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CHRONOLOGICAL, BONE AGE, MATURITY, CORTICAL INDEX AND CORTICAL VOLUME INDEX IN RELATION TO THE FLUORIDE CONTENT IN WATER IN A RURAL COMMUNITY IN KENYA

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ABSTRACT

Growth in children and adolescents is closely related to bone thickness, length and bone maturity as represented by an increase of minerals in the cortical bone and a general reduction in the medulla width. The study aim was to determine the bone age, cortical index, and cortical volume index and relate to the chronological age and the fluoride content in the drinking water. The sample size was determined using the Fisher et al. formula. The mean fluoride concentration in the surface and groundwater was 0.65mg /l and 9.9 mg/l respectively. The 107 children and adolescents selected for the study were born and raised in the study community. The boys and girls aged 6-10 years were 60 (56.1%), while the adolescent boys and girls were 47 (43.9%). The mean chronological age for the whole group was 10.1±3.6 years; mean bone age, bone maturity 1.05±1.04years; cortical index, 0.38±0.09; and the cortical bone volume was 0.065±0.015. The chronological and bone age, cortical index and cortical volume index had a significant negative linear relationship with fluoride content in groundwater. The cortical index and cortical bone volume of the adolescents had a weak linear relationship low fluoride in water. In conclusion bone age, maturity, the cortical index and the cortical volume index had an inverse relation with the high fluoride in water. Also, the bone maturity did not occur at the same rate as bone age, cortical index and cortical volume index.

KEYWORDS: Water, fluoride, bone age, maturity, cortical index cortical volume index.

INTRODUCTION

Chronological age: The chronological age is defined as a measure of the period since birth. It is used in psychometric for determining individual behaviour variables and intelligence. Also, it has been used to establish emotional age and categorise individuals as early, average or late maturers. Chronological age assists clinicians to predict an individual's emotional and physical growth.[1] However, age is a poor indicator of a person's physical maturity. It does not provide information nor identify the various stages of growth and development from childhood through adolescence to adulthood. Hence other ages, dental and skeletal age have been used to accurately determine the age of an individual.[2,3,4,5]

Bone age and bone maturity: Bone maturity is a measure of the size, shape, and degree of bone mineralisation. Greulich and Pyle were the first to develop an atlas of the skeletal age of the hand and wrist on bone assessment.[6] Tanner and Whitehouse (TW2) adopted Greulich and Pyle developed an index which scored the hand wrist bones. The bone scoring index had a numerical value given to each discrete stage (A, B, C…I, ROI). Numerical scores associated with each bone stage were added up to obtain the overall maturity score[7] Fluoride, an inorganic anion of fluorine occurs naturally in the rocks, soil, water, plants, foods, and air.[8] Skeletal fluorosis is a disease condition caused by the prolonged excess ingestion of fluoride in endemic fluoride areas.[9] Age, gender, and calcium intake are factors that influence the development and severity of fluorosis and diet, dose, duration of fluoride intake and kidney efficiency in handling fluoride.[10]

Chronic ingestion of high concentrations of fluoride affects the dental and musculoskeletal tissues. There is increased bone pain; fractures decrease birth rates, increases urolithiasis, impairs thyroid function and lowers intelligence. Juvenile skeletal fluorosis which affects children during times of bone growth may manifest as deformities of the joints and long bones. The malformations occur, particularly in the weight-bearing bones. Studies have also shown that skeletal fluorosis is more prevalent in boys than girls, however; the exact reason for this remains unclear.[11,12,13,14] Though the prevalence of skeletal fluorosis is not well documented, it is believed that it affects millions of people around the

world. The World Health Organisation recommends intake of fluoride to be a maximum concentration of 1.5 mg per litre. The recommended fluoride concentration is supposed to prevent the possible formation of skeletal changes as well as dental Fluorosis.[15] Other sources of fluoride include industrial processes and dental care products. Environments that are fluoride-rich are mainly linked with Precambrian basements areas and those affected by recent volcanism.[16] The most significant sources of fluoride exposure are located in the East African rift valley. In particular Kenya, the high fluoride in the underground water is associated with historical volcanic activity.[17] Groundwater samples tested throughout Nairobi, the Rift Valley, and Central Province in Kenya. The high fluoride concentrations reach up to 30 to 50 mg/litre in domestic water while some of the lakes the fluoride are 2800mg /litre.[18]

Fluoride enters the human body by ingestion, inhalation, and in extreme cases through the skin. Waterborne fluoride is more rapidly absorbed than food-borne fluoride.[19] The ideal concentration of fluoride varies according to climatic conditions with a range of 0.5–1.0mg/litre.[11] Quality clean water is scarce for domestic use, and large communities are using underground water with high fluoride content. Bone and teeth are two organs in the human body with a high mineral content in particular calcium in the form of hydroxy appatite crystals. Fluoride has a high affinity for calcium as it tends to displace the hydroxyl group in the hydroxyapatite crystal to form calcium fluoride and other fluoride complexes. Few studies have reported on the impact of chronic intake of high fluoride in water on bone age, maturity and the cortical index and cortical volume index development and growth of children in Kenya.

MATERIALS AND METHODS

A multistage stratified sampling of boreholes in Thika county was done based on a Ward as the administrative unit. All boreholes with high fluoride were identified from the borehole data was classified and from this one borehole was selected. The selected borehole was sunk in the fifties, and the documented fluoride in the water was 10 ppm. A visit to the community revealed the community also had surface water a dam which was used for irrigation purposes. The study community located twenty-five kilometres from the city of Nairobi, and it is located in the southern part of central Kenya.

The identified community had a total of 148households in which 500 persons lived. Out of whom 332 were adults aged between 20-90 years of age. The infants, toddlers' children and adolescents aged 0-19 years were 168 in total. The 148 households were in clustered around two water sources. Four households with seventeen infants' children and adolescents used surface water whose was confirmed to be 0.65 in a pilot study. Those involved in the survey were children and adolescents aged between 6-17 years who attended the primary school which was in the community. The study took six months, and it was descriptive and cross-sectional.

Sample size and sampling: A productive borehole in the from the ministry of water database for boreholes in Juja county documented to have a fluoride content of 10ppm was selected based on multistage random from other boreholes in the area. The borehole was located twenty-five kilometres northeast of Nairobi. The selected community lived in houses clustered around the water source. It was also noted that there was a surface water source in the community which was used by few households. Dental fluorosis was used as a parameter to select the children for skeletal fluorosis. According to Wenzel et al.; individuals ingesting fluoride of the magnitude of 3.6pp are afflicted with dental fluorosis are reported to be afflicted with skeletal fluorosis. Reported a prevalence for dental fluorosis of 89%. Hence, a single sample group sample size was calculated based on the formula $n= \underline{(Z_{1-\alpha})^2}\ [P\ (1-p)]/\ C^2$; where n was the required sample, Z as the critical value associated with the level of significance, p was the estimate of proportion, C was the level of the marginal error. Z= 1.96, Fluorosis prevalence of 89% according to Nair et. al[20] hence p=0.89 and C=0.05. The calculated sample size was $n= (1.96)^2\ [0.89(1-0.89)/\ (0.05)^2$ =150, ten percent was added for attrition giving a sample size of 160 children. However, 168 children were examined for dental fluorosis. From the 168 examined for dental fluorosis, those aged 6-17 years were selected for examination for skeletal fluorosis.

The Inclusion criteria were that the child must have been born, raised and raised in the community up to the time. The rationale for these criteria was to minimise the confounding factors such as exposure to different contents of fluoride during the period of tooth development which could influence the severity of fluorosis. Nine children aged 6-19 were excluded as there was a high inter-cluster migration. Adolescents aged 18-19 years were excluded as they had already exited the primary school according to the Kenya education system. Also, there was a cut off age for the skeletal maturity, cortical index and cortical volume index children aged below six years were excluded so that the results may be compared to similar studies in the same age group.

Data collection: A questionnaire was used to gather the demographic data for the parents/ guardians and the children.

Collection of water samples: Twenty-four water samples one from the underground source and a second from the surface water source were collected per month for twelve months. Each sample upon collection was transferred to a cooler then transported to Nairobi and were stored in a deep freezer. Fluoride was determined using the Orion fluoride Ion Selective electrode (Orion Research Inc) by the author while the full chemical

profile for the twenty-four months was analysed by the ministry of water Laboratories Kenya government.[21]

Skeletal age was assessed based on the Turner and White (TW2) method, and it involved 107 children aged 6-17 years.[7] In this approach, each of the 20 biologically selected hand and wrist bones was scored on 1 – 9 well-defined rating scale.

Chronological age of the child was obtained by using the date of birth as was recorded in the class register and this was a more reliable method as most of the children did not have a birth certificate. The skeletal age was then obtained by adding the points for every stage. Skeletal maturity was obtained by subtracting bone age from chronological age. The data was recorded on a specially designed form.

The cortical index (D-d/D) was obtained by measuring at the midpoint of the second metacarpal index of the right-hand wrist as is described by Barnett & Nordin.[22] The total cortical bone and medullary width were measured along the maximal length of the 2nd metacarpal.

