Fluoride Exposure Via Drinking Water on Adolescents and its Associated Health Effects

By

Ahmad Khan

Registration No. 02312013013

DEPARTMENT OF ENVIRONMENTAL SCIENCES

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

ISLAMABAD, PAKISTAN

2020-2022

Fluoride Exposure Via Drinking Water on Adolescents and its Associated Health Effects

This work is submitted as a dissertation in partial fulfilment for the award of the degree of

Master of Philosophy

In

Environmental Sciences

By

Ahmad Khan

Registration No. 02312013013

DEPARTMENT OF ENVIRONMENTAL SCIENCES

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY, ISLAMABAD

To my family for their faith, endless love, support, and encouragement.

DECLARATION

I, **“Ahmad Khan” (Registration No. 02312013013)** hereby declare that my M.Phil. thesis titled as **“Fluoride exposure via drinking water on adolescents and its associated health effects”** is all my own effort done in Ecotoxicology and Environmental Biology Laboratory, Department of Environmental Sciences, Quaid-i-Azam University, Islamabad. All the investigations, findings, results, conclusions of this research have neither been previously presented anywhere nor published in any local or international forum.

Ahmad Khan

PLAGIARISM UNDERTAKING

I, Ahmad Khan, hereby state that my M.Phil. Thesis titled **“FLUORIDE exposure via drinking water on adolescents and its associated health effects”** is solely my research work with no significant contribution from any other person. Small **c**ontribution/help whatever taken has been duly acknowledged and that complete thesis has been written by me.

I understand zero tolerance policy of the HEC and Quaid-i-Azam University, Islamabad, towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any form of plagiarism in the above titled thesis even after the award of M.Phil. degree, the university reserves the right to withdraw/revoke my M.Phil. degree and that HEC and the university has the right to publish my name on the HEC/University website on which the names of students are placed who submitted plagiarism.

Ahmad Khan

CERTIFICATE OF APPROVAL

It is to certify that the research work presented in this thesis, titled "**Fluoride exposure via drinking water on adolescents and its associated health effects"** was conducted by **Mr. Ahmad Khan (Registration No.: 02312013013),** under the supervision of **Prof. Dr. Riffat Naseem Malik (T.I).** No part of this thesis has been submitted elsewhere for any other degree. This thesis is submitted to the Department of Environmental Sciences, in partial fulfilment of the requirements for the degree of Master of Philosophy in the field of Environmental Science, Department of Environmental Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

Ahmad Khan (M.Phil. Scholar)

____

Supervisor:

Prof. Dr. Riffat Naseem Malik (T.I.)

____

Department of Environmental Sciences,

Quaid-i-Azam University Islamabad.

Chairman:

Department of Environmental Sciences,

____

Quaid-i-Azam University Islamabad.

TABLE OF CONTENTS

List of abbreviation

RBS	Random Blood Sugar
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
S. creatinine	Serum creatinine
T. Bilirubin	Total Bilirubin
ALP	Alkaline Phosphatase
F^-	Fluoride
BMI	Body Mass Index
WHO	World Health Organization
USA	United State of America
CKD	Chronic Kidney Disease
eGFR	estimated glomerular filtration rate.

Acknowledgement

First and foremost, I thank Allah Almighty the most Beneficent and the most Gracious, who showered his blessings upon me throughout my life. I offer my praises to Hazrat Muhammad (S.A.W.W), whose life is a role model for whole Humanity.

I pay sincere gratitude to my supervisor **Professor Dr. Riffat Nasim Malik (T.I.)** Her professional experience and wide knowledge of science helped me to carry out my research. She has been a source of motivation and courage for me. She has taught me some real-life lessons like integrity, honesty, and courage. Her unconditional and sincere guidance and advice helped me to complete my work. Without her patience and assistance, I would not have been able to complete my research. The training. inspiration, affection and confidence and the things I have learnt from her will always be in my heart. Her mentorship and training will always be helpful for me.

I would also like to pay the deepest gratitude to Ami, Abu, Jibrail bhai, Mohammad and my beloved sisters for their prayers and constant support. Their faith and emotional support were a great source of strength for me.

I'm grateful to Jahanzeb, Zaheer Bugti, sabih-ud-din, Laiba Gul and rest of my friends, for their support throughout my degree. I'm also grateful to my all-lab mates, Muhammad Saeed, Maryam Mehboob, Nosheen shabbir, Rubab, Farman Ullah, Aimal Khan and our favorite lab in-charge Zulqarnain Haider. Special thanks to my batchmate Chaudhry Muhammad Saad Bashir, Amen Sana Rai, and Sana Rubab for their support. I'm thankful to my senior Hajra Batool for her moral and intellectual support throughout this time.

Ahmad Khan

Abstract

The issue of underground water contamination by fluoride has gained significant global attention due to its detrimental effects on public health. Geological processes, such as the presence of minerals like cryolite, fluorite, and other fluorine-bearing minerals, contribute to the deterioration of underground water quality. Extensive research conducted in various regions worldwide has revealed several health risks associated with fluoride exposure, including dental and skeletal fluorosis, neurotoxicity, and adverse effects on liver and kidney function. This growing concern emphasizes the necessity for comprehensive scientific investigations and appropriate interventions to mitigate the risks linked to fluoride contamination in underground water sources.

The impacts of elevated fluoride levels on various regions in Pakistan have been observed, particularly regarding the toxic effects of fluoride on the liver and kidneys of adolescents. For this study, rural areas in the districts of Lahore and Kasur were selected. The study encompassed a total of 119 participants who were categorized into two main groups: the exposed group and the control group. Within the exposed group, participants were further divided into four subgroups based on the fluoride concentration in the water: low (≥1.5 mg/L), medium (≥4 mg/L), high (≥6 mg/L), and very high (≥8 mg/L). Ninety-three participants resided in areas with high fluoride concentrations, while twenty-five participants belonged to the control area of the study.

A questionnaire was administered to the participants, collecting information on general demographics (age, gender, duration of residence in the area), presence of dental fluorosis, and family history of liver and kidney diseases. The fluoride levels in the water were measured on-site using a Hanna Low range fluoride meter, while fluoride levels in urine were determined using a fluoride ion selective electrode. Blood samples were obtained from the participants for the analysis of liver and kidney parameters, which were analyzed using a chemistry analyzer. Urine samples were also collected to measure fluoride levels.

The results indicated elevated levels of fluoride in both water and urine samples. Dental fluorosis was found to be prevalent in the area. Statistical analysis demonstrated that dental fluorosis was not significantly associated with gender or age variations. Furthermore, the findings revealed that fluoride accumulation had an impact on hepatic biomarkers, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in the participants belonging to the very high fluoride exposure group. However, no significant relationship was observed between nephrotoxicity and gender or age of the participants. Notably, statistical analysis indicated significant differences in urea, serum creatinine and estimated glomerular filtration rate (eGFR) levels among the various fluoride exposed groups. It should be noted that the present study did not include smokers; however, a few participants had secondhand smoke exposure. A comparison between participants with secondhand smoke exposure and those without smoking exposure revealed a statistically significant difference in urea levels between the two groups.

Considering the health impacts observed in this study, there is an urgent need to educate the population and implement effective policies to address water pollution in these areas.

Key Words: Dental fluorosis, skeletal fluorosis. Neurotoxicity, adolescents, Liver, Kidney

CHAPTER I

1. Introduction and literature review

Water is the utmost constituent of life on earth. It is decisive for all kinds of life. It makes the earth livable for humans and other living things. The importance of water is beyond description. There is no denying that water is essential for drinking, cooking, irrigation, agriculture, households, livestock, and personal hygiene (Ali et al., 2021).

From the beginning of human life, groundwater has been a primary water source. A big chunk of humans depends on groundwater to live on earth. Ground water is the single largest source of drinking water. It constitutes 97% of global fresh water. In most regions, it is the only economical and viable water source(Ali et al., 2016). This makes the availability of safe drinking water essential. Because water guarantees life in different spheres of human life.

However, the growing population, rapid industrialization, urbanization, and excessive and unskilled use of water resources have not only caused degradation of water quality but also significantly shrunk the per capita water availability (Aslam et al., 2019). Various natural and anthropogenic factors are polluting the water. Few polluting sources are deep percolation from cultivated fields, solid and liquid waste from industries, sewage, and surface impoundments. During its flow, water goes through different geological formations. These interactions lead to the contamination of shallow aquifers (Amini et al., 2011).

