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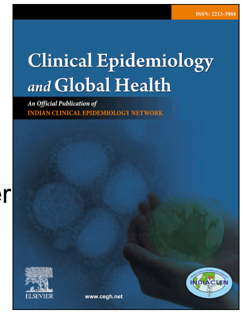


# Linear regression approach for predicting fluoride concentrations in maternal serum, urine and cord blood of pregnant women consuming fluoride containing drinking water

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## ABSTRACT

**Purpose:** The most common source of fluoride is from drinking water. Research comparing fluoride levels in maternal and cord blood have proved that fluoride crosses the placenta. But these results have not been correlated with the source of fluoride.

**Objective**: Firstly, to assess and compare the fluoride concentrations in urine, serum and cord blood of pregnant women consuming low/optimum and high fluoride concentrations in drinking water. Secondly, application of the linear regression analysis model equation for the prediction of fluoride concentration in urine, maternal serum and cord blood of pregnant women.

**Methods:** Based on the fluoride concentration in drinking water the subjects were divided into low/optimum and high fluoride groups. Each group consisted of 90 pregnant women. They were enrolled approximately one month prior to the due date. Fluoride was measured in their drinking water, urine, maternal serum, and cord blood.

**Results:** The low/optimum fluoride concentration in drinking water as compared to urine, serum and cord blood correlated significantly (r= 0.458, 0.529, 0.473). The strength of the correlation was found to be high in high fluoride group when compared to low/optimum fluoride group (r= 0.868, 0.943, 0.695). The β values in urine, maternal serum and cord blood in low/optimum group was 0.247, 0.025, 0.017 respectively and in high fluoride group 0.773, 0.080, 0.060 respectively.

**Conclusion:** As the fluoride concentration increases more than 1 ppm in drinking water the transfer of fluoride through urine, maternal serum and cord blood also increases. Linear regression analysis also displayed similar results.


**Key words**: Fluoride, pregnant women, urine, cord blood, linear regression

**Title: Linear regression approach for predicting fluoride concentrations in maternal serum, urine and cord blood of pregnant women consuming fluoride containing drinking water.**

**Introduction:**

Fluoride [$F^-$], the inorganic anion of fluorine, is commonly found in the natural environment. It is highly reactive due to its electronegativity resulting in the formation of organic and inorganic compounds. The sources of fluoride are natural and chemically synthesized. The natural sources are volcanic activity, water, food and soil. Chemically synthesized sources of fluoride include water (fluoridated drinking water, bottled water, perfluorinated compounds), food, soil, air and dental products like fluoridated toothpastes & mouthrinses.(1)

The daily intake of fluoride for optimum dental health benefits is 0.05 to 0.07 mg/kg body weight as per the American academy of Pediatrics(2). Consumption of fluoride in excess over a long period of time leads to detrimental effects like skeletal and dental fluorosis. Millions of people worldwide are affected by fluorosis and this endemic has manifested across all the continents. (3)

Fluoride prevalence, in India, was reported in 230 districts of 19 States earlier. As per recent data from Ministry of Drinking Water and Sanitation, there are 14,132 habitations (as on 1.4.2014) from 19 States which are yet to be provided with safe drinking water. The population at risk, based on population in habitations with high fluoride in drinking water is 11.7 million(4). The National Programme for Prevention and Control of Fluorosis (NPPCF) was a new health initiative during 11th Five Year Plan, initiated in 2008-09 and is being expanded in a phased manner. 100 districts of 17 States were covered during 11th Plan, further 11 districts were taken up during 2013-15 (over 19 States) and additional 84 new districts are to be taken up during the remaining period of 12th Plan(5).

Interventions taken by National Health Programs for Maternal Health to control the effects of fluoride include the promotion of improved supplementary nutritional support during pregnancy and lactation (ICDS), Improved antenatal care - including health and nutrition counseling, Enhanced maternity protection through the effective implementation of PMMVY(Pradhan Mantri Matru Vandana Yojana) and increasing awareness regarding harmful effects of fluoride(6).