The cortical thickness (D-d), was obtained by use of a pair of callipers and a clear ruler was used to measure the total carpal diameter "D" and medullary width “d.” Then (D-d) was divided by the carpal diameter “D”. The determination of the cortical index (D-d/ D) and cortical bone volume index, (D2 – d2/ DL, were determined based on Exton-Smith et al.[23]

Intra-examiner variability: Ten radiographs were randomly selected by the author and then re-read by a consultant radiologist who had taken the wrist-hand radiographs for the children. The intra-examiner variability was blindly assessed for bone age, cortical index and cortical bone volume and reproduce-ability was 90%, 89% and 94% which was acceptable.

Ethical clearance: The relevant ethical clearance committees approved the research proposal before the start of the survey. The informed verbal consent was obtained from the community elders the heads of the households. The children and adolescents who accepted to be examined were recruited into the study. The parents had to consent, and the children had to accent to participation hence, ten children whose parents did not give consent due to faith beliefs were excluded.

Data analysis: The data was cleaned and analysed using the SSP version 17.

RESULTS

Water: The mean annual fluoride content in groundwater a borehole was 9.23±1.48 mg/L (range 06-11.10 mg/L of fluoride) while the fluoride content in the surface water from a dam was 0.60±0.3mg/L (ranged from 0.550 to 0.68 mg/L of fluoride), Figure 1.

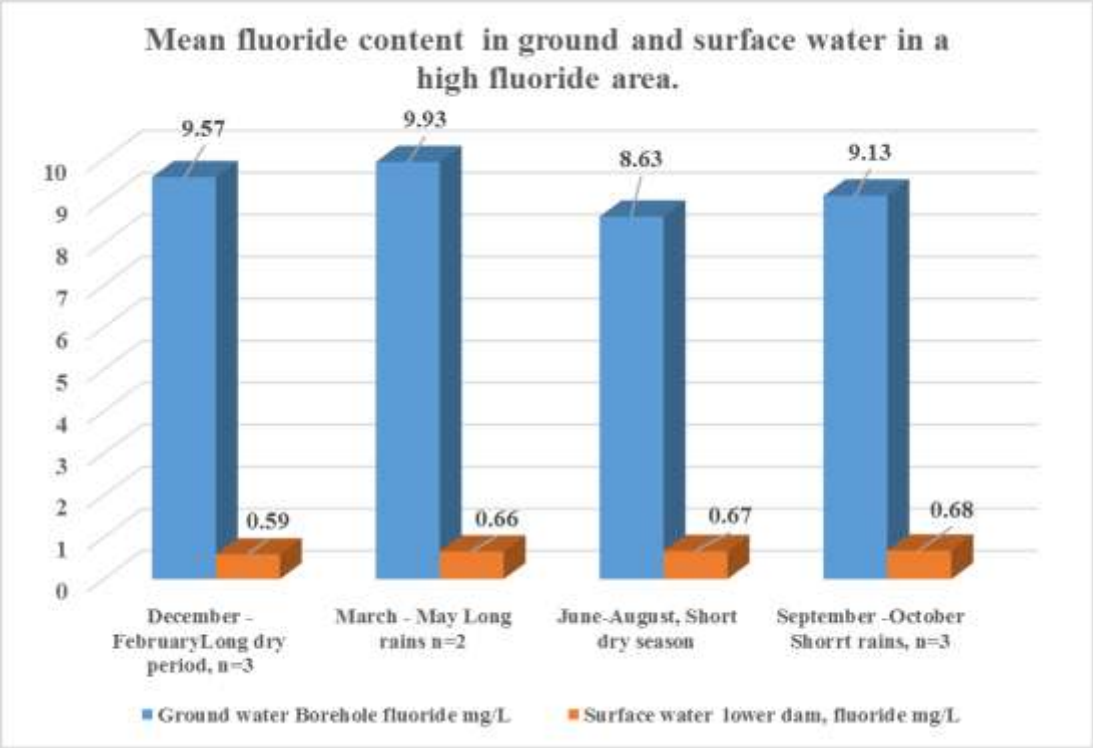

Figure 1: The mean fluoride content by source and variation by season.

Boys and girls aged 6-17: A total of 107 children and adolescents who used the borehole water and exhibited varying degrees of severity of dental fluorosis were selected for the radiological study. The mean chronological age for the 107 children and adolescents was 11.2±3.24, and it ranged between 5.2-17.4 years

Figure 2. The respective mean bone age, bone maturity, cortical index and cortical volume index were 10.1±3.6 (range 5.5- 17) years; 1.05±1.04; 0.38±0.09 (range 0.-0.06- 5.2 years); and 0.065±0.015 (range 0.033- 0.1) Figure 2, 3.

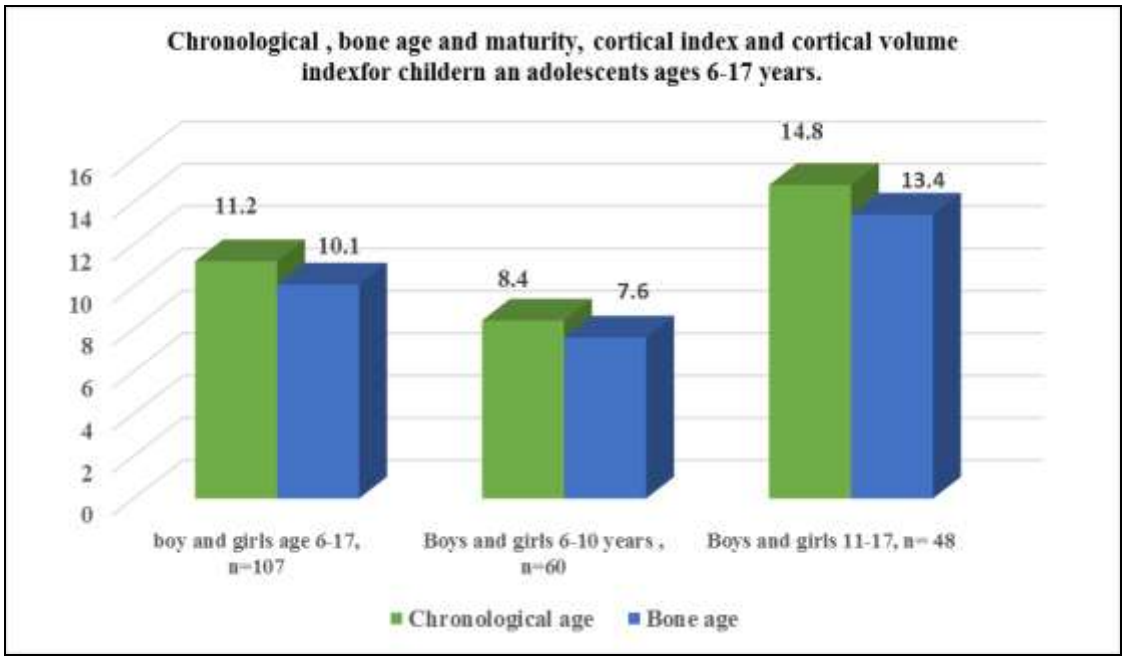

Figure 2: Chronological and bone ages for boys and girls aged 6- 17 years, n=107.

Boys and girls aged 6-10: The boys and girls aged 6-10 years were 60 (56.1%) with a mean chronological age was 8.4±1.4 years and ranged from 5.5-10.67 years. The mean bone age was 7.6±1.4 years (range 4.5-10.3 years); while the bone maturity was 0.74±0.76 years (range -0.06 to 3.03 years). The mean cortical index was 0.35±0.07 (range 0.19-0.62); while the cortical volume index was 0.061±0.012 (range 0.033 to-0.099) Figure 2, 3.

Boys and girls aged 11-17: The adolescent boys and girls were 47 (43.9%) and they had a mean chronological age for 47 (43.9%) adolescents was 14.7±2.2 years (range 11-17.92). The mean bone age was 13.3±2.8 years (range 7-17years) while bone maturity was 1.4±1.2 (range 0.01 to 5.2 years). The mean cortical index was 0.42±0.09 (range 0.25 to 1.0) while the cortical volume index was 0.072±0.017 (range 0.038 t0 0.10) Figures 2, 3.

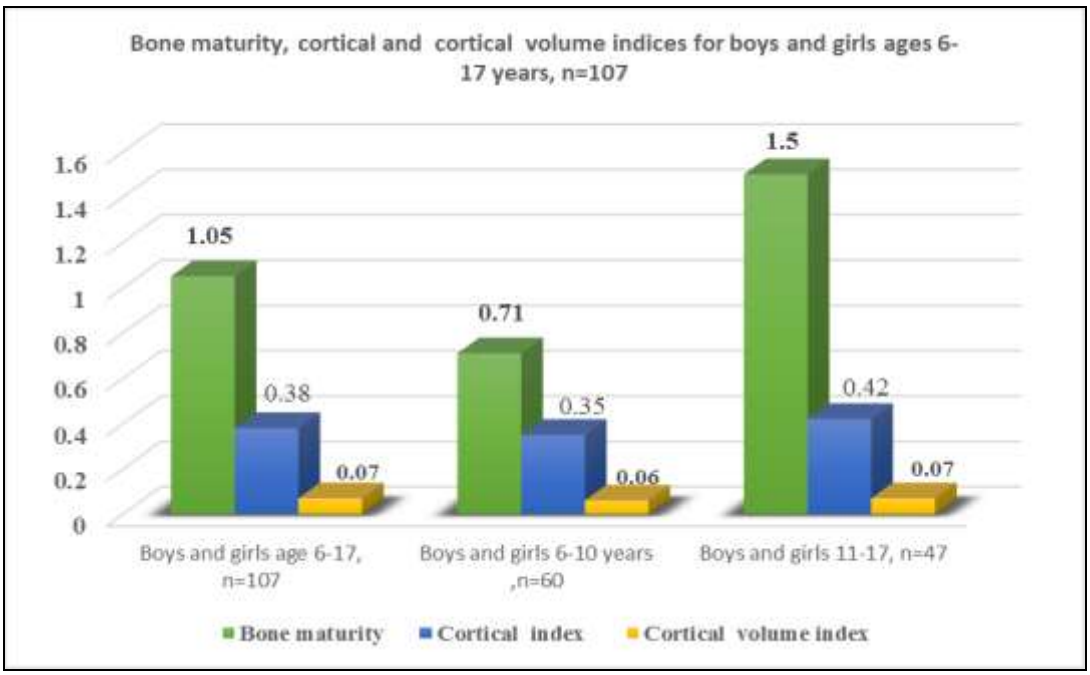

Figure 3: Bone maturity, cortical and cortical volume indices for boys and girls Aged 6-17 years of age, n=107.