There are different hazardous contaminants, like fluoride, arsenic, heavy metals, nitrate, sulfate, pesticides, etc., in groundwater of different areas of Pakistan(Khattak et al., 2022). Polluted water is causing different diseases throughout the country. It has been established through literature that drinking water sources of some of the areas of

Pakistan have high fluoride than the permissible limit of WHO(Saeed et al., 2021). The impact of fluoridated water has not been well explored in Pakistan yet. Therefore, there is a dire need to research and assess the future impact of the excessive amount of fluoride in drinking water.

Table 0-1: Effects of fluoride on human health

Effects	References
Effects on intelligence	(Sexena et al.,2012)
Effects on IQ	(Saeed et al.,2021)
Adverse effect of fluoride on animals	(Choubisa&Choubisa,2016)
Structural changes and disinfection in the thyroid gland	Kheradpisheh et al.(2018)
Nausea, Vomiting and gastric pain	Nabavi et al.(2013)

1.1. Chemical Profile

Elemental fluorine is electronegative and reactive among the elements. It requires just one electron to complete its configuration of inert gases. It is pale irritating yellow-green gas in color. Fluorine has a sharp odor. It does not commonly occur in its elemental shape naturally. Fluorine combines itself with almost all of the elements except inert gases. It forms a firm electronegative bond. It is enlisted in group VII A of periodic table and it is the 9th elements on periodic table with 18.9984 atomic weight. It has been rated thirteenth in abundance and constitutes about 0.3g/kg of the earth’s crust. Fluorine produces elements like HF and Naf (ionic compounds) in water and disassociates itself from negatively charged fluoride ions (Helte et al., 2021).

1.2. Sources of fluoride and its availability

1.2.1. Natural Sources

Water, food, air, are the natural sources of fluoride through which it enters the human body. Fluorite is the natural source of the chemical in the soil. it is also the primary mineral of fluorine in nature (Younas et al., 2019). Fluorite occurs mostly in granite rocks. There is a 20-3600 ppm concentration of fluoride in granite rocks. Certain types of clays and villiaumite like apatite($Ca_5(PO_4)_3F$), amphiboles($Mg_7Si_8O_{22}(OH)_2$,hornblende$(Na,K)_{0-1}Ca_2(Mg,Fe,AI)_5Si_{6-7}Al_{2-1}O_{22}(OH,F)_2$,muscovite($KAl_2AlSi_3O_{10})(F,OH)_2$,),biotite($K(Fe^{2+}/Mg)_2(AI/Fe^{3+}/Mg/Ti)([Si/Al/Fe]_2Si_2O_{10})(O/F)_2$), micas($Al_2K_2O_6Si$), also contains fluorine.

The Punjab plains of Pakistan are situated west of the Indo-Gangetic basin. This alluvial plain is considered as largest alluvial plain in the world (Tahir and Rasheed, 2013). The accumulation of the sediments forms it. These sediments are the results of Himalayas erosion(Rezaei et al., 2017).

1.2.2. Presence of Fluoride in Soil

Fluoride presence in soil is widespread and normally ranges between 200-300 ppm(Amini et al., 2011). Fluoride is retained by soil due to its strong association with soil components. It is not normally leached from the soil. The presence of fluoride content is directly proportional to the depth of soil(Ali et al., 2019). It means as depth increases, fluoride content increases. Soil chemistry, climate, rate of deposition, and chemical form decide the fate of organic fluoride, which has been released into the soil(Vithanage and Bhattacharya, 2015). In Acidic soil, fluoride is present with its complexes like Aluminum or iron. By displacing the hydroxide, fluoride binds itself with clay. This adsorption process mainly depends upon the pH and concentration of fluoride. This is significant between 3-4 pH, and it goes down when pH is above 6.5. Use of fertilizers for irrigation purpose increase the fluoride level in the presence of CL-, SO42-, NO3-, and F- in groundwater(Saha, 2000). Due to alkalinization, high fluoride level was present in groundwater of irrigated land(Maria et al., 2003).

1.2.3 Food and beverages

Most food items contain at least a trace amount of fluoride. It can be easily observed in the environment. It has entered the human food chain using wheat, spinach, tea, cabbage, carrots, and other food. The primary sources of fluoride in these products are the intensive use of agricultural products. In the Indian city of Andhra Pradesh, agricultural products contained fluoride between 0.20-11.0 mg/kg. Tea plants take almost 97% fluoride, which is accumulated in tea leaves. In tea leaves, fluoride content is very soluble. Instant tea prepared in distilled water showed 3.3 mg/L of fluoride (Jorfi et el., 2020).

1.2.4. Anthropogenic sources of Fluoride

Different manufacturing and processing industries are introducing fluoride into the environment. Aluminum smelters, hydrofluoric acid manufacturing plants, manufacturing of phosphate fertilizers, enamel, glass manufacturing, bricks, and tiles industry, dyeing if textile products, and different plastic manufacturing industry are introducing fluoride into the environment (Jorfi et el., 2020). Thermal power plants, industrial effluents of semiconductors, and integrated circuit manufacturing industries. Cigarettes significantly contribute to fluoride intake by humans. An average cigarette has 236 ppm fluoride. Teflon-lined cookware is a source of fluoride for humans. In Teflon-coated cookware, the concentration of F^{-} was nearly 3ppm while a decreasing trend was found in aluminum ware. In stainless steel and pyrex ware, fluoride concentration was increasing (Maria et al., 2003).

1.3. Fluoride abundance

Humans' intake of fluoride through breathing is less than intake of fluoride via food and water. Fluoride presence in air is less than 1.0 µg/cu. m. Highest abundance of fluoride in nature has been reported at 2800 ppm which is the highest recorded concentration. It has been reported that 0.1-0.3 ppm of fluoride in India was found in rainwater. Generally, surface water has 0.01-0.3 ppm of fluoride, and seawater contains 1.2-1.5 ppm (Jiménez-córdova et al., 2019).

`1.4. Fluoride in Human

In humans and animals' hydrofluoric acid entered through skin absorption. The absorbed F^{-} is then distributed through blood circulation. 99% of this F^{-} is accumulated in teeth and bones. Fluoride is absorbed from the stomach through diffusion. It has a negative relation to pH. The absorption also occurs by a rapid diffusion process through the small intestine after gastric emptying. Presence of calcium or calcium carbonate in Food reduces fluoride absorption. The accumulation does not take place in soft tissues, but hydrogen fluoride can enter intra cellular fluid. That reflects the presence or concentration of fluoride in blood (Kabir et al., 2019). Fluoride accumulates in kidney tubules, and it has a significant cquantity than plasma. Therefore, it can be established that the kidney can be the potential target of chronic toxicity of fluoride. It is due to exposure of the kidney to the high concentration of fluoride. The placenta can also regulate the transfer of fluoride from maternal blood to fetal blood. 5-10 µg/L of fluoride levels in human milk have been measured. Salva also excretes fluoride, which is approximately 1%. It has also been reported that human sweat contains low fluoride levels, approximately 20% of plasma levels. It has been estimated that renal removal of fluoride is 35-70% of intake in adult humans. Therefore, urine, salvia, and plasma can be parameters of fluoride exposure. Fingernails can also be used as a parameter for exposure because several literature has reported a direct relation between children and fluoride concentration in drinking water and fingernails. But it has limitations fingernails only reflect 3-6 months old ingestion of fluoride (Khan et al 2018).

The ingestion of fluoride through food is 0.02-0.048 mg/kg/day for residents living in areas with a 1 mg/L F^{-} concentration in drinking water. In those areas where F^{-} concentration is less than 0.03 mg/L in water, dietary intake for residents of these people ranges between 0.0014-0.014 mg/kg/day. Children's dietary intake ranges between 0.03-0.06 mg/kg/day in fluoridated water areas and 0.01-0.04 mg/kg/day in areas without fluoridated water. Minimum adverse effect of fluoride is 0.25 mg /kg/day in humans, and the no-observed-adverse-effect is 0.15. These levels are under scientific discussion (Kumar et al., 2019).

1.5. Geochemistry profile of Fluoride

The interaction between the g round water and rocks leads to water pollution which ultimately causes widespread health problems. There is enhancement in these problems, specifically in developing countries. Deep boring wells are a primary source of water and these problems. Fluoride distribution is mainly impacting the people living close to the natural environment. These people use deep boring water for drinking and grow their food in that vicinity (Kumar et al., 2019). The primary exposure of fluoride to humans is drinking water. This makes it essential to understand the geochemistry profile of water.

