in adrenal function. However, Das and Susheela (1991) reported low corticosteroid levels in fluorotic humans, suggesting adrenal hypo function. Serum potassium is an indicator of cell damage. Increased levels suggest cell deterioration.

The routine classical evaluation of nephropathy (Any type of renal problems) includes the identification of glomerular and tubular markers in the patient’s serum and urine. The normal individual doesn’t contain this content elevated in their urine or in serum samples. These glomerular and tubular markers include: transferring, IgG, antitrypsin, β-2-microglobulin and angiotensin converting enzyme (ACE). Recent
studies also have demonstrated that, there were tubular components in renal complications of disease conditions as shown by the detection of renal tubular enzymes and low molecular weight proteins in the urine as well as in serum. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine (Catalano et al., 1993).

Thus studies were conducted to evaluate the glomerular and tubular marker in urine as well as in serum of the control and fluoride affected people. Table-13 shows the analysis of serum glomerular and tubular markers in the control and test samples. Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow. The blood transferrin level is tested for diverse reasons like to determine the cause of anemia, to examine iron metabolism (for example, in iron deficiency anemia) and to determine the iron-carrying capacity of the blood. Low transferrin can impair hemoglobin production (since to make hemoglobin, you have to have iron) and so lead to anemia. Low transferrin can be due to poor production of transferrin by the liver (where it's made) or excessive loss of transferrin through the kidneys into the urine. Here in the present study the level of transferrin seems to be low when compared to that of control (Table-13). This indicates the chance of anemia due to fluoride toxicity. Low levels of IgG
occur in macroglobulinemia. In this disease, the high levels of IgM antibodies suppress the growth of cells that produce IgG. Other conditions that can result in low levels of IgG include some types of leukemia and a type of kidney damage (Nephrotic syndrome). Here we can find the low levels of serum IgG, but with in the normal range indicating the altered renal function (Table-13).

Alpha 1-antitrysin (A1AT) is produced in the liver. Accumulation of this in liver causes lower levels of A1AT in blood, resulting in the development of liver cirrhosis. Excessive excretion of A1AT through urine indicates the loss of renal function. In present case there was no difference with the control value. It seems to be almost equal, that indicates the normal functioning of liver (Table-13). Beta 2-microglobulin is a protein found on the surface of many cells. Testing is done primarily when evaluating a person for certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and multiple myeloma or kidney disease. In our study it was very interestingly rapid enhancement of B2M was noticed. When kidney disease is suspected, comparing blood and urine levels helps identify where the kidney is damaged. Beta₂-microglobulin normally is filtered out of the blood by the kidney's glomeruli (a round mass of capillary loops leading to each kidney tubule), only to be partially reabsorbed back into the blood when it reaches the kidney's tubules. In glomerular
kidney disease, the glomeruli can't filter it out of the blood, so levels increase in the blood and decrease in the urine. In tubular kidney disease, the tubules can't reabsorb it back into the blood, so urine levels rise and blood levels fall. After a kidney transplant, increased blood levels may be an early sign of rejection.

Increased urinary levels are found in people with kidney damage caused by high exposure to the heavy metals cadmium and mercury. Periodic testing of workers exposed to these metals helps to detect beginning kidney damage. Alterations in low molecular weight proteins in serum and urine were also observed under fluoride toxicity (Gulay Ciftic et al., 2010).

B2M is normally cleared by the kidneys at a rate comparable to GFR (Karlsson et al., 1980; Gautier et al, 1984), then reabsorbed and catabolized in the tubules, and serum levels are inversely related to GFR (Kamsson et al., 1980). Clearance by conventional dialyzers is negligible as these membranes are impermeable to β2m. Production of β2m in normals is 9 mg/hr/70 kg (Karlsson et al., 1980). Production may be increased in proliferative disorders (Schardun et al., 1980) and rheumatoid arthritis (Manicourt et al., 1978) as indicated by high serum levels in the presence of normal renal function.
The routine classical evaluation of nephropathy (any type of renal problems) includes the identification of glomerular and tubular markers in the patient’s serum and urine. The normal individual doesn’t contain this content elevated in their urine or in serum samples. These glomerular and tubular markers include: transferrin, Ig G, antitrypsin, β-2-microglobulin and angiotensin converting enzyme (ACE). Recent studies also have demonstrated that, there were tubular components in renal complications of disease conditions as shown by the detection of renal tubular enzymes and low molecular weight proteins in the urine as well as in serum. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine (Catalano et al., 1993).