Boys aged 6-17: The boys aged 6-17years were 59 (55.1%) and had a mean chronological age of 11.0±4.1 (range 5.5-17,25 years). The bone age was 10.0±3.4 years (range 4.5-17.0 years) and maturity of 1.0±0.93 (range 0.010 to 4.7 years). The mean cortical index was 0.38±0.08 (range 0.190-0.54 while the cortical volume index was 0.068±0.016 (range 0.033 -0.10) Figure 4 and 5.

Girls aged 6-17: The total number of girls aged 6-17 in the study was 48 (47.1%), and their mean chronological age was 11.4±3.8 years with a range of 6-17, 92 years. The bone age was 10.7±4.0 years (range 5-17 years) and maturity of 1.2±1.1yeasr (-0.06 to 5.2 years). The cortical and the cortical volume indices were 0.41±0.092 (range 0.25-o.54) and 0.06±0.016 (range 0.038-0.062) respectively, Figure, 4 and 5.

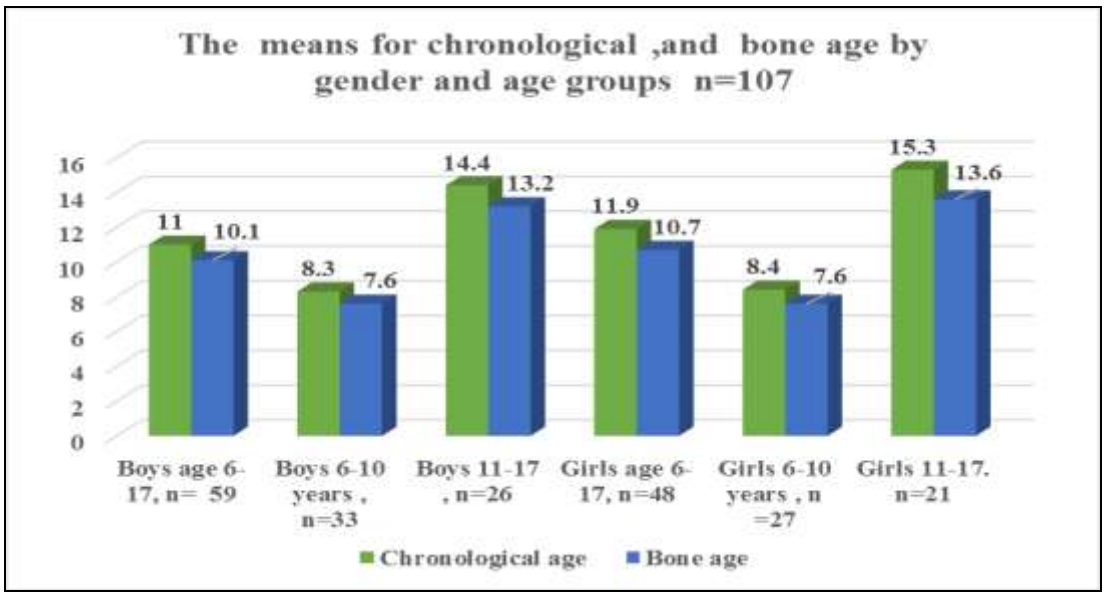

Figure 4: Chronological, bone age by age and gender n=107.

Boys 6-10: Boys aged 6-10 were 33(30.8%); they had a mean age of 8.3±1.3 (range 5.5-10.25 year) with a mean bone age of 7.6±1.3 years (range 74, 48-9.96) while bone maturity was 0.64±0.68 years (range 0.01-2, eight years). The cortical index was 0.32±0.054 (ranged between 0.19-0.42) and a mean bone volume index of 0.062±0.013 (range 0.33-0.99), Figure 4, 5.

Girls 6-10: The girls in the 6-10-year age group were 27 (25.2%) with a mean age 8.4±1.5 and ranged between 6.0-10.7 years. The bone age was 7.6±1.5 years (range 5.0 to 10.3 years) while bone maturity was 0.80±0.85years (range -0.060 to 3.03 years). The mean cortical index was 0.38±0.077 (range 0.250 to 0.250) while the bone volume index was 0.058±0.011 (0.038 to 0.058), Figures 4 and 5.

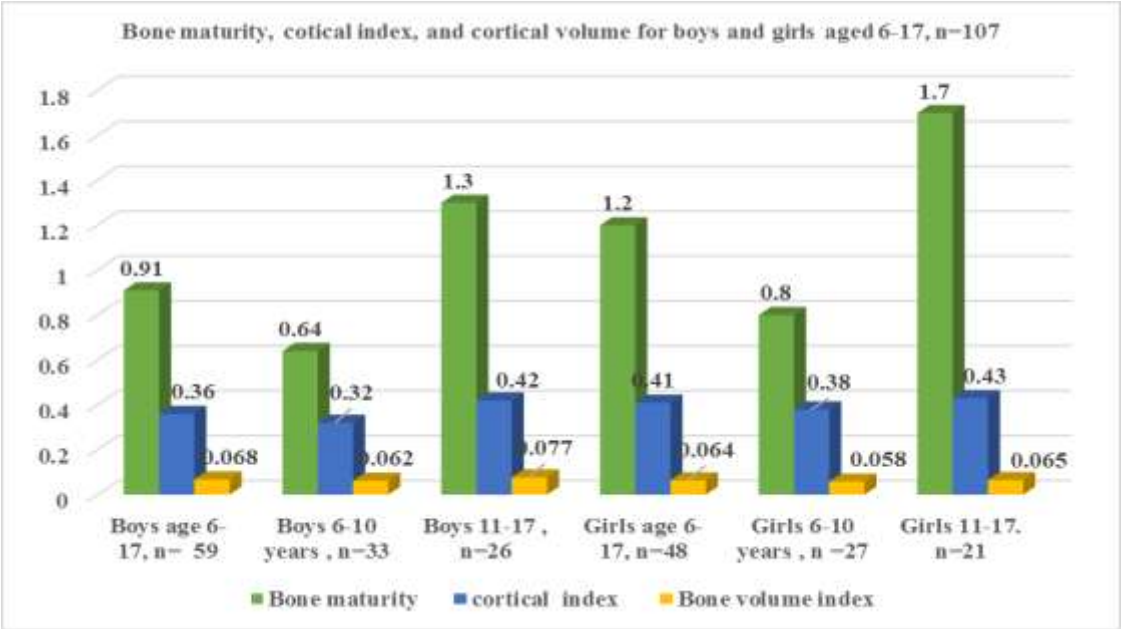

Figure 5: The means for bone maturity, cortical and cortical volume index by age group and gender, n=107.

Boys 11-17: Adolescent boys aged 11-17 were 26 (24.3%), and their mean age was 14.4±2.1, and the range was 11.0-17.3 years. The mean bone age was 13.2± 2.7years (range 8.4-17.00 years), and bone maturity was 1.3±1.1 years (range 0.01-4.4 years). The values for the cortical index were 0.42±0.8 and ranged between, while the cortical volume index had values of 0.26 to 0.54 and 0.077±0.17 (range 0.045-0.1), Figures 4, 5.

Girls 11-17: The adolescent girls aged 11-17years were 21 (19.6%) had a mean age of 15.3 ±2.4 years (range 11.7-18.4years. The mean bone age was 13.6±3.2 years (range 7.8- 17.9); with a bone maturity of 1.7±1.3 year (range 0-.070 -5.17 years). The cortical index was 0, 43±0.1 (range 0.250- 0.570) and the bone volume was 0.065±0.015 range 0.038 -.086), Figures, 4, 5.

Statistical analysis

The fluoride concentration in relation to chronological age, bone age bone maturity, cortical index and cortical volume index by age and gender

There was a negative correlation with Pearson r=-0.557, p= 0.03 between chronological age and the fluoride content in the borehole water whose annual mean fluoride was 9.9. However, bone age, bone maturity, cortical index, and cortical volume index had no association with the high fluoride in water. For the surface water, bone age correlated with the low fluoride content in water with a positive Pearson correlation r=0.448 p= 0.045. However chronological age, bone maturity, cortical; bone index and cortical volume index no relationship with the low fluoride in surface water.