The rainwater is enriched with CO_2 after falling on land. Different reactions, air, soil, organic matter, and bacteria are responsible for this contamination. Different salts like, Na_2SO_4 Nacl and $NaHCO_3$, are also the source for contamination. There may be different proportions of compounds which may contain fluoride in that soil where phosphate fertilizers are used. Below is an ion exchange reaction with exchangeable cations of soil

Eq.1 $CaX_2 + 2\ Na^+_{(aq)} \leftrightarrow 2\ NaX + Ca^{2+}_{(aq)}$

In this reaction X denotes the mineral which has clay causes breakdown of CO_2 which increase the hydrogen ions in groundwater. If there is $CaCO_3$ present, it also gets dissolved.

Eq.2 $C02 + H2O \rightarrow H2CO3$

Eq.3 $H2CO3 \rightarrow H+ + HCO3-$

Eq.4 $HCO_3^- \rightarrow H^+ + CO_3^{2-}$

Eq.5 $CaCO_3 + H^+ + 2F^- \rightarrow CaF_2 + HCO_3^-$

Eq.6 $CaF_2 \rightarrow Ca^{2+} + 2F^-$

F^- is mobilized by alkaline water from the soils, CaF_2 and weathered or eroded rocks precipitate $CaCO_3$ as

Eq.7 $CaF_2 + 2\ NaHCO_3^- \rightarrow CaCO_3\ 2Na^+ + 2F^-\ H_2O + CO_2$

If there is abundance of sodium bicarbonates in water, then dissolution of fluoride will be more. The whole activity can be expressed as.

Eq.8 $CaF_2 + 2\ NaHCO_3^- \rightarrow CaCO_3 + 2\ Na^+ + 2F^- + H_2O + CO_2$

The solubility product of CaF_2 would be.

Eq.9 $Ksp^{19} = [F-]^2[Ca^{2+}] = 4.0 \times 10^{-11}$

The presence of low level of calcium content is associated with high levels of F^-fluoride (Maheshwari et al., 2006). It has also been observed that the presence of F^- fluoride mainly depends on the fluoride containing minerals. Dissolution, decomposition, and rock water interaction also plays an important role. Alkaline water environment which has pH 7.6-8.6 and with high bicarbonate provides more favorable conditions for the dissolution of F^- fluoride in groundwater. pH of the ground water plays an important role in determining the F^- content in water. Therefore, it can be established that erosion and leaching of fluoride bearing minerals are the main source of F^- in groundwater (Meyer et al., 2022). The eq. 9 explains that the solubility of fluorite is low, then the water with minimum level of calcium always an elevated concentration fluoride. Groundwater with sodium bicarbonate and bicarbonate chloride always indicates an increase in concentrations of fluoride.

1.6. Global condition of Fluoride

Endemic fluorosis has spread in several parts of the world, specifically in those areas which are situated at mid-latitude. Skeletal fluorosis has also been reported globally. Endemic fluorosis has spread to at least 25 nations globally (Maheshwari et al., 2006). Ground water of Middle east, south Asia, Africa, and China, have high fluoride concentration. Eastern Africa's rift from Eritrea to Malawi is known as the high fluoride belt. Afghanistan, Iraq, Iran, Thailand, China, and Turkey are also another belt which has high concentration of F^{-} in groundwater. India, and China are two heavily populated states (Ozsvath et al., 2009). The consequential effects of high concentration of fluoride in ground water are felt badly in these two countries. In 1995, endemic fluorosis approximately impacted one tenth population of China. In 2004, approximately 26 million people suffered from dental fluorosis and almost one million people suffered from skeletal fluorosis in the same year. In year 1997, low lying areas of China like Zhuiger basin, and Kuitan had 21.5 ppm concentration of fluoride in ground water. It has been reported that Gysers and Hot spring of yellow stone national Park of USA have 25-50 ppm fluoride concentration in drinking water. Other USA areas like Southern California lake land has 3.5-5.3ppm, West deep aquifers of USA have 5-15ppm fluoride. Almost 60% of Mexican population have been affected by fluoride. A large number Canadian communities are also using drinking water from natural resource which has 4.3 ppm fluoride concentration. In some European countries like Czech Republic, Finland and Poland, have fluoride concentration up to 3ppm. Fluoride concentration in Ethopia's rift valley has been reported between 1.5-177ppm. Different African countries like Uganda, Ethiopia, Uganda, Central Argentina, Northern Mexico, North Algeria, Senegal, Ivory coast, are severe affected countries (Rezaei et al., 2017).

1.7. Scenario of Fluoride in Asia

About a quarter of the world's population lives in South Asia. It is almost home of 43% of world's poor. This region of the world is facing serious water pollution. Ganges river of India, and yellow river of China are severely facing serious pollution problems and clogging. These both rivers are on the list of extremely polluted rivers. Largest cities of Asia and Africa use surface water. People living in rural areas of Asia and semi-urban communities use ground water. This is the most vulnerable population for water borne diseases. It has been estimated that two third of Asian population does not have access to clean drinking water. The intrusion of F^- in ground water worsens the situation (Rasool et al., 2015 and Maheshwari et al., 2006).

1.8. Fluoride scenario in Pakistan

In 1951, Pakistan had 5000m^3 per annum water availability. It has decreased to 1100. It is more than the globally recognized scarcity rate. It has been estimated that water availability will be below 700 m^3 by 2025. The over exploration of ground water is decreasing the quantity of ground water and deteriorating the quality. The results of different studies conclude that underground water has toxic substances. The Concentration of toxic substances have been accelerated by human activities. It has been also found that 40% of diseases in a country prevail due to poor water quality (Saeed et al., 2021).

Fluoride is quite an important parameter of water. It has beneficial effects on humans. The low concentration of fluoride has beneficial effects on teeth while excessive concentration may lead to adverse impacts. It has been investigated that drinking water of Lahore has fluoride in drinking water in permissible limits while the adjacent area like mangamandi has more than the permissible limits of WHO. The excessive fluoride level has led to dental and skeletal fluorosis among the residents of the area. PCWR report about water quality of 23 metropolises of Pakistan concluded that fluoride contribution in contamination of ground water is 5%. While biological contamination is accounts for 68% which is highest among other contaminants (Rasool et al., 2015).

1.9. Dental impacts of Fluoride

1.9.1. Tooth decaying or dental caries.

Decaying of tooth is multifactorial and infectious disease. Dental caries is caused by demineralization of inorganic compounds. Dissolution of organic substance also leads to dental caries. Unhygienic oral condition leads to bacterial growth. This ultimately leads to dental caries (Sanders et al., 2018). The formation `of lactic acid, propionic acid, and acetic acid causes black spots on teeth or cavities. These tiny organisms enter the denting and start increasing in soft tissues. Streptococcus mutans and lactobacilli are the bacteria which are responsible for the disease (Saxena et al., 2019).

Fluoride controls bacterial activity, prevent the demineralization, and increases the remineralization of the dental enamel. Both enamel and dentin are composed of calcium and phosphate minerals. These minerals are embedded in matrix of protein or lipids Cariogenic bacteria take the fluoride due to fermentation process. It produces different kinds of acid. Fluoride enters into bacterial cells; it then changes the enzymatic activity of bacteria. These bacterial activities produce acid. This results in reduction of demineralization process of dental mineral. Adsorbed fluoride attracts the calcium ion which is present in saliva. Calcium and phosphate ions take part in the reaction due to fluoride. It produces crystals which have less solubility in acid when compared with minerals of tooth (Singh et al., 2007).