Increased fluoride content in drinking water will cause fluorosis of teeth and bone, toxicity of fluoride, increased risk of hip fracture among the elderly, renal damage, osteoporosis, decreased IQ among children, hypothyroidism and cancer(7). High levels of serum fluoride damage the kidney while long term drinking of fluoridated water can be a risk factor in development of type 2 diabetes. Fluoride also appears to be mutagenic, genotoxic and causes chromosomal abnormalities.(8)

The supply of nutrients to and the removal of metabolites from the fetus is provided by the placental transport. Literature search suggests, the passage of fluoride across the placenta into the fetus is existent. Studies indicate transplacental passage of fluoride is about 30% to 87%.(9)

The most common source of fluoride is from drinking water. Fluoride content in drinking water may differ from one region to another. Research comparing fluoride levels in maternal and umbilical cord venous blood plasma have proved that fluoride crosses the placenta. But these results have not been correlated with the source of fluoride. One of the debatable issues could be as to what extent of fluoride will pass through the placenta in pregnant women consuming drinking water with low and high fluoride concentrations. Hence the present study was undertaken to assess and compare the fluoride concentration in urine and serum of pregnant women just before delivery and cord blood after delivery in low/optimum and high fluoride drinking water pregnant women. The linear regression analysis model equation for the prediction of fluoride concentration in urine, maternal serum and cord blood of pregnant women consuming drinking water with fluoride was applied.

**Methodology:**

Based on the fluoride concentration in drinking water the subjects were grouped into low/optimum fluoride group (fluoride concentration in drinking water ≤1 ppm) and high fluoride group (fluoride concentration in drinking water > 1ppm). The grouping was done in accordance with the values of fluoride concentration in drinking water by the Indian bureau of standards(10). Each group consisted of 90 pregnant women recruited from JSS hospital, Mysore. The pregnant women were enrolled approximately one month prior to the due date. The inclusion criteria comprised of primiparous healthy mothers between 18-25 years (non-smokers) with singleton fetus & uneventful full term pregnancy. The exclusion criteria was, women with complications during pregnancy and pregnant women not willing to give consent for enrolment into the study. Ethical clearance was obtained from the institutional ethics committee JSS dental college and hospital.( Ethical clearance certificate number –JSS/DC/Ethical/2016-17/1768) Written informed consent in accordance with the Declaration of Helsinki was submitted by all the subject.

**Fluoride analysis:**

The drinking water consumed by women during the course of their pregnancy was collected. Just before the delivery serum and urine fluoride levels of the pregnant women were assessed. Post delivery cord blood fluoride levels were assessed. A standardized instrument was used to measure the fluoride levels in line with the American Public Health Association (APHA) guidelines. A 10ppm standard fluoride solution and 0.5ml of Total Ionic Strength Adjustment Buffer (TISAB III) was used to sensitize the 9609BNWP fluoride electrode. The electrode was immersed in the electrolyte solution for 20 minutes before calibration and then the fluoride levels were assessed.

**Data management and statistical analysis:**

Data was collated into Microsoft excel sheet. Statistical analysis was carried out using SPSS-23 version. Descriptive analysis involved calculations for mean, standard deviation, frequency and percentages. Inferential statistics was obtained after application of unpaired't' test for comparison of two independent groups with data following normal distribution and non parametric Mann-Whitney test was applied to compare two independent groups with data following skewed distribution. The normality of the data was tested by Kolmogorov –Smirnov test and Shapiro Wilk test. Pearson Chi-square test was used to compare the frequencies and percentages. The association between the groups was established by Spearman's correlation test.

For mathematical equation prediction, simple linear regression analysis was performed. To run the analysis, drinking water fluoride levels of pregnant women were used as an independent variable and urine, maternal serum and cord blood fluoride levels were used as dependent variables. $R^2$ was calculated to assess the level of the variability of dependent variable with changes in independent variable.