Under-ground water fluoride 9.9mg/L: A Linear regression analysis was carried out between the annual mean fluoride content in the underground where the fluoride content was the controlling factor. Chronological, and bone age, bone maturity, cortical and cortical volume index were the dependent factors by age and gender. The chronological age for boys aged 6-10 years; bone age; cortical index and cortical volume indices showed weak negative significant linear relationships with underground water. The respective statistical values were chronological age, beta=-0.502, t=-2.557, p=0.022 at 95% CL.; bone age beta=-0.555, t=-3.063, p≤0.008 at 95% CL; cortical Index beta = -0.538, t=-3.187, p≤0.006 at 95% CL., and cortical volume Index beta =-0.450, t=-2.191 and p≤0.045 at 95% CL. Bone maturity for the boys had no relationship with the different annual fluoride content in groundwater. For the girls aged 6-10years, only chronological age had a strong and negative relationship beta=-0.550, t=-3.132, p≤0.007 at 95% CL, with the annual varying fluoride concentrations in the borehole water.

The other variables bone age, maturity; cortical and cortical volume indices showered a negative nonlinear relationship: The variables for the adolescent boys aged 11-17 which showed a linear relationship with water with a high fluoride content were chronological and bone maturity, cortical and cortical volume indices. The respective negative linear associating values were chronological age beta=-0.520, t= -2.700, p≤0.16 at 95%CL., bone age beta=-0.555t=-2.696, p≤0.017 at 95%CL. Cortical index had beta= -2.635, t=-2.635, p≤0.019; and cortical bone volume beta= -0.529; t=-2.415; p≤0.029 at 95%CL. However, there was a negative nonlinear relationship between bone maturity and the fluoride content in the underground water beta=0.374, t=1.560, p≤0.140 at 95%CL. For the adolescent girls the variables which had a linear relationship with the fluoride content in the groundwater were chronological age beta=-0.529, t=-2.948, p≤0.010 at 95%CL.; bone age beta= -0.445; t=-2.303; p≤0.036, bone maturity the cortical index beta=-0.446, t=-2.312, p≤0.035 and the cortical volume index beta=-0.445, t=-2.283 and p≤0.037at 95%CL.

Surface water fluoride 0.65mg/L: However, fewer variables for the boys aged 6-10 had a linear relationship with the different seasonal, annual fluoride content in the surface water. The variable for the boys which associated with the fluoride content was cortical Index beta= 0.534, t=2.527 and p≤0.022 at 95%CL. The girls in the same age group had chronological age associated with the fluoride content in the water with a beta value of 0.450, t=2.563, p≤0.022 at 95%CL. 0.488, t=2.239 and p=0.040 at 95%CL. The adolescent girls aged 11-17 years, the chronological age had a positive linear relationship with the low fluoride content with beta = 0.454, t=2.532, p≤0.023 at 955 CL; while the cortical volume index beta =-0.555, t=-2.666, p≤ .017 at 95%CL.

Differences in bone age, maturity, cortical index and cortical volume: The 107 boys and girls aged 6-17 had significant differences for ANOVA for cortical index and cortical volume index, and the respective values were F=5.447, DF=1 p≤0.022 and F= 4.126, DF=1 p≤0.045 at 95%CL where gender as the selection factor. In the children aged 6-10 years their significant differences between boys and girls for cortical bone index F=13.403, df=1 and p≤=0.001 at 95%CL. However, chronological, bone age, bone maturity and cortical volume index had no differences. For the adolescents, there were no differences between genders for all the variables. When chronological age was used as the selection factor for ANOVA, there was the difference in bone age between and within the groups for 107 boys and girls in the 6-17 age category with F=28.841, d. f=74, p≤0.001. Bone maturity differences were also significant F=1.934, df=74, p≤0.020. The cortical index F=7.036, DF=74, p≤0.001; while cortical volume indexes the differences were significant with F= 3.641, d. f=74, p≤0.000. However, when bone age was used as the selection factor, only chronological age and cortical index showed significant differences in the means of the variables. The respective values being chronological age F=17,296, DF=32 and p≤0.000, while the cortical index, F =3.886, DF=92 and p≤0.003. However, there were no differences

in the means for bone maturity and cortical volume index.

Differences in variables were observed for 60 (56.1%) boys and girls aged 6-10 years using chronological age as the selection factor. Bone age F=3.372, DF=40, p≤0.003at 95%CL, cortical index F=5.110, df=40, p≤0.000 at 95%CL. However, the differences in bone maturity and cortical volume index were not significant. When the bone age was used as the selection factor for boys and girls aged 6-10years no differences were observed in the means for all variables. As chronological age F= 1. 747d.f=25, p= 0.229, bone maturity F=0.458, df=25, p=0.930; cortical index F=1.709, df=25, p=0.239; and cortical volume index F=1.916, d. f=25, p=0.191.

For 47 (43.9%) adolescent boys and girls age 11-17 years differences in the variables within and between the groups was significant when chronological age was the selection factor. The bone age F= 22.679, d. f=33, p=0.001, bone maturity, F=2.667, d. f=33, p=0.031. Cortical bone, F= 23.739, d. f=33, p=0.001 and cortical volume index F=6.513, d. f= 33, p= 0.001. However, when bone age is the selection factor no differences were observed in chronological age with F= 66.688, d. f=40, p=0.001, cortical index differences F= 282.032, p=0.001 and the cortical volume index was F=16.146, d. f=40, p=0.001.

Chronological age, bone age, bone maturity, cortical bone index and bone cortical volume index paired t-test: For 107 children aged 6-17 the mean age 11.2±3.6years while the bone age was 10.1±3.6years and the differences were significant with paired t-test=10.595, d. f=106, p≤0.001. Sixty (56.1%) boys and girls aged 6-10 years had a mean chronological age of 8.4±1.4, and the bone age was 7.6±1.4years. A paired test showed significant difference t= 7.7, d. f=59, p≤0.001. Forty-seven 43.9% of adolescent boys and girls age 11-17 the mean chronological age was14.8±2.2 and the bone age was 13.3±2.8. The differences were significant t= 8.238, d. f=46, p≤.001 at 95%CL.

Pre-adolescent boys and girls 6-10 years vs adolescent boys and girls 11-17years: The mean values for chronological age were 7.09±1.2 years, bone age 7.4±1.2year, bone maturity 0.63±0.75, cortical bone volume 0.33±0.064, and cortical volume index 0.057±0.011 for twenty -seven preadolescent boys and girls. The mean values for twenty- one adolescent girls mean age 14.7±1.2 years for bone age, 13.3±2.8 years while bone maturity 1.5±1.2 years; cortical bone volume, and cortical volume index 0.07±0.017. The differences in between the variables for the pre-adolescent and the adolescent boys and girls were significant with the paired t-test for chronological age t=-44.645, d. f=46, p≤0.001. The differences for bone age was t=-19.617, d. f=46 p≤0.001, bone maturity t=-4.005, d. f=46 p≤0.001, cortical bone index t=-9.529, d. f=46, p≤0.001 and bone volume, and cortical volume index t=-6.476 d. f=46, p≤0.001 at 95%CL.

There was the difference between the chronological age and the bone age of fifty-nine 55.1%, boys aged 6-17 whose mean chronological age was 11.0±3.5years and the bone age was 10.1±3.4 years, and the difference was t=7.60, d. f=58, p≤0.001. Similarly, for 48 (44.9) pre-adolescent and adolescent girls with the mean chronological age of 11.4±3.8 and bone age of 10.2±3.7 years had differences which were significant, t=7.282. d.f=47, p≤0.001.

A comparison of the various variables of bone age, bone maturity, cortical bone index, and cortical bone volume index between thirty-three boy aged 6-10 whose mean age 7.7±1.1 years was a with that of twenty-six adolescent boys age group 11-17years with a mean age 14.4±2.1 years of showed differences with a paired t-test. The differences values for were chronological t=-31.082, d. f=25. p≤0.001; bone age (7.3±1.2, 13.2±2.7 years) t=-15.049, d. f=25. p≤0.00, bone maturity (0.52±0.56, 1.26±1.09 years) t=-2.935, d. f=25. p≤0.007; cortical bone index (0.31±0.05, 0.42±0.086) t=-9.257, d. f=25. p≤0.001; and cortical bone volume index (0.058±0.011, 0.077±0.017) was t=-6.656, d. f=2, p≤0.001 at 95%CL.

Twenty-seven preadolescent girls aged between 6-10 years had a mean age of 8.0±1.3 years while twenty-one adolescent girls aged 11-17 years had a mean age 15.3±2.4 years. A comparison of the various variables of bone age (7.1±1.3 years, 13.6±3.2 years), bone maturity (0.77±0.93; 1.71±1.33 years), cortical bone index (0.35±0.067 and 0.43±0.10), and cortical bone volume index (0.054±0.009 and 0.065±0.015) had significant differences with a paired t-test. Chronological age t=-28.710, d. f=20, p≤0.001; bone age t=-11.457, d. f=20. p≤0.001; bone maturity t=-2.292, d. f=20. p≤0.033; cortical bone index t=-4.229, d.f=20, p≤0.001; and cortical bone volume index was t=-3.953, d.f=20, p≤0.001 at 95%CL. Thirty-three boys and twenty-six girls showered differences between the chronological age and the bone age with a paired t-test with t=5.509, d.f=26, p≤0.001, However bone age showered no differences between gender with t=1.220, d.f=26, p≤0.234; similarly, bone maturity had no differences t=1.036, d.f=36, p≤0,310. The cortical index had differences with t=6.114, d.f=26 and, p≤0.001; but the cortical volume index did not show differences with a t= -0.511, d.f≤ 26, p=613 at 95%CL. Forty-eight, 44.9% of girls aged 6-17 had the mean chronological age of 11.4±3.8 years and the bone age was 10.2±3.7 years, and the mean differences were t=7.282, d.f =47, p≤0.001.