1.9.2. Fate of fluorides on dental decay

F^{-} Exerts adverse and beneficial impacts on human health, although the consumption levels that are linked to these effects are only slightly different. Scientific evidence disagrees because many study findings are unclear or conflicting. The question of how much F^{-} concentration is too much and whether Fluoride in drinking water is required or important against dental decay. Even in the 1930s, oral fluoride was thought to be a useful way to reduce tooth cavities, and historically, people consuming (Saxena et al., 2019). Those who drank non-fluoridated water were shown to have a substantially higher presence of caries than those who drank fluoridated water. The importance of fluoride in preventing dental cavities has recently been supported by credible evidence from most of the systematic scientific research. According to reports, there reduction in dental caries incidence at a natural setting that is clinically significant Dean's data show a fluoride content of about 1 ppm, and that as well. between the ages of three and seventy-five. Expert Committee for WHO Following researching fluoride's effects on oral health, it was proposed in 1994 an ideal fluoride concentration of 1 mg/L for best protection against tooth cavities. F^{-} was classified by the WHO in 1996 as a "potentially hazardous element, some of which may yet have some necessary activities at low levels" and "possibly carcinogenic." advocated it as a desired or necessary component for people to prevent dental decay Depending on the maximum daily air quality average for the year. WHO established acceptable levels for disease that range from 0.5 to 1.2 mg/L. McDonagh, and colleagues presented a summary of the thorough examination of trustworthy information on the efficacy and safety of addition of fluoride in water. The studies looking into the relationship between fluoride and dental caries between 1969 and 1999 were comprehensively examined in this review. According to the "best available evidence," The frequency of caries is decreased by fluoridating drinking water supplies, both as indicated by the percentage of kids who do not have caries (a decline of 14.8%) and (a decrease of 2.25 teeth). Since F^{-} works best when it's present consistently at low levels, WHO suggested the use of fluoride mouthwash for saliva and acid fluid. rinses for individuals with an elevated risk of dental caries. Also, fluoridated toothpastes (96% of it contains 1000 to 1500 ppm fluoride) was proven cause for the progressive drop in the occurrence of dental cavities in most industrialized nations. It is quite important to mention that most scientific research has failed to explain the positive impacts of F^{-} against tooth decay. It has been recommended that dental caries in children is a bacterial disorder, whose severity fluctuates depending on several conditions, including nutrition, oral bacteria, oral hygiene, educational and economic status of parents. Also, substantial temporal decreases in tooth decay can mainly be attributable to the dietary patterns and immunological condition of populations and that dietary regulation of caries, without the use of fluoride is conceivable as even chewing cheese reduces tooth decay. Additionally, the prevalence of tooth decay is rising in sugary products and other fermentable carbs in food of several developing countries. Dieting is beyond just avoiding sweets, several foods like whole-grain cereals, nuts, and seeds contain no fluorides. diary goods may prevent tooth decay. No sickness can be proved to be caused by its shortage; it is contended that fluoride is not an essential food ingredient for humans. Because more than 85% of bone disintegration takes place on excavations and fissures of the tooth, where F^{-} has always been established ineffecient (Tahir et al., 2013).

1.9.3. Fluorosis in the teeth

Early indicator of fluoride which is evident to the human eye is dental fluorosis, it is a non-reversible toxic effect on the ameloblasts, these are the cells which make teeth. Clinically, it ranges from hypo calcification on histological examination to from barely noticeable white striations on the teeth to severe flaws and enamel staining. Precise processes through which dental fluorosis develops have not been completely explained (Tahir et al., 2013).

1.9.3.1. Dental fluorosis history

The first dental fluorosis connected to drinking water was recognized in 1925, but it took a while before it was demonstrated that fluoride in water particularly caused it. A 'darmous' animal disease that was seen in horse teeth and bones along the coast of North Africa was later determined to be brought on by fluoride. Fluorosis was first recognised in humans as an occupational disease in 1930, and the development of skeletal fluorosis in Danish cryolite miners as a result published in 1932. Early in the 1930s, farmers in Andhra Pradesh's Nellore District discovered fluorosis among cattle, and the first medical report on the disease was published in the Indian Medical Gazette.in 1937 by Short et al.

1.9.3.2. Dental fluorosis and fluoride

Recent study has mostly worked on the hypothesis that dental fluorosis comes from fluoride-induced effects on crystal development and a delay in the hydrolysis and elimination of amelogenin matrix proteins during enamel maturation. Enamel crystallite formation is inhibited by amelogenins. These are the proteins released by ameloblasts. Amelogenins are extracted from the enamel matrix by amelogeninases during the early maturation stage of tooth development, drastically accelerating crystallite formation. The response to increased fluoride levels seems to be greatest throughout the development of teeth's calcium-rich components, i.e. When teeth are developing, dentin and enamel have a great attraction for fluoride. A calcium concentration gradually decreases in fluorosis teeth from "moderate to severe" condition Fluorides weaken teeth because of this.by the loss of calcium ions. Enamel that has been heavily fluorosis is more porous. Because the well-mineralized zone is extremely vulnerable to mechanical stress, it is pitted, discoloured, and prone to wear and fracture owing to major structural changes, a decrease in mineral content, a disruption in the mineralization of the enamel, and morphological aberrations on the surface of the fluorosis teeth easily breaks apart. The severity of dental fluorosis has been measured using a variety of techniques (Ugran et al., 2016 and Tahir et al., 2013).

1.9.3.3. Dental fluorosis physical symptoms

Dental fluorosis causes teeth to turn from white, yellow, or black to brown. This change in teeth color may occur as streaks or as spots. inevitably horizontal in orientation when further matrix layers are added. horizontally as teeth are growing. Consequently, it will always be visible in pairs. It can never be seen alone in a single isolated tooth and always on a developing pattern. Discoloration develops on the enamel surface distant from the gums. It becomes a permanent component of the tooth matrix. It causes the enamel to become discoloured. lose its brilliance and lustre if the staining is on the gums and along the other factors, such as the teeth's perimeter (Ugran et al., 2016). Having stained teeth from coffee or tea, smoking, and chewing tobacco. Dental fluorosis is always visible as horizontal lines or bands on the surface of the teeth because the enamel lines are put down in incremental lines during the prenatal and postnatal periods never in such straight bands (Younas et al., 2019).

1.9.3.4. Dental fluorosis' psychological and social effects

Teeth have a significant role in the facial skeleton. Teeth contribute to speech, phonation, and aesthetics in addition to their role in the mastication of food. Due to its negative impact on teeth and ultimately on a person's personality, dental fluorosis warrants special attention. A person with dental fluorosis finds it challenging to smile because of their distress and anxiety (Zuo et al., 2018). People with discoloured teeth often experience psychosocial problems. They'll live in the shadow of being "disabled" loss of self-esteem or a negative self-image. They are persistent even at a young age and more likely to encounter humiliation, exclusion, and discrimination. Such Conditions may result in aggravated resentment and fury, which could ultimately which in turn caused severe psychological depression (Younas et al., 2019).

1.9.3.5. Dental fluorosis prevalence

Increased F^{-} intake from various sources has led to an increase in the intensity of dental fluorosis globally. It is well known and shown that there is a direct relation between the frequency of dental fluorosis and the concentration of F^{-} in water. It has been noted that kids and teens tend to Dental caries is more common in children between the ages of 8 and 16. The percentage of adults affected by dental fluorosis ranged from 2–20% in years. Further, the Adults affected ranged from 2 to 30%, with no gender differences. Typically, it is found that fluorosis can arise at varying rates in various homes in various areas with nearly identical F^{-} levels in the drinking water and susceptible to a variety of other factors elements include dietary state, weather, one's susceptibility and biological reaction, the length of F^{-} exposure, and the number of dissolved salts in drinking water (Zuo et al., 2018). Although there is not enough information to conduct a thorough comparison, the review by McDonough et al.

reveals that there has been no statistically significant difference between anthropogenically and naturally fluoridated water. Citing Brown's research and contrasting naturally occurring and synthetic When comparing the fluoridated and control areas, it was suggested that naturally same dental effects to water that has been artificially fluoridated. Additionally, there is no difference between the bioavailability of F^{-} from natural and anthropogenic sources. be anticipated from the perspective of fundamental theoretical chemistry because fluoride ions in water can never be different, whether they are present naturally or intentionally introduced.

1.10. Fluoride's impact on the knee

One of the components of the human skeletal system is F^{-}, and chronic long-term exposure causes skeletal fluorosis, the intensity of which rises almost proportionately to the concentration and length of exposure. When evaluating the negative impacts of skeletal fluorosis and fractures are seen to be the most pertinent outcomes (Zuo et al., 2018).

The age and type of the bone affect the receptivity of F^{-} on the skeletal surface, with young and cancellous bone being more sensitive than old or cortical bone. the concentration of F^{-} in bones changes with gender and age, bone type, and particular bone component are all thought to be indicators of a person's long-term fluoride exposure (Tahir et al., 2013). The observation was made that bones and teeth contain 99% of the fluoride in the body (however some fluoride is present in other body parts) teeth contain very little fluoride compared to bones and the Rest dispersed throughout the circulation and highly vascularized soft tissues. F^{-} is more easily absorbed in the active, expanding, and cancellous areas than within small areas. F^{-} level is different in different types of bones (Saha et al., 2000).

1.10.1. Interaction of fluoride with bones

It is common that ingesting F^- has an impact on the chemical make-up and physical structure of bones. Fluoride's accumulation in the skeleton due to prolonged high exposure and its impact on non-malignant bone disease, notably skeletal fluorosis, are among the most harmful effects on humans and broken bones. Fluoride can change how quickly and how much bone is lost.

Bone mineral metabolism's homeostasis can be impacted by tissue. a combination of various degrees of osteoporosis, osteomalacia, and osteosclerosis the bone lesions' characteristics. Bone metabolism has risen, bone collagen synthesis has been impaired, and calcium absorption has increased. F^- toxicity changes include changes to bone-related hormones, metabolism of minerals, structural alterations such as increased bone mass, exostosis (bony protrusion) at bone surfaces, and elevated density (Saha et al., 2000).