**Results:**

The descriptive characteristics of the study have been summarized in Table 1. The mean age group of low/optimum and high fluoride group were 23.88(3.57) years and 24.13(3.85) years respectively. The difference between age groups was not statistically significant. The socioeconomic status, maternal education, gestation length, sunlight exposure, birth weight and length of newborn were also considered. Among these socioeconomic status and maternal education showed statistically significant differences. The results of the other variables revealed slight difference but were not statistically significant. The mean & SD of fluoride concentrations in drinking water of the low/optimum group was 0.50(0.28) and in high fluoride group 2.65(1.29). The fluoride concentration in urine, pregnant mothers' blood and cord blood was 0.20, 0.014 and 0.0110 respectively in low/optimum group and in high fluoride group, it was 1.91, 0.15 and 0.10 respectively. The comparison of means of fluoride concentrations in drinking water, urine, pregnant mothers' blood and cord blood between the two groups was found to be highly significant statistically ($p<0.000$).

Graph 1 and 2 outline the correlation of low/optimum and high fluoride concentrations in drinking water with urine, serum of the pregnant women and cord blood. The low/optimum fluoride concentration in drinking water as compared to urine, serum and cord blood correlated significantly ($r= 0.458, 0.529, 0.473$). The strength of the correlation was found to be high in high fluoride group when compared to low/optimum fluoride group ($r= 0.868, 0.943, 0.695$).

Table 2 sums up about the linear association between fluoride concentrations in drinking water with urine, maternal serum and cord blood. To find out the association, fluoride concentration in drinking water was used as an independent variable and maternal urine, serum and cord blood were used as dependent variables. Similar to correlation, in regression the β values of low/optimum fluoride group were found to be low when compared to high fluoride group. This indicates that compared to low/optimum group, in high fluoride group as one unit of fluoride concentration in drinking water increases, increased fluoride levels are seen in maternal urine, serum and cord blood. ($y=a+bx$ where a is constant, b is β, y is dependent variable and x is independent variable). $R^2$ explains the degree of variation in passage of fluoride through maternal urine, serum and cord blood. The high fluoride group had 83.7% variability in maternal serum followed by urine at 70.9%. The urine of low/optimum fluoride group had least variability 8.3%

Table 3 describes the overall association between drinking water fluoride and urine, maternal serum and cord blood fluoride. In this both low/optimum and high fluoride groups data were combined and regression analysis was performed. According to the equation $y=a+bx$ if we assume one unit increased fluoride levels in drinking water of pregnant women, we can predict an increase of 0.614 units of fluoride in urine, 0.043 units in maternal serum and 0.031 units in cord blood.

**Discussion:**

The fluoride concentration in pregnant mothers' drinking water was estimated and then the subjects were categorized into low/optimum and high fluoride groups. The other objective was to assess fluoride concentration in urine and maternal serum before delivery and cord blood after delivery in both the groups. The final objective was to compare urinary fluoride and placental transfer of fluoride in low/optimum and high fluoride groups. There are very few studies related to this topic and very minimal efforts have been made to find out the linear relationship between low/optimum and high fluoride drinking water pregnant women with urine, maternal serum and placental transfer of fluoride.

The mean fluoride concentration in drinking water in this study was found to be 0.50(0.28) in low/optimum group and 2.65(1.29) in high fluoride group which is higher than both the Indian standard and the fluoride level suggested by the WHO (1mg/L). The fact that drinking water was the source for the risk factors associated with human fluoride exposure was established by Grinaldo et al (11). An increase in fluoride levels are observed in the maternal blood & urine when the fluoride concentration increases in drinking water, as indicated by this present study. This outcome is in line with the studies previously conducted by Iftekhar Ahmed et al, Lizet Jarquin-Yanez et al and Christine till et al.(12,13,14)

Urine fluoride concentration was 0.20(0.24) in low/optimum group and 1.92(1.19) in high fluoride group. Urinary fluoride levels were significantly lower among women drinking water with low/optimum fluoride levels as compared to high fluoride group. Studies by Christine Till et al, Opydo- Szymaczek and Borysewicz-Lewicka, Lajya Devi Goyal et al have exhibited results similar to this(14,15,16).The same results were seen in adults and children consuming high fluoride drinking water(12,13,17). Morning urine samples can be a good estimator for assessing fluoride exposure. For the same purpose, in the present study, fasting urine were collected from the pregnant women of both low/optimum and high fluoride groups.