Twenty-seven girls aged 6-10 years had a chronological age of 8.4±1.5 years and the bone age were 7.6±1.5 years, the mean difference was significant with t=5.138, d. f=26, p≤ 0.001. Twenty-one adolescent girls had a mean chronological age of 15.3±2.4 years while the bone age was 13.6 ±3.2 years and the differences had t=6.160,

d. f=30, p≤0.001 at 95%CL. There were differences in the chronological age of fifty-nine boys, and forty-eight girls both age category 6-17 were significant with a paired t-test where t= 7.177, d. f=47, p≤0. 001. There were differences between the bone age of the boys and the girls in this same age group with t= 4.366, d. f=47, p≤0.001. Bone maturity had no differences with t=1.338, d. f=47, p≤0.187. The cortical index between gender for the same age group the differences was significant with t=5.951, d. f=47, p≤0.001; however, the cortical volume index had no differences in value t=-0.385, d. f=47, p≤0.702.

A comparison of the various variables for twenty-seven adolescent boys and twenty-one girls showed chronological age had differences between gender with t=8.521, d. f=20 while bone age differences t-2.815, d. f=20 and p≤0.011. Bone maturity had no differences between gender. However, the cortical index had differences which were significant with t= 2.941, d. f=20, p≤0.008; similarly, the cortical volume differences between gender were significant with t= -2.914, d. f=20, p≤0.009.

Paired t-test differences: For 107 children aged 6-17 the mean age 11.2±3.6years while the bone age was 10.1±3.6years and the differences were significant with paired t- test=10.595, d. f=106, p≤0.001 at 95%CL. The 60 (56.1%) boys and girls aged 6-10 years the mean chronological age was 8.4±1.4 and the bone age was 7.6±1.4years a paired test showed significant difference t= 7.7, d. f=59, p≤0.001 at 95%CL. Forty-seven 43.9% of adolescent boys and girls age 11-17 the mean chronological age was 14.8±2.2 and the bone age was 13.3±2.8. The differences were significant t= 8.238, d. f=46, p=0.000 at 95%CL.

When the children were considered b age and gender, fifty-nine 55.1%, boys aged 6-17 had a mean chronological age of 11.0±3.5years and the bone age was 10.1±3.4 years, and the difference was t=7.60, d. f=58, p≤0.000. The boys aged 6-10 years were 33 (30.1%) the chronological age 8.3 ±1.3 was while the bone age was 7.6±1.3, the mean differences were significant, t=5.749, d. f=32, ≤0.001 at 95%CL. The mean chronological age for the adolescent boys was 14.4±2.1 while the mean bone age was 13.2±2.7and the differences had t= 5.555, d. f=25, p≤0.001, at 95%CL.

Forty-eight, 44.9% of girls aged 6-17 had the mean chronological age of 11.4±3.8 years and the bone age was 10.2±3.7 years, and the mean difference had t=7.282, d.f =47, p≤0.001 at 95%CL. Twenty-seven girls aged 6-10 years had a chronological age of 8.4±1.5 years and the bone age was 7.6±1.5 years, the mean difference was significant with t=5.138, d.f=26, p≤0.001 at 95%CL. Twenty-one adolescent girls had a mean chronological age of 15.3±2.4 years while the bone age was 13.6 ±3.2 years and the differences had t=6.160, d.f=30, p≤0.001 at 95%CL.

Comparison between Boys and Girls by age: There were differences in the chronological age of fifty-nine boys, and forty-eight girls both age category 6-17 were significant with a paired t-test where t= 7.177, d. f=47, p≤0. 000. There were differences between the bone age of the boys and the girls in this same age group with t= 4.366, d. f=47, p≤0.000. Bone maturity had no differences with t=1.338, d. f=47, p≤0.187. The cortical index between gender for the same age group the differences was significant with t=5.951, d. f=47, p≤0.001 at 95%CL; however, the cortical volume index had no differences in value t=-0.385, d. f=47, p≤=0.702. Thirty-three boys and twenty-six girls showered differences between the chronological age and the bone age with a paired t-test with t=5.509, d. f=26, p≤0.001 at 95%CL, however bone age showered no differences between gender with t=1.220, d. f=26, p≤0.234; similarly bone maturity had no differences t=1.036, d. f=36, p≤0.310. The cortical index had differences with t=6.114, d. f=26 and, p≤0.001 at 95%CL; but the cortical volume did not show differences with a t= -0.511, d. f= 26, p≤0.613 at 95%CL. A comparison of the various variables for twenty-seven adolescent boys and twenty-one girls showed chronological age had differences between gender with a paired t-test where, t=8.521, d.f=20, p≤0.001 while bone age differences t-2.815, d.f=20 and p≤0.011. Bone maturity had no differences between gender. However, the cortical index had differences which were significant with t= 2.941, d.f=20, p≤0.008; similarly, the cortical volume differences between gender were significant with t= -2.914, d.f=20, p≤0.009. The chronological age for 107 children aged 6-17 years was compared with the bone age, and the differences were significant t= 10.595 d. f=106, p≤0.001 at 95%CL. The differences between chronological and bone age for sixty children aged 6-10 years was substantial with t=7.709, d.f =59 p≤0.001 at 95%CL. Similarly, 47 (43.9%) adolescent aged 11-17 had their chronological age compared with the bone age, and there were significant differences with t=8.238, d. f=46, p≤0.001, at 95%CL.

Boys and girls 6-17 years paired t-test: Differences were noted between gender for 59 (55.1%) boys and 48 (44.9%) girls when a paired samples t-test was performed for chronological, and bone age with the respective values as t=7.717. DF=47, p≤0.001 and t=4.366, DF=47, p≤0.001 at 95%CL. Also, the differences between the cortical bone index and for the boys and girls in the 6-17 age category was significant where t=5.951, d-f 47, p≤0.001 at 95%CL. However, no differences were observed with the paired samples t-test for bone maturity and the cortical bone volume where the respective values were t= 1.338, d. f=47, p= 0.187 and t= -0.385, d. f= 47 and p≤0.702 at 95%CL.

Boys and girls 6-10 years paired t-test: There were differences in the chronological age and cortical index between boys 33 (30.8%) and 27 (25.2%) girls aged 6-10 years based on a paired t-test samples analysis where

t=5.509, d.f =%6, p≤0.000 and t=6.114, d. f=26 p≤ 0.001 at 95%CL. However, there were no differences for the paired t-test sample analysis for bone age, bone maturity, and cortical volume index for gender and the same age group.

Boys and girls 11-17 years paired t-test: Adolescent boys aged 11-17 were 26 (24.3%), while the girls were 21 (19.6%) and significant differences were noted between the chronological ages for boys and girls in the adolescent group when a paired samples t-test, was done where t= 8.521. d.f=20, p≤0.001, bone age t=2.815, d. f=20, p≤0.011; cortical index t=2.941, d. f=20, p≤0.008 cortical volume index t=2.914, d.f =20 p≤0.009 at 95%CL. However, there were no differences in bone maturity between gender with, t=1.185, d. f=20, and p≤0.250 at 95%CL.

Linear regression with chronological age as the predictor: Linear regression was done for the boys categorised as 6-17 while controlling for chronological age. There was a linear relationship between the chronological age, and bone age with beta=0.963, t=27.162, p≤0.001 at 95%C. However, there was no linear relationship between bone maturity and chronological age beta=0.235, t=1.827, p≤ 0.073 at 95%CL. A linear relationship which was positive and robust was observed between the chronological age and cortical index where, beta=0.855, t=12.434, p≤0.001 at 95%CL. Also, the cortical volume index had a significant positive linear relationship with the chronological age beta=0.731, t=8.08, p≤0.001 at 95%CL.

There were 48(44.9%) girls aged 6-17 years whose bone age had a linear relationship with the chronological age beta=0.954, t=21.583, p≤0.001 at 95%CL. However, chronological age had no linear relationship with bone maturity beta=0.250, t=1.753, p≤0.086. Cortical index and cortical volume bone index had linear relationships with the respective beta =0.630, t=5.497 and p≤0.000 and beta=0.659, t= 5.941, p≤0.001 at 95%CL.

Boys and girls 6-10: A linear regression analysis on data for boys aged 6-10years showed that there was a strong linear relationship between chronological age and bone age beta=0.857, t=9.249, p≤0.001 at 95%CL. However, bone maturity had no relationship with chronological age beta=0.290, t=1.687, p≤0.102. The cortical index had a strong positive linear relationship with chronological age beta= 0.833, t=8.368, p≤0.001 at 95%CL; while the cortical volume index there was a strong positive association beta=0.754, t=6.385, p≤0.0001 at 95%CL.