Fluoride is thought to generate hydroxyl Fluor apatite by replacing the hydroxyl ion and possibly the bicarbonate ion associated with hydroxyapatite, a mineral phase during the development of bone. The fluoride ions that are present in the calcium ion plane form altering the structure to one that is more structurally dense and electrostatically stable profile of mineralization leading to increased density and hardness This change might be either as a result of the hyper mineralization of older (denser) fractions or as a result of the enhanced crystallinity of the hydroxyapatite. Therefore, even while the remineralization process improves bone density and results in denser and tougher bones, Long-term, high-dose fluoride administration reduces mechanical strength because bone strength is mostly obtained from the junction between a collagen (Rasool et al., 2015).

1.10.2. Skeletal Fracture with Fluoride Exposure

The fluoride side effect that has received the most research, aside from fluorosis, is skeletal impacts. It is conceivable that fluoride could have either a positive or negative impact on the likelihood of fracture. Contradictory results, though, were reported in study on the relation among F^{-} exposure and the occurrence of broken hips (Ugran et al., 2016).

1.11. Fluorosis of the bones

Skeletal fluorosis is a disorder that causes weak bones with low tensile strength as a result of a protracted fluoride buildup. Along with the bones, it has an impact on the joints. Up until an advanced level, it is difficult to recognize Its early symptoms may be similar to those of arthritis. When most advanced phases, it develops into a disabling illness with significant public health implications and socio-economic effects, which have an impact on millions of individuals (Rezaei et al., 2017).

The restriction of spine motion is the first clinical symptom of skeletal fluorosis. Joints in the neck, spine, knee, pelvis, and shoulder show the most negative impacts. The rigidity gradually increases until the entire spine transforms into a single, uninterrupted column of bone, a phenomenon known as "Poker back," At this point, the spine's different ligaments start to calcify ossified, etc. The limbs' numerous joints start to stiffen very quickly. The Chest mobility is gradually reduced when the ribs become involved breathing, causing the chest to resemble a barrel. Flexibility abnormalities may arise as contractures cause the joints to become more immobile at the patient's hips, knees, and other joints, leaving them immobile (Saeed et al., 2021).

In several reported cases and different surveys of people living in specific parts of globe (India, China, northern, eastern, central, and southern Africa), where endemic skeletal fluorosis is common, Fluoride consumption may be very high due to frequently significant drinking water that has significant concentrations of naturally occurring natural fluoride (Malin, et al., 2019).

The kidneys are the most important excreting organ in humans. Approximately 50–60% of the F^{-} that is consumed, as well as any defect, inhibits the excretion causing a rise in the amount of F^{-} deposited in serum and bones. The condition of the kidneys affects the effectiveness of renal excretion and will get worse as we mature. Renal failure patients may experience a four-fold Fluoride content of the skeletal system rising people face higher risk (Khattak et al., 2022).

1.11.1. Fluorosis of the skeletal cripple

The intense and advanced form of skeletal fluorosis is crippling fluorosis. Fluoride ingestion at high levels and long exposure, malnutrition, physically demanding work, and compromised renal function are all common drinking water fluoridation causes severe skeletal fluorosis.

Symptoms of crippling skeletal fluorosis include, scoliosis (lateral vertebral), a side view of the spine) flexon deformity, column (the act of bending or the condition of being bent) paraplegia (paralysis of the lower body, including the legs and knees), lugs (paralysis of all the four limbs). The stress it generated by osteophytes (bony overgrowth), intervertebral foramen constriction, and expansion of the body's size (Khattak et al., 2022).

This disease progresses slowly but relentlessly, thus neurological abnormalities can occasionally be brought on by a little injury. Since crippling malformations at the knee, hips, and other joints coexist with one another. It becomes challenging to determine whether the problems are brought on by bone malformations or neurological disorders.

These situations cover a broad spectrum, neurological deficiencies, which can show up in either the lower defects in either the lower motor neuron or the motor neuron, or both, which is much more typical. At this time, the cervical spine's anatomical characteristics will be affected individuals may experience marked cachexia because of inactivity of the trunk and limb muscles. Although absolute deafness seldom happens, a gradual high frequency perceptive deafness is also seen. the tightening (Malin, et al., 2019).

Table 1-2 Toxic effects of fluoride on animal kidneys

Experiment	**Observation/Conclusion**	**Reference**
Fed fluorinated compounds with food to rats, pigs,dogs, and cows	In rats, pigs, and dogs' kidney, damage observed	Roholm et al., 2005
Fluoride sensitivity examination on rats cells	Kidney cells being the most sensitive type	Hamming et al., 2004
Fed 100 and 375 ppm of NaF to rabbits and growingalbino rats	Serious damage to the kidneys observed	Kawahara et al., 2006
NaF in drinking water to old rats	Renal tubule hypertrophy and hyperplasia were found	Ramsyer et al., 2001

Rabits injected daily with 5, 10, 20, and 50 mg/kg NaF body weight for 15 weeks	At higher doses, degeneration of kidneys including tubular epithelia was observed	Suresh et al., 2000
Fed 10–100 ppm NaF to 100 adult male albino mice for months	Cloudy swelling and necrosis of the tubular cells and atrophy of the glomeruli, and areas of interstitial infiltration of round cells observed	Kaur et al., 2005
First and second generation of rats exposed to 30 mg/L NaF throughout the gestation and the lactation periods	Tubular degeneration on kidneys observed	Karaoz et al., 2004
The renal tubular site of action of fluoride examined in Fischer 344 rats using clearance techniques	The results suggested that NaF and methoxyflurane. alter renal function primarily by inhibiting active chloride transport in the ascending limb of Henle's loop	Roman et al., 2001
Examined the effect of NaF on intracellular calcium mobilization	Fluorides increase the secretion of parathyroid hormone leading to calcification of renal cortical	Murao et al., 2000

in a normal rat kidney epithelial cell line (NRK-52E cells)	thick ascending loop of Henle and distal tubule	
NaF administered intravenously as a 2% aqueoussolution in the young rats (75 days old)	Single doses of NaF (20 and 30 mg/kg,) produced a mild necrosis of the tubular epithelium in the inner third of the cortex	Taylor et al., 2000
Wistar rats acutely exposed to fluoride at a plasma concentration of 0625 mg/mL	Acute exposures to low concentrations of NaF induced a significant tubular dysfunction, resultingin diluted urine, impaired protein reabsorption, and increased calcium and phosphate urinary excretion	Santoyo et al., 2013
Apoptosis of renal tubular cells induced by NaF at different levels	The results suggested that NaF induces the apoptosis in renal tubules via activation of the Bax expression and Bcl-2 suppression	Xu et al., 2006

Table 3: Toxic effects of fluoride on human kidneys

Adverse effects/Observations	Reference
Fluoride exposure disrupts the synthesis of collagen and leads to the breakdown of collagen in bone, tendon, muscle, skin, cartilage, lung, kidney, and trachea	warren et al., 2006
Individuals with kidney disease have decreased ability to excrete fluoride in urine and are at risk of developing fluorosis even at normal recommended limit of 0.7–1.2 mg/L	Berth et al., 2003
Patients with fluorosis had damaged kidneys	Ramseyer et al., 2001
Consumption of optimal amount of fluoride in drinking water does not increase the risk of developing CKD	Ludlow et al., 2007
Human kidneys concentrate fluoride as much as 50-fold from plasma to urine. Portions of the renal system may, therefore, be at higher risk of fluoride toxicity than most soft tissues	Ludlow et al., 2007
Inorganic fluoride exerts a direct and dose-dependent cytotoxic effect on human proximal tubular epithelial (HK-2) cells	Ludlow et al., 2007
Nephrotoxicity was reported in patients received methoxyflurane anesthesia for surgery. Causative agent was shown to be flouride ion shown to be fluoride ion	Mazze at al., 2006

Renal failure due to fluoride	Lanth et al., 2009
An experiment on fluoride ion toxicity in human kidney collecting duct cells has shown that the mitochondrion is a target of fluoride toxicity	Cittanova et al., 2009
An experiment with 210 children on toxicity due to fluoride in drinking water showed toxicity of fluorides to human kidney at 2 mg/L level	Xiong et al., 2007

1.12. Effect of diet and malnutrition

Non-skeletal fluorosis results from the interaction and involvement of soft tissues, organs, and other biological systems with F^{-} in addition to teeth and bones. Affected human tissues include the thyroid gland, spermatozoa, gastro-intestinal mucosa, ligaments, and skeletal muscles damaged. Actin and myosin filaments in muscular tissues are destroyed. Fluoride ultimately causes muscle energy to be depleted. Therefore, fluorosis directly affects the skeletal muscle. This is the principal cause of muscular deterioration and the ensuing loss of muscle energy in patients who have been fluorosis, making them unsuited for daily duties (Joseph wt al., 2009).