The results of this study show lesser transfer of fluoride through placenta in cases of low fluoride concentrations in drinking water, and more placental transfer of fluoride in high fluoride concentrations in drinking water of pregnant women. An inference that can be drawn here is that the placenta does not act as a complete barrier to the passage of fluoride into the foetus (cord blood) in both the groups. These results are consistent with studies done by Jopydo-Szymaczek, M Borysewicz, Iftekhar Ahmed et al (12,15). The correlation values were high in both maternal serum and cord blood in high fluoride group as compared to low/optimum fluoride group. Similarly linear association of β values was much higher in maternal serum and cord blood. Gupta et al noted that the placenta acted as a selective barrier when the concentration of fluoride in the mother's blood exceeded 0.4 ppm (18). The values of r and β of the present study indicate that as the fluoride concentration increases in the drinking water of the pregnant women placental transfer of the fluoride also increases.

Low uterine blood flow at the placental bed leads to lower transplacental transfer due to maternal age (19). Hence in this study, pregnant women of similar age were selected for both the groups. Excess fluoride exposures can lead to deleterious effects on the expecting mother and fetus and is associated with adverse pregnancy outcomes as concluded by Lajya Devi Goyal et al(16)**.** The effect of high fluoride on the IQ levels in children is still debatable. The long term effects of high fluoride concentrations in drinking water consumed by pregnant women on the fetus needs to be further investigated in future. Documentation of the results of the study would aid future health care workers in dealing accordingly with pregnant mothers from low/high fluoride containing drinking water regions (20). Hence, the present study recommends that necessary documentation should be done for all the complications associated with fluoride in pregnant women.

The conclusions of the present study was that as the fluoride concentration increases more than 1 ppm in drinking water the transfer of fluoride through urine, maternal serum and cord blood also increases compared to low/optimum levels of fluoride. Linear regression analysis also showed similar results. Careful review of the scientific literature and consultation with local and national experts has identified no evidence for adverse health impacts associated with drinking water fluoride at recommended levels. Dietary guidelines should be formulated according to the fluoride present in drinking water. Prevention via innovative technologies and implementation of these policies through government to provide safe drinking water and strict follow up should be done at frequent intervals.