Adolescent boys and girls aged 11-17: Chronological age for the girls in this age group had a linear relationship with bone age, cortical index, and cortical volume index. The respective regression values were beta=0.837, t=7.654, p≤0.000; beta=0.828, t=7.374, p≤.0001 at 95%CL, beta=0.844, t=7.871, p≤0.000 at 95%CL. However, bone maturity insignificant and non-linear relationship with chronological age with beta=0.263, t=1.364, p≤0.185 at 95%CL.

Linear regression with bone age as the predictor: Bone as the predictor for a boy aged 11-17 years there were strong positive linear relationships between chronological age for the boys and the variables for bone age beta=0.839, t=7.569, p≤0.000 no relationship between chronological age and bone maturity beta=0.138, t=0.683, p≤0.501. The cortical index had a linear relationship, beta= 0.810, t=6.774, p≤ 0.0001 at 95%CL; while cortical bone volume index beta=0.714, t=5.001, p≤0.001 at 95%CL. When bone age for the girls aged 11-17 was the controlling factor the parameters, chronological age, bone maturity, cortical index and cortical volume index had strong positive associations with chronological age. Bone age beta=0.929, t=, 10.981, p≤0.001 at 95%CL; bone maturity had a strong negative linear association beta= -0.632; t=-3.556, p=0.002, cortical index beta=0.932; t=11.219 and p=0.001 at 95%CL. Bone maturity was used as the predictor factor for ages 6-17 6-10 and 11- 17 in linear regression and it was observed than bone maturity had a linear relationship with the chronological age, bone age, cortical index, and cortical volume index for age group 11-17 years for combined gender, boys 11-17, and girls aged 11-17.

Linear regression with bone maturity as the predictor: One hundred and seven children aged 6-17 the bone maturity in relation to the other variables with linear regression with bone maturity was chronological age beta=0.231, d.f =2.436, p≤0.017; bone age beta=0-.032, d.f =0.333, p≤0.740; cortical index beta=0.267, d.f =-2.842, p≤0.005 at 95%CL, and cortical volume index beta=0-.267, d.f =-2.842, p≤0.005 at 95%CL.

The boys and girls combined gender 59 (55.1%) boys and girls ages 6-10 years and chronological age had a weak positive linear relationship with bone maturity with beta =0.279, t=2.210 and p≤0.031; bone age had a negative none linear relationship which was not significant with s beta value = -0. 251, d. f=-1.972, p≤0.053. The cortical index had beta= -0.150, d. f=-1.156, p≤0.252 while the cortical index beta =0-.226, d. f=-1.765, p≤0.083at 95%. The combined gender 47 (43.9%) boys and girls 11-17 years chronological age beta=-0.424, d. f=-3.136, p≤0.003at 95%CL; Bone age beta=-0.709, d. f=-6.748, p≤0.001 at 95%CL. The cortical index values for beta=-0.698, d. f=-6.540, p≤0.001 at 95%CL; cortical volume index beta= -0.706, d. f=-6.696, p≤0.001 at 95%CL.

Thirty-three (30.8%) boys age 6-10, linear regression with bone maturity was chronological age beta=0.27, d.f =2.210, p≤0.031, bone age beta=-0.251, d.f =-1.972, p≤0.053; cortical index beta=-0.150, d.f =-1.156, p≤0.252 and cortical volume index beta=-0.226, d.f =-1.765, p≤0.083 at 95%CL.

The linear regression for boys aged 11-17 with bone maturity as the predictor for chronological age, beta= -0.326, d.f =-1.688, p≤0.104; beta=-0.654, d.f =-4.235, p≤0.000; beta=-0.639, d.f =-4.066, p≤0.001; beta=-0.326, d.f =-1.688, p≤0.104 at 95%CL.

A linear regression for forty eight (44.9%,) girls age 6-10, where bone maturity was used as the controlling while the other variables the dependen,t hence chronological age beta=0.250, d.f =1.753, p≤0.086, bone age beta=-0.015, d.f =-0.105;, p≤0.917; cortical index beta=-0.400, d.f =-2.963, p≤0.005 and cortical volume index beta=-0.341, d.f =-2.457, p≤0.018 at 95%CL.

Twenty seven girls aged 6-10 years the chronological age did not have a linear relationship with bone maturity beta=0.263, d.f=1.364, p≤0.185, bone age value were beta =-0.295, d.f=-1.545, and p≤ 0.135, The cortical index beta =-0.284, d.f=-1.479, and p=0.152; and cortical volume index beta =-0.272, d.f= -1.411, and p=0.171 at 95% CL. Twenty (18.7%) girls aged 11-17 years chronological age beta=-0.632, d. f=-3.556 p≤0.002; bone age, beta=-0.811, d.f -6.046, p≤0.001 at 95%CL. The cortical index -0.806, d.f = -5.931, p=0.001 at 95%CL, and cortical volume = -0.783, d. f= -5.488, p≤0.001 at 95%CL.

DISCUSSION

The borehole water throughout twelve months had a mean fluoride content of 9.9mg/L while the surface water had a mean fluoride content of 0.655. The high fluoride in water had an inverse relationship with chronological age p=0.001. Bone as an organ has the highest content of calcium which forms hydroxylapatite crystals in the bone trabeculae found in the bone matrix. In the presence of fluoride, the hydroxyl in the appatite crystals is displaced, and calcium fluoride crystals are formed. The calcium fluoride bond is a strong bond which is not easily broken hence there is an accumulation of fluoride in the bone. The skeletal tissues are also affected when exposed to high concentration of fluoride for prolonged periods leading to skeletal fluorosis, a crippling disease that is characterised by discomfort, pain and rigidity of the bones and joints of the body. Osteoporosis, osteomalacia and osteopenia occur in severe cases \ and the condition has a very high incidence of morbidity.[28] The high fluoride content may be associated with the amount of uptake associated with cellular growth and development. At the embryonic stage when bone formation is taking place, and the cells are multiplying and differentiating fast there may be a higher uptake of the fluoride by the cells for mineralisation. At birth and as the child passes through adolescence, the fluoride uptake by the cells is reduced based on the fact that there is a reduction in bone matrix mineralisation and less bone matrix is being laid down as the child matures. Also, the predetermined mineral content by tissue and organ type may have acquired full the predetermined organ mineral content saturation. In the case of the low fluoride, the small quantities are taken up for a longer period hence low fluoride content may be beneficial in the early stages of development. The lack of association between the cortical index and the cortical volume index, the two indices may not be sensitive as they are dependent on the bigger represent in bone width and the smaller diameter representing the medulla of the bone. The high fluoride in water may be associated with the observations of children whose bone appeared to have osteoporotic lesions, and they did not complain of pain. Also, there was bowing of the leg, but the joints were not deformed. However, only two siblings who had a history of genu vulgar whose joints were swollen painful and bowered, and the fluorosis in their dentition was TF scores 8-9. Other studies have associated bone deformities with nutritional deficiencies', hormonal imbalances in children and adolescents.[12] In the X-rays the delineation between the cortical bone and the medullar was not clear, it was like a merging of the two sections thus giving the bone a "moth" eaten bone structure appearance. The bone with a moth-eaten appearance would not be adequately strong to support the increasing weight in a growing child or adolescent, and this would result in the bowing without pain which was observed in some children. In this study osteoporosis and sclerosis in the bone structure was not displayed. A 'moth' eaten appearance may be associated with osteoid bone which has been inadequately mineralized. Although fluoride has been used in low doses to enhance tooth and bone health.[25] However large doses of fluoride in an expectant mother have been documented to cross the placental barrier reaching the cells of the embryonic foetus through fetal blood. Fluoride has been reported to affect the human embryonic stem cell. Low fluoride of about 2mmM/dl concentration in the form of sodium fluoride (NaF) has been reported to affect the human embryonic stem cells (hESCs. The effect of fluoride occurs during the embryonic stage when there is cell proliferation, differentiation and viability. In the presence of the minute fluoride concentrations, there is a compromise in the cell pluripotency. However, when the fluoride dose was increased above 2mM/dl, the hESCs greatly reduced in proliferation, differentiation and viability. Higher doses occasioned cell death by affecting the hESCs at a subcellular level where there is an alteration in the mitochondria membrane potential (BMP). It also affects caspase as it activates the cell oxygen reactive process where there is an increase in phosphor-kin-N-terminal kinase.[26,27] Also, a child born and living in the high fluoride are is still exposed to high fluoride doses particularly during the high-velocity bone growth increments.[26] The difference in between chronological age and bone age which were significant with a paired t-test with p=0.001 may be due to for dietary factors associated with fluoride in the drinking water which was 9.9m/L. current studies have reported fluoride to interfere with metabolic processes in the human body.

The chronological age 11.2± 3.6years for combined gender for children age 6-17 was different from the bone

age 10.1± 3.5 years while the bone maturity was 1.04±1.05 years. The differences between the bone age and the chronological ages were significant, paired t-test p=0.001 the mean cortical index was bone, 0.38± 0.09 and the cortical volume index was 0.065± 0.015. The mean age for 6-10-year-old boys was 8.4±1.5 years, and it was advanced when compared to the bone age of 7.6±1.5 with a mean bone maturity of 0.80± 0.85 years. The difference was significant paired t-test, with =0.001. The cortical index for the boys was ±0.0540.32 while the cortical volume index was 0.062 ±0.013.