A significant drop in serum levels was noticed by Susheela and Jethanandani amounts of testosterone in people with skeletal fluorosis. Fluoride can result in pathological alterations including DNA damage and lipid peroxidation. It has reported that it suppress the immunological system in humans. Although the overall body of research does not prove fluoride to be either genotoxic or allergic in humans, some genotoxic effects cannot be completely ruled out (Rasool et al., 2015).

1.13 Significance of the Study

The issue of deteriorating water quality is a matter of great concern worldwide, with numerous factors contributing to the problem. Among these factors are the use of phosphate fertilizers, irrigation water, and other foodstuffs, which all contribute to the deterioration of crop quality. In the rural areas of district Lahore and Kasur, it has been established that endemic fluoride areas are contributing to the worsening of the water quality. The residents of these areas, who are predominantly poor and illiterate, are exposed to high concentration of F^- through water and are generally unaware of the potential health impacts of chronic F^- exposure. Particularly children who are more vulnerable to the adverse effects of fluoride due to their underdeveloped immune systems. The unhygienic conditions of the villages and the lack of access to clean drinking water further exacerbate this situation. While a substantial amount of literature exists on the contamination cause by F^- in the district Lahore and Kasur, there is limited evidence and literature on the risk assessment of fluoride toxicity on adolescents, as well as on the assessment of health impacts on children in these areas. Furthermore, no literature is currently available on the impact assessment of fluoride toxicity on the liver and kidney function of adolescents in these areas.

Therefore, there is a pressing need to address this research gap and assess the chronic impact of F^- on kidney and liver function of adolescents residing in these rural areas. This study will contribute to a better understanding of the health impacts associated with fluoride contamination in these areas and will aid in the development of effective interventions to mitigate the risks associated with chronic fluoride exposure.

1.14 Problem statement:

The presence of F^- in groundwater in Pakistan can be primarily ascribed to geological and industrial processes (Khattak et al., 2022). Extensive research has revealed that elevated fluoride levels can lead to adverse health effects, particularly concerning kidney and liver function in children (Xiong et al., 2007). Nevertheless, there exists a notable dearth of studies examining the potential influence of fluoride toxicity on liver and kidney biomarkers among adolescents residing in the rural areas of district Lahore and Kasur. Consequently, it is imperative to undertake comprehensive investigations to evaluate and comprehend the potential long-term ramifications of fluoride exposure on liver and kidney biomarkers within this specific population.

1.15 Objectives of the study:

The objective of the current study was to address the existing research gaps in rural settlements of district Lahore and Kasur where high concentrations of F^- in water have been observed and reported. Specifically, the following objectives were developed:

1. The objective of this research was to assess the prevalence of dental fluorosis among adolescents and determine the community fluorosis index in the affected areas.
2. The objective of this study was to investigate the potential nephrotoxicity and hepatic toxicity induced in adolescents resulting from prolonged exposure to elevated levels of fluoride in drinking water.
3. The objective was to explore any potential variation in liver and renal biomarkers of passive and non-passive smokers in these communities affected by elevated fluoride levels.
4. The objective of the present study was to find any relation between fluoride toxicity and gender and age variation.

Structure of Thesis:

Chapter 1: It presents the introduction and extensive literature review related to chronic fluoride toxicity on human and animals' health. This chapter also includes literature review about chronic impacts of fluoride toxicity on human health.

Chapter 2: It has the details about study area, sampling strategy, sampling process, questionnaire. This chapter also includes how dental fluorosis assessment was carried out. Chapter 2 also explains how clinical analysis on blood was performed and how fluoride assessment on urine was carried out. Moreover, this chapter includes information about the statistical analysis.

Chapter 3: This chapter presents the findings of the present study. It also includes the discussion of fluoride toxicity.

Chapter 4: In includes the conclusion of the study. This also presents suggestions to improve the current scenario of areas.

CHAPTER 2

2. Materials and Methods

2.1. Site description

The sampling sites were opted on based on previously reported high concentrations of high F^{-} than permissible limit of WHO in underground water. It has been reported that these areas of Lahore and Kasur. Due to elevated level of fluoride range between 0.38-21.1 ppm rural areas of district Lahore and Kasur are facing endemic fluorosis (Farooqi et al., 2007). Another study conducted in these areas also concluded that an elevated level of F^{-} in drinking water was expressing neurotoxic effects on children ((Saeed et al., 2021). While another study conducted across the Punjab revealed that some of the rural areas of district Lahore and Kasur have an elevated level of fluoride (Khattak et al., 2022). Considering these scientific evidence, rural areas of district Kasur and Lahore were opted for a cohort-based study. Adolescents from rural sites: Kot Asadullah, kalalawala, Kot Maigha and chah Fateh wla were selected. These adolescents were termed as exposed groups. While the adolescents from Gopi Rai were termed as controlled group.

Kalanwala is sub-urban area of district Lahore, situated at fertile land near Manga Manndi. It is located at flood plain of Ravi River and has position of 31.276824° in north and 74.049128°in east. The elevation level of Kalalawala is 657 ft. Kalalwala is 45 KM away from Lahore (Farooqi et al., 2007). Lahore is the capital of Province Punjab of Pakistan. It is second largest city of Pakistan. The total area of Lahore is 1772 KM^2 with a population of 11.12 million (Census 2017) with $84 billion GDP. It has semi-arid climate with 24.1 °C (Hong Kong Observatory, 2010). The average precipitation rate Lahore is 607mm (Climate-data. ORG, 2016). It is situated on the east bank of Ravi River. Ravi River and Sutlej are also main sources of draining for district Lahore. In rural areas of the district lahore groundwater is main source of drinking water. In rural areas almost 80% of water is obtained using hand pumps in shallow ground water (Farooqi et al., 2009).

Kot Asad ullah is another ancient village situated at flood plain of river Ravi with elevation 655 ft. It has a position of 31.277783° in North and 74.049881° in east.

Kot Maiga is village of district Kasur with position of 31.230923 North and 73.953786 East. It has an elevation of 663 feet. Chah Fateh wala is another small village of Kasur with position of 30.955241, 74.028258 at elevation of 515 feet.

Whereas Gopi Rai was opted for controlled group. Gopi Rai is adjacent to Lahore city with position 31.471508 North and 74.222543 South. It is approximately 24 KM away from exposed sites.

Control group was added for comparative analysis between the results of exposed group and controlled group. The control areas were unexposed to fluoride and detection quantity of F^- in drinking water was below the detection level or having not more than permissible limit. This was opted on the based on a previous study conducted across the Punjab to assess the F^- in drinking water (Khattak et al., 2022).

