Summary and Conclusions
The present study was undertaken to assess the prevalence of dental and skeletal fluorosis in the population of selected villages i.e., Sarampet and Vaillapally. Further, fluoride status, bone mineral status, biochemical markers of bone metabolism status, hormonal status, oxidative and antioxidant enzyme status were also assessed on the randomly selected 132 fluorotic adults of two sex groups and 88 well matched non-fluorotics adults, who served as controls. The data was discussed with comparison between fluorotics and non-fluorotics, between males and females and between two levels of fluorosis i.e., Fluorotic group I (5.1-6.4 ppm) and Fluorotic group II (7.2-8.2 ppm) based on drinking water fluoride levels.

The following are the salient features of the present study:

The prevalence of dental fluorosis in selected villages, 97.2% were affected with dental fluorosis of different grades ranging from I to V. 98.8% of males and 95.1% females were affected with dental fluorosis. Males also showed a higher severity of the disease than females. In higher severity grades of 'moderate' and 'severe', the percentages for males were 12.5% and 81.8% as against females whose percentages in the respective severity grades were 16.3% and 76.3%.

The prevalence of dental fluorosis was higher in fluorotic group II i.e., 98.2% than the fluorotic group I i.e., 96.0%. With regard to the grades of severity of the disease, fluorotic group II showed higher prevalence of highest grades of 'moderate' and 'severe' fluorosis, which were 14.4% and 81.9% compared to the fluorotic group I with prevalence rates of 13.8% and 76.4% respectively.

In adults, among males, the prevalence of dental fluorosis was the highest in the age group of 51-90 yrs i.e., 100% followed by 21-50 yrs i.e., 100% and 7-20 yrs i.e., 94.3%. Among females, the prevalence of dental fluorosis was the highest in the age group of 51-90 yrs i.e., 100% followed by 21-50 yrs i.e., 96.8% and 7-20 yrs i.e., 84.4%.

Fluorotic group II males showed higher prevalence i.e., 99.4% compared to fluorotic group I males i.e., 98.2%. Similarly fluorotic group II females showed
higher prevalence i.e., 96.7% compared to fluorotic group I females i.e., 93.2% respectively.

In the present study none of the children below 15 yrs showed skeletal fluorosis and it was observed from the age group of 16 yrs.

The prevalence of skeletal fluorosis was found to increase with age. The prevalence of skeletal fluorosis was higher in the age group of 50-90 yrs males i.e., 100% followed by 21-50 yrs males i.e., 92.4% and 16-20 yrs i.e., 61.5%. Similarly the prevalence in females was higher in the age group of 50-90 yrs i.e., 100% followed by 21-50 yrs i.e., 82.0% and 16-20 yrs i.e., 55.5%. Further when the sexes were compared males showed higher prevalence of skeletal fluorosis i.e., 80.3% than the females i.e., 72.3%.

Fluorotic group II males showed higher prevalence i.e., 83.0% compared to fluorotic group I males i.e., 77.2%. Similarly Fluorotic group II females showed higher prevalence i.e., 74.8% compared to fluorotic group I females i.e., 69.4% respectively.

Fluoride levels of drinking water sources in fluorotic and non-fluorotic areas were a range of 5.1 to 6.4 ppm in Sarampet; 7.2 to 8.2 pm in Vaillapally and 0.21 to 0.62 ppm in Hyderabad.

A significant difference was found between the fluorotic and non-fluorotic males and females with respect to water (p<0.01).

Mean water fluoride intake of fluorotics was found to be higher when compared to non-fluorotics. The differences found in the fluoride intake between the fluorotics and non-fluorotics for the two sex groups were found to be significant (p<0.01) at 1% level.

Mean serum fluoride and urinary fluoride levels were found to be higher in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum fluoride and urinary fluoride levels between the fluorotic and the non-fluorotic for the two sex groups were found to be significant (p<0.01) at 1% level.
The differences in water fluoride intake, serum fluoride and urinary fluoride levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group; between fluorotic group I and fluorotic group II were found to be significant (p<0.01) at 1% level.

Correlation between water fluoride intake and serum fluoride levels was found to be positive and significant (p<0.01) at 1% level; between water fluoride intake and urinary fluoride levels was found to be positive and significant (p<0.01) at 1% level.

Mean height, weight and BMI were found to be lower in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in height between the fluorotics and non-fluorotics for the two sex groups were found to be not significant for the two sex groups and the differences found in weight and BMI between the fluorotics and non-fluorotics for the two sex groups were found to be significant (p<0.01) at 1% level.

The differences in weight and BMI between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group were found to be significant (p<0.01) at 1% level. However, the difference in weight and BMI between fluorotic group I and fluorotic group II was found to be not significant.

The differences in height between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group; between fluorotic group I and fluorotic group II were found to be not significant.