Similarly, for the girls in the same age category the chronological age 8.4 ±1.4 years was not the same value as the bone age 7.6 1.5 with a bone maturity of 0.80 ±0.84 The delay in bone age was significant with a paired t-test p=0.001, The mean cortical index for the girls was 0.38± 0.07 and the cortical volume index was 0.058± 0.011.

A comparison of the mean chronological age for the combined boys and the girls both aged 6-10 showed differences with the paired t-test p=0.001. Similarly, the bone age between the same age group was different, p=0; 001. However, there were no differences between bone maturity between gender with a paired t-test, p=0.187. Comparison of the cortical index for boys and the girl's age group 6-10 showed differences which were significant with p=0.001. However, there were no differences in the cortical volume index, p=0.702.

The mean age for the combined gender for an adolescent was 14.8± 2.2years while the bone age was 15.3± 2.8 years and bone maturity1.5± 1.2 significant differences were noted between chronological age and bone age with a paired t-test where p= 0.001. The cortical index was 0.42± 0.09 while the cortical volume index was 0.07±0.017.

For the adolescent boys, the chronological age was more advanced than the bone age. Hence the bone age lagged behind the chronological age by 1.3±1.09years, and the difference was significant with a paired t-test, p=0.001. The cortical index for the adolescent boys was 0, 41±0.086and the cortical volume index 0.077±0.017.

The boys and girls aged 6-10 years their variables of chronological age, the cortical index had significant differences with p= 0.001. However, there were no differences in bone age p=0.234, bone maturity with p=310 and in the cortical volume index p=0.613.

The mean age for the adolescent girls was 15.3 ±2.4 while the bone age was 13.6±3.2 and bone maturity of 1.17±1.13. Significant differences were observed between bone age and the chronological with a paired t-test, p=0. 001. The mean cortical index for the adolescent girls was 0.172±1.33, and the cortical volume index was 0.433±0.10 while the cortical volume index was 0.065±0.016.

A comparison of the various variables between adolescent boys and girls showed a difference in chronological age was different p=0.001. There were differences in bone age paired t-test, =0. 000. Cortical index showed differences p=0.008 and cortical volume, p=0,009. However, there were no differences with bone maturity=0.250. Bone maturity in all the age groups when the boys were compared with the girls showed no difference even when there were significant differences between the bone ages in both boys and girls.

There was a broad range in bone maturity from minus -0.06 years up to 5.2 years may be an also individuals whose bone maturity was ahead of the chronological age by five years. This wide range in bone maturity may be an indication of a delay or interferences in bone mineralisation associated with fluoride interferences in development and metabolic processes. Also, it may be associated with the differences in adequacy of dietary intake based on household or the individual child and family genetic predisposition to fluoride toxicity. However, when the group was categorised by gender and into age, it was noted that bone age for the pre-pubertal girls lagged behind the chronological age by 0.8±0.85 years (9.6 months). Studies have reported a delayed bone age for 254 pre-pubertal African American.[28] The sixty pre-pubertal boys and girls in the current study had a mean bone age of 8.4 ±1.4 and a bone maturity of 0.7 ± 0.75; there was no correlation between bone age and maturity with a Pearson's correlation r=- 0.251, p= 0.053. The cortical index for the pre-pubertal girls and boys was 0.305±0.07 while the cortical volume index was 0.060±0.012. Bone age and cortical index correlation r= .837, p=0.001 and while the relationship between bone age and the cortical volume index was r=0.869, p=0.001. These findings are not in agreement with Walker et.al.; where they reported no significant difference.[29]

Thirty-three pre-adolescence boys had the respective a mean chronological 8.3 ±1.3; a bone age of 7.6 ±1.3 years and a mean bone maturity was 0.6 ± 0.7. The differences between the chronological age and bone age in all age groups were significant t-test p=0.000. These substantial changes between chronological age create a problem in cases where the treatment of a child is based on age. In particular, children whose bone age is delayed may have higher treatment relapses compared to children whose bone age and chronological age may be close. Delayed bone age may also create challenges in forensics when the age of the child is unknown particularly when age is a need for identification purposes the individual may be a low age. The differences in bone age with chronological age may be a particular problem for countries handling refuges and identification such as passports; identification cards are needed. The bone age though slightly delayed it is close to the bone age and maturity for pre-adolescent African American boys. The mean bone cortical index was 0.32±0.05 while the cortical volume index was 0.062±0.013. The bone

maturity of sixty-five pre-pubertal African American boys has been reported to be 0.12± 0.68. Studies have indicated that the Greulich and Pyle method under reports up to 0.3yrs the males in this study is 2.5 times delayed when compared to the African American.[28] The pre-pubertal girls had the mean chronological of 8.4 ±1.5; a bone age of 7.6 ± 1.5 years and a mean bone maturity was 0.80±0.84. The bone age for the girls seemed to lag behind the chronological age by half a year. When compared to the was 0.05 ±0.64, the girls in the present study are 16 times behind the African-American girls.[28] The differences may be associated with dietary intake and the prepubertal African American girls whose bone maturity periods of onset of menarche and puberty. Also, in the current methodology the Tanner and White methodology while in the African American the Greulich and Pyle method was used. Though the sample was African American, the children in the current study were drawn from the Bantu ethnicity which may be different from the African American.[28]

In the current study of twenty-one girls whose mean chronological age was 13.7± 2.0 years had a bone age and maturity were 11.9 ± 2.1 and 2.0±1.3 years respectively. The average bone age for the adolescent girls is within the range reported by Wenzel et al. as 11.6 ±1.26 and 12.3±1.47years for two groups of thirty-nine and thirty girls age between 11-15 years. The fluoride content in the water in Arusha in Tanzania was 3. 6ppm.[30] However, the mean bone maturity of the girls in the present study is 2.0±1.3years delayed compared to the Wenzel et al. study where the girl's reported mean bone maturity was 0.3 ±1.164 and 0,4±1.128.[30] In the current study who used water with a fluoride content of 10ppm had a bone maturity that was 5-6.7 times retarded when compared to the Arusha girls who were using water with a fluoride content of 3.6ppm.

In the current study when the children have categorised into pre-adolescents and adolescents, the boys had a mean bone age of 8.0 ±1.7 and a bone maturity of 0.2±2.4 years. Thou, the bone maturity for both the males and the girls, were close to what was reported or healthy African American European boys and girls the standard deviation was extremely broad varying between minus 2.4 and 2.4 years. The wide variation in bone maturity may be an indication that bone age and bone maturity were not isochronized distributed and there may be other underlying factors.

Bone age and maturity are significant in establishing the stages of growth of individuals in pediatric dentistry and orthodontic treatment. Hence, for appropriate treatment to be given at a suitable time, the correct age has to be used. An adolescent with delayed growth may result in a relapse of the treated dental condition. Mora et al. reported the bone maturity for sixty-five and thirty-nine pre-pubertal and pubertal groups of African American boys to be 0.12± 0.68 years and minus 0.32 ±1.14 two years.[28]

The mean chronological age for the 6-17 children and adolescents was 11.2±3.24 while the bone age was 9.84±2.79 giving a bone maturity of 1.36 years. This show that the bone maturity for the children aged 6-17 was delayed by 1.36 years (16.32 months). The cortical bone thickness for the preadolescent girls was aged 6-10 was compared with the cortical bone index girls aged 11-17. The increase in the cortical bone index between girls aged 6-10 and those aged 11 to 17 were found to differ with paired t-test p=0.001. When bone volume index for the preadolescent girls was compared with that of the adolescent girls, it was noted that the differences were significant with p=0.001 at 95%CL. The differences in the cortical index for pre-adolescent and adolescent were significant p=0.008 at 95%CL.

Similarly, a comparison of the preadolescent and adolescent bone volume shoved significant differences p=0.009. The cortical index is a measure of the thickness of the cortical bone region, and several risk factors are known to affect healthy cortical factors. The reported cortical bone index values for Caucasians boys is 0.10 to 0.53 while for the girls it ranges between 0.46-62.[29,31] The normal values for cortical volume index for both boys and girls were reported as 0.095.[23]

In this study the cortical bone index for the boys aged 6-17 whose mean age was 11.4 was 0.36, the 6-10 age group had a mean age of 8.4 years with a cortical index of 0.32 while the adolescent boys with a mean age of 14.4 years the cortical index was 0.42. There was an increase in the cortical bone index between the preadolescent boys and the adolescent with a p=0.001.

The adolescent boys aged 14.4 years in the current study had a cortical index of 0.42 which was close to the cortical index of 14 years old boys with dental fluorosis from Kruidfontein South Africa whose index was 0.41. However, the cortical index for the adolescents in this study of 0.42 was lower by between 6-25% than what was reported for the Bantu urban, rural and Caucasian adolescent boys in South Africa whose respective cortical index was 0.45, 0.45 and 0.56. Similarly, the current study adolescent index of 0.42 was lower by 32% of what Garn 1961 reported for Caucasian boys whose cortical index was 0.62.[31] The bone volume index for the preadolescent boys was 0.069 for boys aged 6-17years, 0.063 for 6-10 and 0.077 for the adolescent boys. The increase in cortical bone volume from 0.063 to 0.077 for pre-adolescence to adolescence was significant with paired t-test =0.001. No studies are reporting on the cortical bone index for pre-adolescents in a high or low fluoride area. The findings of the cortical volume index of .077 for adolescent boys whose mean age was 14.4 was lower than that adolescent boy from Kruidfontein who had dental fluorosis whose cortical volume index was 0.081.