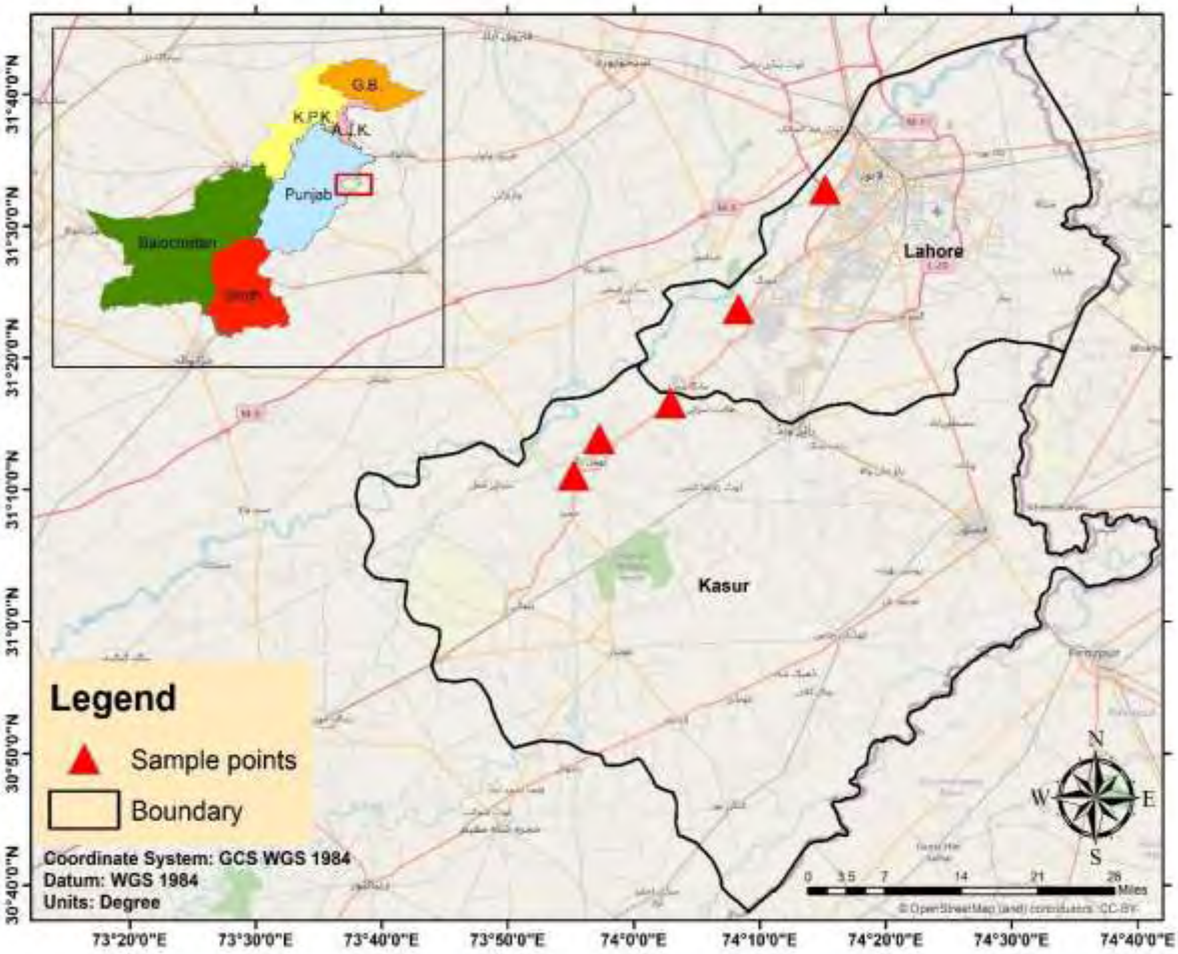


Figure 1 Geospatial position of the study Areas

2.2. Water Analysis

Water samples were collected from the home of each participant. Water samples were analyzed to assess the concentration of F^-. The fluoride level in water was measured at site with Low Range Fluoride meter (Hanna 729). Water samples with concentration more than 2 ppm were diluted using deionized water and measured.

2.3. Specimen collection, preservation and analysis

Sterilized containers were used for the collection of urine samples. Urine samples from participants collected under non-fasting conditions. Gel and clot activator yellow top vials were used for collection of blood samples. A ward boy from a local hospital was hired for extraction of blood. After collection of samples, urine sterilized containers were tightly closed, sealed, and were properly tagged. Disposable syringes were used to collect blood samples. The skin of the participants was cleared using alcohol swabs. The samples were kept in a cold cooler during the field. Urine samples were transferred to the environmental health and toxicology laboratory for examination. While blood samples were sent to hematology lab of Tehsil quarter hospital alipur for analysis.

2.4. Questionnaire

Participants were given questionnaires for collection of general information and some other fluoride specific questions. Participants were asked about general demographic data (age, weight, sex, height), socioeconomic status, dietary habits, disease history of participants and their family. Several gastrointestinal discomforts like nausea, vomiting, diarrhea and abdominal pain have been reported due to fluoride toxicity. Questions about tea consumption, vitamins intake were asked to assess any other possible source of fluoride intake than water (Vithanage and Bhattacharya, 2015). Tendency to urinate, and physical activity routine and dental fluorosis were asked to assess the chronic impacts of the fluoride accumulation in human body (Choubisa and Choubisa, 2016). The questions about daily habits of the adolescents like exercise, routine of brushing teeth and playing hours of participants were also asked (Ugran et al., 2017). To assess the any other potential source of fluoride, question about the tea intake of participants was also asked (Kabir et al., 2020). It was also asked that if there have been any family member suffering from any kind of liver or kidney disease (Perera et al., 2018).

2.5 Dental fluorosis examination:

Dental fluorosis was examined by using dean's index of fluorosis 1942. The examination of the participants was done by observing two most affected teeth of individual. After assessment participants were categorized according to dean's index of fluorosis as presented in table 2.1.

Community fluorosis index is used to assess the severity, and presence of dental fluorosis among the public. The value of community Fluorosis index explains the significance of public health, i.e. 0.0-0.4 (Negative), 0.4-0.6 (borderline), 0.6-1.0 (Slight), 1.0-2.0 (medium), 2.0-3.0 (Marked) and above 3.0 (very Marked) (saravanan et al., 2008). CFI assessment was done on results of dental fluorosis results.

Community fluorosis index was assessed in public health aspect. Community fluorosis index was assessed based on results extracted from dental fluorosis examination.

Community Fluorosis Index (CFI) = $\sum$ (Scores$\times$ No of participants) / No of total cases examined

Table 2-1 Categorizes of dental fluorosis and grade points according to dean index (Dean, 1942)

Groups	Characters	Points
Normal	Surface of the teeth is smooth and pale creamy white color	0
Questionable	Ranging from few white flecks to occasional white spots	0.5
Very Mild	Approx. 10-25%of teeth surface with white opaque scattered areas	1
Mild	About half of teeth surface having scattered white opaque areas	2
Moderate	Brown stains present. Whole surface of teeth is affected	3
Severe	Brown satins are widely spread. There is discrete pitting. General form of tooth may be disappeared	4

2.6. Fluoride determination in Urine

The F^- concentration in Urine was determined using method 8308 (Tolos). Urine samples were filtered to separate it from any kind of impurities by using Whatman filter paper. An aliquot of 5 ml urine samples was taken in 50 ml of falcon tube. It was mixed with TISAB II (HI 4010-00) buffer in 1:1 i.e., equal proportion. The samples were then analyzed using Fluoride Ion selective electrode (Model HI-522).

2.7. Blood parameters Analysis:

The blood samples were sent to the clinical laboratory of Tehsil Head quarter hospital Alipur for clinical analysis. The analysis was performed by highly skilled professionals. First the plasma and serum were separated using centrifuge. After the preparation of serum reagents were added in the sample. Several dyes are used for analysis. Serum biochemical biomarkers were measured with colorimetric assay kit for T- AC. ALP, AST, ALT, Urea and Creatinine were measured by kinetic method(Rives, 2020). The results were then quantified by chemistry analyzer (Saatkamp and Silveira, 2023). Chemistry analyzer (Micro Scan 300) was used for the analysis of blood samples.

While the eGFR was measured a race free question (CKD-EPI, 2021 eGFR creatinine equation) given by national kidney foundation and American society of nephrology(Project, n.d.).

2.8 Quality Control

All experiments were performed using strict quality control procedures. During clinical analysis, standard operating procedures of hospital were strictly followed. Each sample was separately analyzed with precision and competency. The glass-wares used for measurement of F^{-} in urine were properly cleaned which included 24 h soaking in HNO_3. Glass-wares were also rinsed with deionized water. To eliminate any analytical error each batch included a blank and standard reference. The LOD of F^{-} ion selective electrode was 0.03mg/L while with reagent blank value it was 0.06mg/L. The accuracy of the F^{-} ion selective electrode measurement was ranged between 95.2 to 100. It was relative to the standard F^{-} solution. It was along with errors of measure less than ±5. While the calibration slop of Ion selective electrode ranged from -30 mV to 37 mV.

2.8. Statistical Analysis

IBM statistical package for science version 25 was used for statistical analysis of data. The descriptive statistical summary of the data was measured.

2.9 Analysis of Variance (ANOVA)

The ANOVA (Analysis of Variance) test was conducted IBM statistical package for science version 25 was used for statistical analysis of data.

T – test

The t-test was performed using IBM statistical package for science version 25.

2.10 Correlation Heatmap

A correlation heatmap is a graphical representation of the correlation matrix, which displays the correlations between multiple quantitative variables in the form of a color-coded matrix.

A correlation heatmap was performed on quantitative data, also known as continuous data. This type of data consists of numerical measurements that can take any value within a specific range.

The purpose of creating a correlation heatmap was to visually explore the relationships between multiple variables and identify patterns of correlation. Each cell in the heatmap represents the correlation coefficient between two variables, and the color scale is used to indicate the strength and direction of the correlation.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Demographic characteristics and socio-economic status

At each site adolescents were randomly selected and were clearly explained the content of the study. They along with their parents were also told the possible outcomes and requested their involvement. The adolescents which participated were interviews and consent was taken by their parents or guardian and document was signed. A total of 125(93=exposed, 25=controlled) adolescents aged between 11-19 years were selected to investigate. There were 13 females in exposed and 1 female was from controlled group. The parameter for selecting as target participant was permanent resident of area, non-smokers, aged between 11-19 years, using ground water as drinking water source and willingness to provide biological specimen.