The angiotensin-converting enzyme test is used to measure the blood level of angiotensin-converting enzyme, which converts angiotensin I to angiotensin II and controls blood pressure. Angiotensin-converting enzyme and ACE2 are highly expressed in the kidney. The role of ACE in the development of renal damage is generally accepted. Here in the present study the ACE level seems to be decreased when compared to that of control individuals (Table-13). Control individuals having a concentration of 44.97, and fluoride affected people are showing a concentration of 37.07 indicating a significant (P<0.001)
decrease. This indicates the accumulation of angiotensin I. Angiotensin-converting enzyme and ACE2 are highly expressed in the kidney (Jaganmohan et al., 2010d). The impairment of ACE was also observed by Naresh kumar et al in male rabbits (Naresh Kumar et al., 2010). The role of ACE in the development of renal damage is generally accepted (Dzau et al, 2001). Individual differences in renal ACE activity predict the susceptibility for proteinuria-associated renal damage in experimental conditions (Huang et al, 2001 and Rook et al, 2005). Furthermore, ACE II is increased in damaged tubules and is suggested to be a possible mediator of renal damage in experimental and human renal disorders (Wolf and Ritz, 2005; Ruiz-Ortega et al., 2006). Blockade of the actions of Ang II by ACE inhibitors or AT1 receptor blockers has been proven to effectively reduce blood pressure and proteinuria (Anderson et al., 1986), thereby providing renoprotection. A disrupted balance between intra renal ACE and ACE2 with consequent low levels of Ang II and high levels of Ang (1–7) might contribute to the Reno protective mechanisms of ACE inhibitors (Campbell et al., 1991; Ferrario and Iyer, 1998; Ferrario et al., 2005; Kocks et al., 2005).

In recent years a vast amount of data has been published on the association between the insertion/deletion (I/D) polymorphism of the gene coding for angiotensin-converting enzyme and renal disease. It has become clear that the polymorphism does not affect the prevalence
of renal disease. However, data on the association with progression of renal disease and therapy response are still appears to be contradictory. Moreover, sufficient data on the physiological significance of this polymorphism was still lacking. Hence, this present contribution provides an overview of the available studies and the potential pitfalls in interpreting the data. And it was also attempted to discuss the putative mechanisms for the association between the DD genotype and progression of renal disease and suggest directions for the future that might be employed to further clarify the role in renal pathophysiology.

Renal failure is an outcome of complex pathophysiological process resulting from multiple etiologies with contribution from both genetic and environmental factors. A large variation abounds in the frequencies of ACE I/D polymorphism in different ethnic groups. It is was evident from the present study that the D allele frequency of our controls was intermediate (Jaganmohan et al., 2010e) to most reported Caucasian (Tarnow et al., 1995; Chowdhury et al., 1996; Marre et al., 1997; Schmidt and Ritz, 1997; Hubacek et al., 2000) and Asian (Oh et al., 1996; Hsieh et al., 2000; Gesang et al. 2002; Tamaki et al., 2002; Ergen et al., 2004; Wang et al., 2005) populations. However, two Caucasian (Doria et al., 1994; Powrie et al., 1994) and an Asian (Tamaki et al., 2002) population were reported to have comparable allele frequencies. The failure to find statistically significant differences
in the distribution of ACE gene I/D genotypes and their allele frequencies between the fluoride mediated nephropathy patients and the controls suggest that this polymorphism was not a risk factor for the development of renal failure in the studied population. These observations were found similar to the work of Tamaki et al. (2002) and Ergen et al. (2004). In conclusion, our study suggests that the ACE I/D polymorphism is not associated with prevalence of advanced form of renal failures due to the intake of fluoride within the selected regional population (Jaganmohan et al., 2010e).