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Conflict of interest : none

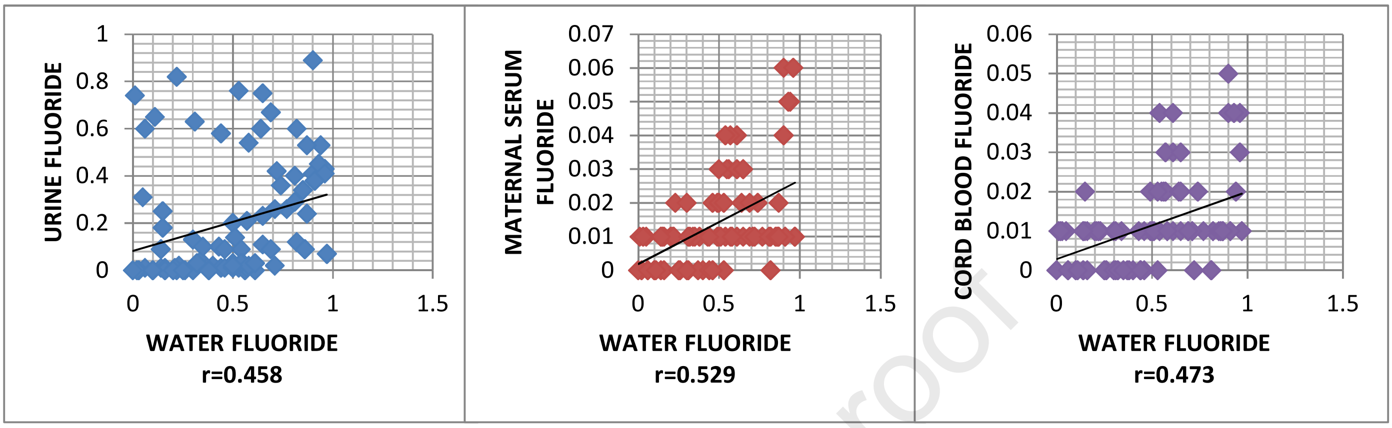
References:-


1. Sources of Fluoride Chart by the International Academy of Oral Medicine and Toxicology, IAOMT; www.iaomt.org Chart last updated 7/27/17, https://www.greenfacts.org/glossary/def/fluoride.htm.
2. Thippeswamy H M, Nanditha Kumar, Anand S R, Prashant G M, Chandu G N – Fluoride content in bottled drinking waters, carbonated soft drinks and fruit juices in Davangere city, India – Indian Journal of Dental Research – Nov-Dec 2010;21(4):528-530
3. World Health Organization (WHO), in: K. Bailey, J. Chilton. E. Dahi, M. Lennon. P, Jackson, J. Fawell (Eds.). Fluoride in Drinking-water. WHO Press. Switzerland. 2006.
4. Maternal Health :: National Health Mission nhm.gov.in
5. NPPCF new guideline by GOI.pdf cghealth.nic.in
6. Nutrition_Strategy_Booklet.pdf niti.gov.in
7. Thippeswamy, H.M., Devananda, D., Nanditha Kumar, M. *et al.* The association of fluoride in drinking water with serum calcium, vitamin D and parathyroid hormone in pregnant women and newborn infants. Eur J Clin Nutr (2020). https://doi.org/10.1038/s41430-020-00707-2
8. Maheshwari N, Qasim N , Anjum R, Mahmood R Fluoride enhances generation of reactive oxygen and nitrogen species, oxidizes hemoglobin, lowers antioxidant power and inhibits transmembrane electron transport in isolated human red blood cells Ecotoxicology and Environmental Safety *DOI: 10.1016/j.ecoenv.2020.111611*

9. Gurumurthy Sastry M, Shruti Mohanty, Sapna Vyakaranam, Aparna Varma Bhongir, Pragna Rao. Transplacental Transport of Fluoride, Calcium and Magnesium. NJIRM 2011; Vol. 2(3). July- September eISSN: 0975-9840 pISSN: 2230 - 9969 5.
10. https://www.geographyandyou.com/paid-articles/drinking-water-standards/
11. M. Grimaldo, V. H. Borja-Aburto, A. L. Ramirez, M. Ponce,M. Rosas, and F. Diaz-Barriga, "Endemic fluorosis in San Luis Potosi, Mexico. I. Identification of risk factors associated with human exposure to fluoride," *Environmental Research*, vol. 68,no. 1, pp. 25–30, 1995.
12. Iftekhar Ahmed, Tahir Rafique, Syed Khakan Hasan, Nazeer Khan, Masood Hameed Khan, Tanzil Haider Usmani. Correlation of fluoride in drinking water with urine,blood plasma, and serum fluoride levels of people consuming high and low fluoride drinking water in Pakistan. Fluoride 45(4)336–340 October-December 2012
13. Lizet Jarquín-Yañez, José de Jesús Mejía-Saavedra,Nelly Molina-Frechero, Enrique Gaona, Diana Olivia Rocha-Amador,Olga Dania López-Guzmán,and Ronell Bologna-Molina Association between Urine Fluoride and Dental Fluorosis as a Toxicity Factor in a Rural Community in the State of San Luis Potosi. Scientific World Journal. 2015;2015:647184. doi: 10.1155/2015/647184. Epub 2015 Feb 19.
14. Christine Till, Rivka Green, John G.Grundy, Richard Hornung, Raichel Neufeld, E. AngelesMartinez-Mier, Pierre Ayotte, Gina Muckle, and BruceLanphear. Community Water Fluoridation and Urinary Fluoride Concentrations in a National Sample of Pregnant Women in Canada. Environmental Health Perspectives 126(10) October 2018