Correlation between water fluoride intake and weight was found to be negative and significant (p<0.01) at 1% level; between water fluoride intake and BMI was found to be negative and significant (p<0.01) at 1% level. Between water fluoride intake and height was found to be negative but not significant.

Mean serum calcium and phosphorus levels were found to be lower in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum calcium levels between the fluorotics and non-fluorotics for the two sex groups were found to be significant (p<0.01) at 1% level and the differences found in the serum phosphorus levels
between the fluorotics and non-fluorotics for the two sex groups were found to be significant \((p<0.05)\) at 5\% level for males and \((p<0.01)\) at 1\% level for females.

Mean serum alkaline phosphatase levels were found to be higher in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum alkaline phosphatase levels between the fluorotics and non-fluorotics for the two sex groups were found to be significant \((p<0.01)\) at 1\% level.

The differences in serum calcium and alkaline phosphatase levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group; between fluorotic group I and fluorotic group II were found to be significant \((p<0.01)\) at 1\% level.

The differences in serum phosphorus levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group were found to be significant \((p<0.01)\) at 1\% level. However, the differences in serum phosphorus levels between fluorotic group I and fluorotic group II were found to be not significant.

Correlation between serum fluoride and serum calcium levels was found to be negative and significant \((p<0.01)\) at 1\% level; between serum fluoride and serum phosphorus levels was found to be negative and significant \((p<0.01)\) at 1\% level. Between serum fluoride and serum alkaline phosphatase levels was found to be positive and significant \((p<0.01)\) at 1\% level.

Mean serum bone specific alkaline phosphatase, tartrate resistant acid phosphatase-5b and urinary C-terminal telopeptides of type-I collagen levels were found to be higher in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum bone specific alkaline phosphatase, tartrate-resistant acid phosphatase-5b and C-terminal telopeptides of type-I collagen levels between the fluorotics and non-fluorotics for the two sex groups were found to be significant \((p<0.01)\) at 1\% level.

The differences in serum bone specific alkaline phosphatase and tartrate resistant acid phosphatase-5b levels between fluorotic group I and non-fluorotic
group; between fluorotic group II and non-fluorotic group; between fluorotic group I and fluorotic group II were found to be significant (p<0.01) at 1% level.

The differences in urinary C-terminal telopeptides of type-1 collagen levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group were found to be significant (p<0.01) at 1% level. However, the differences in urinary C-terminal telopeptides of type-1 collagen levels between fluorotic group I and fluorotic group II were found to be not significant.

Correlation between serum fluoride and serum bone specific alkaline phosphatase levels was found to be positive and significant (p<0.01) at 1% level; between serum fluoride and serum tartrate resistant acid phosphatase-5b levels was found to be positive and significant (p<0.01) at 1% level. Between urinary fluoride and urinary C-terminal telopeptides of type 1 collagen levels was found to be positive and significant (p<0.01) at 1% level.

Mean serum vitamin D levels were found to be higher in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum vitamin-D levels between the fluorotics and non-fluorotics for the two sex groups were found to be not significant.

Mean serum parathyroid hormone levels were found to be higher in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum parathyroid hormone levels between the fluorotics and non-fluorotics for the two sex groups were found to be significant (p<0.01) at 1% level.

The differences in serum vitamin D levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group were found to be significant (p<0.05) at 5% level. However, the differences in serum vitamin D levels between fluorotic group I and fluorotic group II were found to be not significant.

The differences in serum parathyroid hormone levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group;
between fluorotic group I and fluorotic group II were found to be significant (p<0.01) at 1% level.

Correlation between serum fluoride and serum 25-OH vitamin D levels was found to be positive but not significant. Between serum fluoride and serum parathyroid hormone levels was found to be positive and significant (p<0.01) at 1% level.

Mean serum thyroid-stimulating hormone, triiodothyronine and thyroxine levels were found to be lower in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum thyroid-stimulating hormone, triiodothyronine and thyroxine levels between the fluorotics and non-fluorotics for the two sex groups were found to be not significant.

The differences in serum thyroid stimulating hormone, triiodothyronine and thyroxine levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group; between fluorotic group I and fluorotic group II were found to be not significant.

Correlation between serum fluoride and serum thyroid stimulating hormone levels was found to be negative but not significant; between serum fluoride and serum triiodothyronine levels was found to be positive but not significant. Between serum fluoride and serum thyroxine was found to be negative but not significant.

Mean serum malondialdehyde levels were found to be higher in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum malondialdehyde levels between the fluorotics and non-fluorotics for the two sex groups were found to be significant (p<0.01) at 1% level.

