Summary and Conclusions
Unlike bacteriological pollution, the effect of the excess chemical constituents (that are present in groundwater, like fluoride) on human health is chronic in nature and manifest after consuming the water over a long period of time. Long term ingestion of drinking water having fluoride beyond a limit of 1.5 ppm will lead to dental and skeletal fluorosis as well as non skeletal manifestations. Up to now there are plenty of studies related to fluoride poisoning through drinking water and skeletal and dental manifestations. But studies related to altered renal functions in the fluoride affected people are very scanty. Hence the present study was initiated to find out the association of fluoride poisoning with renal failures in the selected area in Andhra Pradesh, India. The people of Nellore district in Andhra Pradesh, particularly in the area of Udayagiri mandal are frequently suffering with renal problems or even deaths due to renal failures. Present study was conducted in the selected ten villages of the Udayagiri mandal. After the study and analysis, the results were summarized and were provided as follows:

1. The study was performed in ten selected villages of Nellore district in Andhra Pradesh, for assessing the relationship of fluoride poisoning with renal failures.
2. It was observed that the water samples from different bore wells of ten villages showed fluoride concentration of maximum range from 2.37 to 6.74 ppm by SPADNS method.

3. In addition to the fluoride levels, total water analysis was made from the ten villages. All these parameters show abnormality in all the selected areas.

4. Among the ten villages selected, three villages are showing high levels of fluoride content in their drinking water (ranges 4-7 ppm).

5. The three villages namely, Varikunta padu (6.74 ppm), Kolangadi palli (5.12 ppm) and Gangireddy palli (4.43 ppm) were taken for the further analysis in the present study.

6. Detailed information has been collected from the villagers of the three selected villages.

7. This has helped to omit the people suffering with hypertension or diabetes, as there was a possibility for occurring renal failures with the hypertension or diabetes.

8. After screening of the data we have selected a sum of 90 people, who have never suffered with hypertension or diabetes.

9. Even though the selection was done to 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 threat individuals.
10. The study reveals that there was a drastic increase in serum creatinine, which was almost doubled when compared with control value, indicates the loss of renal function and symptoms of renal failure.

11. Selected objects blood samples were collected and used for the complete blood analysis and observed for altered haematological parameters.

12. It was observed that an 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.

13. In case of lipid parameters, from the results it was clear that there was a drastic enhancement in all lipid parameters except in HDL.

14. Liver function studies had shown that there was a minute enhancement when compared with the control values. But there was
no one parameter showing abnormal change. All most all the parameters were in normal range.

15. Analysis of serum sodium and potassium levels of the selected subjects observed to be increased. This clearly suggests that the fluorine was interacting with the electrolytes and altering the sodium and potassium levels.

16. Further studies were conducted to evaluate the glomerular and tubular markers in urine as well as in serum of the control and fluoride affected people.

17. Serum analysis of glomerular and tubular markers observed to be decreased when compare to their controls except the B2M.

18. The control subjects showed 3.03 g/ml, where the fluoride affected people showed a maximum increase of B2M to 10.60 g/ml. That shows a significant increase, which indicates the altered renal activity in the fluoride affected people.

19. Urine glomerular and tubular markers are shown to be increased abnormally when compared to their controls.
20. An increased level of B2MG as well as ACE indicates the kidney failure. But to understand the actual mechanism, further studies have been made in insertion and deletion polymorphism of ACE genes.

21. The DNA samples from 90 fluoride mediated nephropathy persons and 60 normal healthy control persons were amplified for I/D polymorphism in the ACE gene and the results were analyzed.

22. The preferential amplification of the D allele and inefficiency of the amplification of I allele may result in the mistyping of ID heterozygotes as DD homozygotes.

23. Therefore, in order to increase the specificity of DD genotyping, all samples, identified as DD after initial amplification were reconfirmed with an insertion-specific primer pair, as mentioned in material and method section.

24. The presence of insertion sequence was revealed by the amplification of a 275 bp fragment, while DD homozygotes failed to amplify due to the lack of annealing site.
25. The frequency of D allele and DD genotype was only marginally higher in fluoride affected patients as compared to the normal controls.

26. The observed and expected genotypic frequencies were in Hardy-Weinberg Equilibrium.

27. The present study suggests that the ACE I/D polymorphism is not associated with advanced form of renal failures due to fluoride intake within the selected regional population.

From the present study, it can be concluded that, there was a great need for more basic physiological studies that investigate the consequences of ACE I/D polymorphism in renal pathophysiology. Only then one can understand the impact of ACE I/D polymorphism on the onset and course of renal disease and eventually develop treatment strategies specifically adapted to certain genetic risk profile. Finally, it is also necessary to take into account that the impact of certain exogenous factors involved in gene-environment interactions as many exogenous factors have great impact on the development and course of disease. Such gene-environment interactions should certainly need more attention in future studies. The present study is an attempt to address these questions and it is an attempt to provide comprehensive
and over view information on this burning issue and problem in this area and to provide a possible mechanism for contemplating the situation in these affected areas.