15. J Opydo-Szymaczek,a M Borysewicz-Lewickaa Urinary fluoride levels for assessment of fluoride exposure of pregnant women in Poznan, Poland. Fluoride 38(4)312–317 November 2005
16. Goyal LD, Bakshi DK, Arora JK, Manchanda A, Singh P. Assessment of fluoride levels during pregnancy and its association with early adverse pregnancy outcomes. J Family Med Prim Care 2020;9:2693-8.
17. Sunil Kumar, Suman Lata, •Jyoti Yadav, J. P. Yadav Relationship between water, urine and serum fluoride and fluorosis in school children of Jhajjar District, Haryana, India. Appl Water Sci (2017) 7:3377–3384
18. Gupta S, Seth AK, Gupta A, Gavane AG. Transplacental passage of fluorides. *J Pediatr*. 1993;123(1):139-141. doi:10.1016/s0022-3476(05)81558-6
19. Malhotra A, Tewar A, Chawla HS, Gauba K, Dhall K. Placental transfer of Fluoride in pregnant women consuming optimum Fluoride in drinking water. J Indian Soc Pedod Prev Dent 1993; 11 (1): 1-3.
20. Khan MA, Nilima N, Prathibha J, Tiwary B, Singh M. Documentation compliance of in-patient files: A cross sectional study from an east India state. Clinical Epidemiology and Global Health 8 (2020) 994–997

Graph 1:-The correlation between drinking water fluoride and urine fluoride, maternal serum fluoride and cord blood fluoride among low/optimum fluoride group pregnant women



Graph 2:-The correlation between drinking water fluoride and urine fluoride, maternal serum fluoride and cord blood fluoride among high fluoride group pregnant women