Mean serum catalase and glutathione-s-transferase levels were found to be lower in fluorotic males and females compared to non-fluorotic males and females respectively. The differences found in the serum catalase and glutathione-s-transferase levels between the fluorotics and non-fluorotics for the two sex groups were found to be significant (p<0.01) at 1% level.
The differences in serum malondialdehyde, catalase and glutathione-s-transferase levels between fluorotic group I and non-fluorotic group; between fluorotic group II and non-fluorotic group; between fluorotic group I and fluorotic group II were found to be significant (p<0.01) at 1% level.

Correlation between serum fluoride and serum malondialdehyde levels was found to be positive and significant (p<0.01) at 1% level. Correlation between serum fluoride and serum catalase levels was found to be negative and significant (p<0.01) at 1% level; between serum fluoride and serum glutathione-s-transferase levels was found to be negative and significant (p<0.01) at 1% level.

Conclusion:

The degree of severity of fluorosis increased with age, which is also indicative of age dependent accumulation of fluoride in the body.

The prevalence and degree of severity of fluorosis increased with water fluoride levels as evidenced in fluorotic group II compared to fluorotic group I.

Males showed higher prevalence of dental and skeletal fluorosis compared to females.

Fluoride status parameters i.e., water fluoride intake, serum fluoride and urinary fluoride levels were significantly higher in fluorotics than non-fluorotics. Significant positive association was found between water fluoride intake and serum fluoride and urinary fluoride levels. The differences in serum fluoride and urinary fluoride levels between the two sex groups of fluorotics were found to be significant.

Anthropometric parameters, height, weight and BMI were significantly lower for fluorotics than the non-fluorotics. Significant negative association was found between water fluoride intake and weight and BMI. The differences in weight and BMI between the two sex groups of fluorotics were found to be significant.

In fluorotics, bone mineral markers i.e., serum calcium and serum phosphorus levels were significantly lower and serum alkaline phosphatase levels were significantly higher than non-fluorotics. Significant negative association was found between serum fluoride and serum calcium and phosphorus levels. Similarly
significant positive association was found between serum fluoride and alkaline phosphatase levels. The differences in serum alkaline phosphatase levels between the two sex groups of fluorotics were found to be significant.

In fluorotics, biochemical markers of bone metabolism i.e., serum bone specific alkaline phosphatase, serum tartrate resistant acid phosphatase-5b and urinary C-terminal telopeptides of type-1 Collagen levels were significantly higher than non-fluorotics. Significant positive association was found between serum fluoride and serum bone specific alkaline phosphatase and tartrate resistant acid phosphatase-5b levels. Similarly significant positive association was found between urinary fluoride and C-terminal telopeptides of type 1-collagen levels. The differences in serum bone specific alkaline phosphatase, tartrate resistant acid phosphatase-5b and urinary C-terminal telopeptides of type-1 collagen levels between the two sex groups of fluorotics were found to be significant.

With regard to calcitropic hormonal status, the fluorotics showed significantly elevated parathyroid hormone levels than non-fluorotics. Significant positive association was found between serum fluoride and serum parathyroid hormone levels. The difference in serum 25 (OH) vitamin D levels between two sex groups of fluorotics was found to be not significant and the differences in serum parathyroid hormone levels between the two sex groups of fluorotics were found to be significant.

In fluorotics, thyroid status i.e., serum thyroid stimulating hormones, triiodothyronine and thyroxine levels were lower than non-fluorotics. The differences in serum thyroid stimulating hormone, triiodothyronine and thyroxine levels between the two sex groups of fluorotics were found to be not significant.

In fluorotics, serum malondialdehyde levels were significantly higher while their catalase and glutathione-s-transferase levels were significantly lower than the non-fluorotics. Significant positive association was found between serum fluoride and serum malondialdehyde levels. Similarly significant negative association was found between serum fluoride and serum catalase and glutathione-s-transferase levels. The differences in serum malondialdehyde, catalase and glutathione-s-
transferase levels between the two sex groups of fluorotics were found to be significant.

From the foregone conclusions it can be inferred that there is a need to overcome this problem following certain strategies:

- Public water works department can set up a defluoridation plant by using Nalgonda Technique and involve community participation in order to make people aware of the benefits of defluoridation of water and extend their sustained support to the plant.

- Governmental and non-governmental agencies can take up the supply of anti-fluoride factors like the following through either PHCs or schools especially in rural areas affected with fluorosis.
  
  - Nutrient mixtures with right combination of calcium, vitamin C and vitamin D3
  
  - Tamarind pulp bar (with jeera+salt) or pulp pill.

- On a long-term basis, it is necessary to determine RDA (Recommended Dietary Allowances) for calcium and vitamin C in different degrees of fluorosis as these nutrients have been shown to counteract fluoride effect and bring in beneficial results.

- The compulsive inclusion of tamarind pulp to the extent of 10 g and more per day in the diets in the form of chutneys, soups, rasam etc., may be promoted, as it entraps fluoride ions and decreases fluoride absorption.