Similarly, the current study index of 0.077 is lower by between 8-23% than that of the urban and rural Bantu

whose index was 0.084 while the Caucasian adolescent had a cortical volume index of 0.1 in the South African study. It was also lower than what was reported by Exon-Smith whose study reported a cortical volume index for Caucasian adolescents of 0.095.[29] The mean cortical volume index for the girls aged 6-17 with a mean age of 11.4 years was 0.40, girls aged 6-10 had a mean age of 8.4 years, the index was 0.38. The adolescent girls with a mean age 0f 15.3 years had an index of 0.57. The difference in the cortical volume index between the preadolescent and the adolescent girls were significant p=0.00.

In the current study, the preadolescent girls had a cortical bone index of 0.38 which was lower than the adolescent cortical index of 0.57 and the difference was significant paired t-test, p=0,001. There were no research findings on the cortical bone index on girls aged 6-10 whose mean age was 8.4 years whose cortical index was 0.38 to compare with currently. The mean cortical index of 0.57 for the adolescent girls whose mean age was 15.3 years was the same as what was reported by Walker et al., for girls aged 14 years who had dental fluorosis in Kruidfontein in South Africa. However, it was lower by 33% of the cortical index as reported for the Kruidfontein adolescent girls who had dental fluorosis. However, the cortical bone index of 0.57 for the adolescent girls in the current study was higher by 5% than the cortical index of 0.46 and 0.54 for Bantu girls in the rural and urban communities respectively, in the South African study. The adolescent girls in this study had their cortical index lower by 8% compared to what has been reported for Caucasian American girls whose index was 0.62 and the South African Caucasian girls whose index was 0.61.[29,31]

The mean respective cortical volume for the 6-17 years 0.100, 6-10 years 0.058 and 11-17 years 0.065. The differences between the cortical bone volume for the pre-adolescent and the adolescent girls were significant paired t-test=0.001.

However, the cortical bone volume of 0.065 for the adolescent girls whose mean age was 15.3years was lower than the cortical volume of 0.086 for the adolescent girls with dental fluorosis from Kruidfontein South Africa 24%. The cortical volume index was also lower than that of the Bantu rural, and urban l adolescents whose cortical volume index was 0.083 and 0.094 respectively by between 21-33%. The cortical volume index 0.095 reported by Exon Smith was also higher by 32% than the current report of 0.065.[23,29] The cortical index was 0.37±0.006 while the bone volume index was 0.22±0.07. The metacarpal indices in these study are lower than what Alexander et al. reported South African Bantu boys and girls children aged 14, and from anon, fluoride areas had low cortical index 0.45± 0.07 for the boys, 0.54±0.06 for urban girls and 0.57±0.10 rural girls.[23] The respective values for cortical index and bone volume index for the boys were 0.35±0.06, and 0.07±0.01 which are lower than the girls mean cortical and bone volume indices of 0.39± 0.05 and 0.059±0.004. The cortical and bone volume indices for both genders are found to be lower than that of the South African Bantu adolescents without dental fluorosis and those with dental fluorosis.

The mean bone age was 11.4 ± 2.6 and bone maturity was 1.9 ±2.4, this value showed that the boys from this high fluoride area had a bone maturity which was 6.3 times behind the mean bone age for healthy European African America whose bone maturity was 0.3 years. In this study, the average bone maturity of 2.0±1.3 years for the adolescent girls lacked behind by ten times that of the healthy European African American girls. The adolescent girl's cortical index was 0.38± 0.06 while the bone volume was 0.057 ±0. 008. The cortical and bone mass indices in this study are lower than what was reported healthy urban and rural adolescent girls with dental fluorosis and those without dental fluorosis. There were strong positive correlations between chronological age and cortical index where r= 0.783, p=0.000, chronological age and bone volume index r=0.7056, p=0.002. Bone age and bone volume index r=0.08499, p=0.000 with p at 95%CL. It has been documented that juvenile skeletal fluorosis occurs during the period of bone growth and may present as deformities of the long bones. The bone deformity is prevalent in the weight-bearing bones. Bear weight and this may be accentuated during the period of adolescence due to an increase in a growth spurt. The growth spurts in adolescence are related to an increase of the growth hormones.

The number of children whose chronological age was between 6 – 10 was twenty-three, but when the bone age was considered, the number increased to 28. A delay in the bone maturity resulted in the older children being counted in the pre-adolescent age group when bone age and maturity were considered.

The cortical volume index of the adolescent in this study was 66% lower than for the girls from Kruidfontein who had dental fluorosis and urban Pretoria South African Caucasians whose values were 0.56 and 0.010. Also, the cortical index o the boys were lower by when compared to the Bantu.[23]

The chronological age had no association with the cortical index also the bone age had any relationship with the cortical index? However, the bone volume index showed a strong correlation with and bone age with p=0.000. The strong association was observed for both the pre-adolescent and the pubertal group. Perhaps this may be an indication that the bone volume index which is a measure of the bone microstructure may be a more reliable index in determining bone age than the cortical bone thickness.

Skeleton fluorosis is reported to be more common in adolescent males than females. However, in this study

pre-adolescent girls were more affected than the boys as their mean skeletal age was 7.7 ± 1.3 compared to the boys whose bone age was 8.0 ±1.7. In this case, the differences may not be associated with hormonal changes but differences in possible dietary intake. A balanced diet with adequate calcium has been advocated to minimise the toxic effects of fluoride on bone tissue.[12,23]

This community had three existing sources of water with low levels of fluoride ranging between zero point two and zero point six five. The village therefore only needed education on the consequences of drinking water with a fluoride content of 10pp on the health of the community as a whole. The low fluoride sources were within WHO recommend a dose of zero point five to one point five mg per litre; hence there was no need for fluoride removal in the water with 0.65 ppm.

Earlier, Opinya and Imaligant reported on an adolescent from the same community had been diagnosed as genu valgum. The adolescent had had corrective surgery of the limbs at age nine years, but the condition had recurred. The situation got worse at the time of the study until the young man had difficulties walking long distances or running.[32]

This difference in severity of bone changes and delayed bone maturity may be associated with the onset of hormonal changes during puberty and particularly girls whose three stages of increased bone growth spurt velocity has been linked with menarche.

Bone is highly dynamic tissue, and it can remodel when the toxic sources of fluoride to the osteoblasts and osteoclasts are removed. The increased bone changes may be observed in the adolescent boys and girls who were siblings of the pre-adolescents had bone ages 11.4 ± 2.6 and 11.9 ± 2.1years respectively. The same was noted for maturity which is close 1.9 ±2.4 and 2.0 ± 1.3 though delayed. In the current study, the delay in bone maturity was associated with osteoporosis which was highest in the pubertal females who had a mean bone age of 7.7 years and a mean chronological age of 13.7± 2.0years.[24] Bone age is a measure of skeletal development which contributes to the cumulative growth of an individual.[26] There are no studies yet which have been done in Kenya and reported on normal skeletal and bone maturity about chronological in Kenyan children.

Bone maturity has been found to be useful in the determination of dental-facial growth about malocclusion (source). The rate of maturation of the bones of the face which consist of the bones of the base of the skull the maxilla and mandible have been associated with both horizontal and vertical growth of the face the hand and wrist bone maturity.[33,34,35] Delayed bone maturation is significant in the practice of dentistry as facial growth changes may influence patterns of tooth eruption and dental treatment. In the case of females living in a high fluoride area and having a daily dose of fluoride which is beyond the recommended may have delayed skeletal maturity which would affect the dental treatment; hence there is need to examine environmental conditions which may influence.

Wenzel et al.; reported that there was no relationship between fluoride in water (3.6 ppm) and bone maturity. However; they documented an association between the degree of severity of bone fluorosis and dental fluorosis.[27] The current community already had three sources of water with low levels of fluoride ranging between zero point two and zero point six five though not near to the households when compared with the high fluoride source which had a public tap close to the houses. The low fluoride sources were within WHO recommend a dose of zero point five to one point five mg per litre; hence there was no need for fluoride removal. The community therefore only needed education on the consequences of drinking water with a fluoride content of 10pp on the health of the community as a whole. Bone is dynamic tissue physiologically, and a change of water source could lead to minimising of the skeletal changes and healthy bone formation.

CONCLUSION

The mean bone age of the pre-adolescent males and females was close to the chronological age. In the adolescents, the bone age lagged behind the chronological age, and the boys were more affected than the girls. The cortical index and bone volume index showed differences between gender and age. Bone age differed from chronological age significantly. The result is significant in the practice of dentistry in particular orthodontics and surgical procedures which may impact negatively on bone growth.

Challenges

The measurement of the medullary diameter and the cortex thickness is dependent on the proper identification of the midpoint of the metacarpal. However, when the midpoint is not at the most hollow point both the cortex and medullary width may be increased and therefore may be a false report in an increase in the medulla. Possible challenges on the developing of radiographs involving 109 individuals there may be changes in the chemical.

Conflict of interest: I have no competing interests.

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