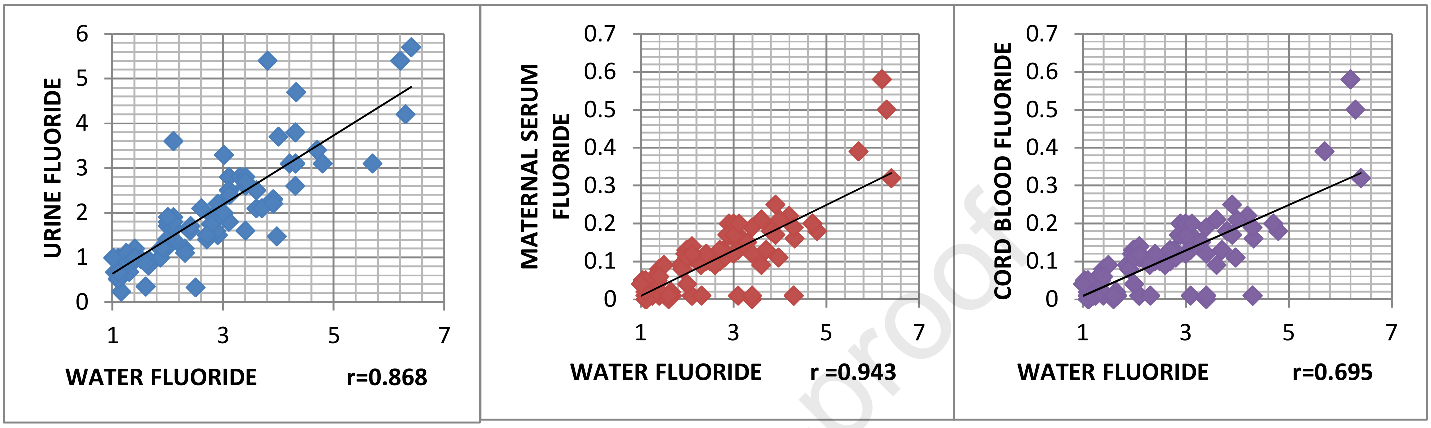


**FIG: 1 - FLOW DIAGRAM OF THE STUDY PROCEDURE**

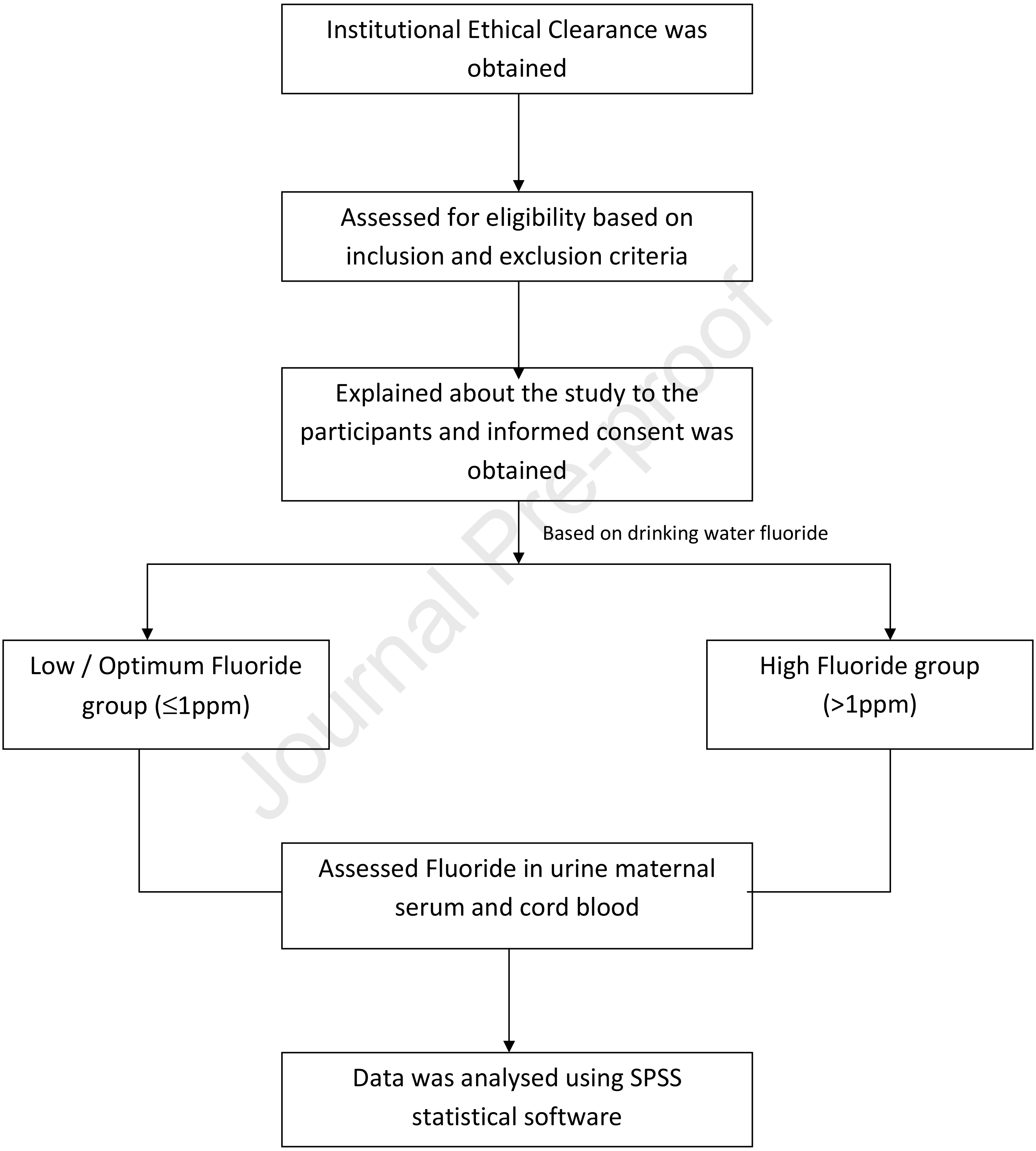


**Table 1: Descriptive characteristics:-**

| **Variables** | **Low/optimum** | **High** | **p-value** |
|---|---|---|---|
| Age (years) | 23.88(3.57) | 24.13(3.85) | 0.645 NS |
| Socioeconomic status | n (%) | n (%) | |
| | Class I-13(14.4) | Class I-3(3.3) | |
| | ClassII-26(28.9) | ClassII-18(20.0) | 0.000 HS |
| | Class III-31(34.4) | Class III-23(25.6) | |
| | Class IV-15(16.7) | Class IV-27(30.0) | |
| | Class V-5(5.6) | Class V-19(21.1) | |
| Education | Illiterate-4(4.4) | Illiterate-10(11.1) | |
| | Primary(1 to 4)-10(11.1) | Primary(1 to 4)-23(25.6) | |
| | Middle(5 to 7)-16(17.8) | Middle(5 to 7)-26(28.9) | 0.000 HS |
| | High school and PUC-32(35.6) | High school and PUC-23(25.6) | |
| | Degree and Diploma-28(31.1) | Degree and Diploma-8(8.9) | |
| Gestation length(weeks) | 38.31(1.13) | 38.47(0.97) | 0.321 NS |
| Gender | Male- 47(52.2)<br>Female- 43(47.8) | Male-44(48.9)<br>Female-46(51.1) | 0.766 NS |
| Birth weight(kgs) | 2.69(0.57) | 2.60(0.56) | 0.274 NS |

| | | | |
|---|---|---|---|
| Birth length(cm) | 49.24(1.38) | 49.14(1.37) | 0.599 NS |
| Drinking water fluoride level ppm | 0.50(0.28) | 2.65(1.29). | 0.000 HS |
| Urine fluoride ppm | 0.20(0.24) | 1.92(1.19) | 0.000 HS |
| Pregnant mothers blood fluoride ppm | 0.014(0.014) | 0.153(0.113) | 0.000 HS |
| Cord blood fluoride ppm | 0.011(0.011) | 0.11(0.10) | 0.000 HS |

**Table 2:-Simple linear regression analysis for low/optimum and high fluoride group:-**

| parameters | Constant(intercept) | | β | 95% confidence interval for β | $R^2$ | P-value |
|---|---|---|---|---|---|---|
| Urine | * | 0.082 | 0.247 | 0.074 - 0.420 | 0.083 | 0.006 |
| | # | - 0.136 | 0.773 | 0.668 – 0.878 | 0.709 | 0.000 |
| Maternal serum | * | 0.002 | 0.025 | 0.015 – 0.034 | 0.246 | 0.000 |
| | # | - 0.058 | 0.080 | 0.072 – 0.087 | 0.837 | 0.000 |
| Cord blood | * | 0.003 | 0.017 | 0.010 – 0.024 | 0.194 | 0.000 |
| | # | - 0.051 | 0.060 | 0.668 – 0.878 | 0.578 | 0.000 |

* = low/optimum fluoride group     # = high fluoride group

**Table 3:- Simple linear regression analysis after combining both low/optimum and high fluoride group :-**

| parameters | Constant(intercept) | β | 95% confidence interval for β | $R^2$ | P-value |
|---|---|---|---|---|---|
| urine | -0.161 | 0.775 | 0.724- 0.826 | 0.834 | 0.000 |
| Maternal serum | -0.027 | 0.070 | 0.066-0.074 | 0.885 | 0.000 |
| Cord blood | -0.020 | 0.051 | 0.046-0.056 | 0.686 | 0.000 |