Participants were asked about their economic status, i.e., low, medium, and high. More than 80% of the participants belonged to low economic status. Economic status provides information about any potential source of fluoride and about dietary habits. It was found that more than 60% of the participants were underweight among exposed participants. While in the controlled group 50% of the participants were underweight. In current study it was also observed most of the participants did not have muscle formation as compared to their age and height. This can be assumed fluoride toxicity might be the reason. Only 1% of the participants showed gastrointestinal discomfort.

Table ***3-1 Demographic characteristics of Participants***

Variables	Control	Exposed
Gender(n)		
Male (105)	25 (96)[a]	80 (86)[a]
Female (14)	1 (3)[a]	13 (13)[a]
Age Years (n)		
≥11-19 Years	29 (19)[a]	93 (78)[a]
Economic Status(n)		
Low (84)	19 (73)[a]	65
Medium (34)	7 (26)[a]	27
High (1)		1
Bod Mass Index(n)		
Under weight (70)	13 (50)[a]	57 (61)[a]
Normal weight (49)	13 (50)[a]	36 (38)[a]
Dental Fluorosis(n)		
Normal (38)	25 (96)[a]	13 (13)[a]
Very Mild (31)	1 (3)[a]	30 (32)[a]
Mild (26)		26 (27)[a]
Moderate (19)		19 (20)[a]
Severe (5)		5 (5)[a]
Kidney Liver Patient in Family(n)		
Kidney (8)	2 (7)[a]	6 (6)[a]
Liver (5)	2 (7)[a]	3 (3)
Toothpaste Name(n)		
Colgate (70)	14 (53)[a]	56 (60)[a]
Medicam (14)	5 (19)[a]	9 (9)[a]
Close Up (2)	0	2 (2)[a]
Sensodyne (3)	1 (3)[a]	2 (2)[a]

Doctor (1)		1 (1)[a]
Do not Use (28)	5	23
Passive Smokers(n)		
Yes (67)	16 (61)[a]	51 (54)[a]
No (52)	10 (38)[a]	42 (45)[a]
Tea (n)		
0 (31)		31 (33)[a]
1 (10)		10 (10)[a]
2 (25)		25 (26)
3 (20)		20 (21)
4 (7)		7 (7)[a]

[a]Percentage

3.2 Fluoride content in Water

The Punjab plains of India and Pakistan represents the largest alluvial plain across the globe. It is formed by accumulation by accumulation of sediments. These sediments are primarily composed of erosion of the Himalayas (Younas et al., 2019). Extensive research has indicated that significant portion of Punjab region consists of alluvial deposition ranging from the Holocene to Pleistocene with a thickness of approximately 300 m. (Khattak et al., 2022). The Punjab plains experience a diverse range of environmental conditions. The climate in this region varies between sub-humid in the northern areas to semi-arid in the southern parts. The northern region receives more than 100 centimeters of rainfall annually, while the southern areas receive over 30 centimeters. One of the challenges faced by various areas within the Punjab plains is the occurrence of frequent floods. These floods impact some regions more severely than others, particularly the low-lying areas. Another noteworthy concern is the elevated levels of fluoride found in the villages of northern Punjab, as highlighted by studies (Khattak et al., 2022). The climate of Punjab region varies from sub-humid in the north to semi-arid in the south. Rainfall in Punjab is >100 cm to the north and it is >30 cm to south region. This region lies on the edge of the monsoon. The agricultural sustenance of the Punjab plains heavily relies on an extensive canal network. However, an unintended consequence of this irrigation infrastructure is the seepage of water from these canals, leading to a rise in groundwater levels(Ali et al., 2019). An elevated presence of fluoride in water sources in the region cannot be solely attributed to the availability of fluorite minerals or other fluorine-containing minerals originating from the Himalayas. Several Local geochemical and hydrological factors, particularly those regulating the concentration of calcium ions (Ca2+), play a significant role in this phenomenon. These processes are primarily accountable for the heightened fluoride levels observed in the saltier

doab region and the southern area of Lahore (Khattak et al., 2022). Quantitative analysis of water samples, as presented in Table 3, reveals the mean± S.D. and range of fluoride concentrations in different groups: low (2.94±0.71 mg/L), medium (4.8±0.56 mg/L), high (6.78±0.57 mg/L), and very high (8.55±0.83 mg/L). The fluoride levels in water exhibited statistically significant differences among all groups. Furthermore, a notable distinction is observed between the controlled group and the exposed group, with the latter displaying significantly higher fluoride concentrations than the former. This study unequivocally demonstrated fluoride level in water is considerably higher to the control group. Notably, concentration of F^{-} in water is 1.60 mg/L in group 1, whereas it reached 4.10 mg/L in group 2, 5.20 mg/L in group 3, and 6.60 mg/L in group 4. In contrast, the control group showed a minimum F^{-} concentration of 0.50 mg/L in water. Additionally, the Pearson correlation analysis indicated a firm relation between high fluoride levels in water and the presence of dental fluorosis (p = .000), as presented in Table 3. This finding aligns with a previous study which showed a positive and strong association between elevated level of fluoride and water. (Das and Mondal, 2016).

3.3 Fluoride concentration in Urine

The urinary fluoride level serves as a valuable indicator for assessing fluoride exposure and its significance differs significantly among control and exposed. Measuring F^{-} concentrations in urine provided a reliable means of evaluating fluoride exposure. The present study established a statistically significant distinction among exposed and controlled groups in reference to urinary fluoride levels. One way ANOVA values has been illustrated in Table

3, which presented the p-value indicated the association between the control and exposed groups.

Within this study, the mean urinary fluoride concentrations for the exposed groups, categorized as low, medium, high, and very high, were determined as 1.84 µg/L, 1.72 µg/L, 1.56 µg/L, and 1.70 µg/L, respectively. In comparison, the control group exhibited urinary fluoride concentration mean 0.88 µg/L. According to the WHO, the permissible limit of fluoride in urine should not more than 1.0 µg/L (Singh et al., 2007). The study findings also reveal the fluoride mean concentration in the different groups as follows: low (0.87±2.78 µg/L), medium (1.0±2.89 µg/L), high (0.03±3.80 µg/L), and very high (0.74±2.29 µg/L). The minimum and maximum values for urinary fluoride concentration were recorded as 0.87±3.80 µg/L. Furthermore, a positive correlation between urinary fluoride concentration and the presence of dental fluorosis was observed (p=0.00). Figure 3.2 represents simple linear regression analysis where water was independent variable and urinary fluoride as dependent variable. A statistically significant relationship of the water was found with urinary fluoride level. These results suggested that high concentration of fluoride in urine can be associated to a higher concentration of fluoride in drinking water. Similar findings have been reported in a previous study conducted in the same geographic area (Saeed et al., 2021).. Figure 3.3 illustrated the relationship between fluoride concentrations in water and urine. It was observed that Kot Maigha exhibited the highest fluoride concentration in water, while simultaneously displayed the lowest concentration of fluoride in urine. A similar pattern was observed in Kalalan Wala. This disparity can be attributed to the fact that residents in these areas were not using well water during the study period. Instead, they relied on treated water sources. Conversely, Kot Asad Ullah residents demonstrated a higher fluoride concentration in urine alongside a lower concentration in water compared to Kallan Wala and Kot Maigha. While the residents of Nathy wala were also exposed to elevated levels of fluoride. This occurrence suggested that participants from Kot Asad Ullah might have other potential sources of fluoride, such as tea or milk intake. While Gop Rai was a control area which showed the level of fluoride was under the permissible limit. These findings align with a previous study (Green et al., 2020). Additionally, the study found a non-significant relation between age variation and urinary fluoride levels. In human plasma, fluoride exists in both ionic and non-ionic forms. Ionic fluoride does not get attach with plasma proteins, leading to its easy excretion through

urine. It has been reported that the amount of fluoride excreted through urine reflects the amount of ingested fluoride, and this excretion primarily occurs through the kidneys (Singh et al., 2007). Previous research has documented that urinary excretion accounts for 32-80% of the total fluoride in body (Singh et al., 2007). It is important to note that fluoride excreted through human waste is not absorbed fluoride within the body (Ekstrand et al., 2009). Fig.2 presents a graphical comparison of fluoride levels in urine and ware. Kot Maigha exhibits the highest fluoride concentration in water among the groups, despite having the lowest concentration of fluoride in urine. However, the presence of dental fluorosis indicated that people are still utilizing water sources with elevated fluoride levels.

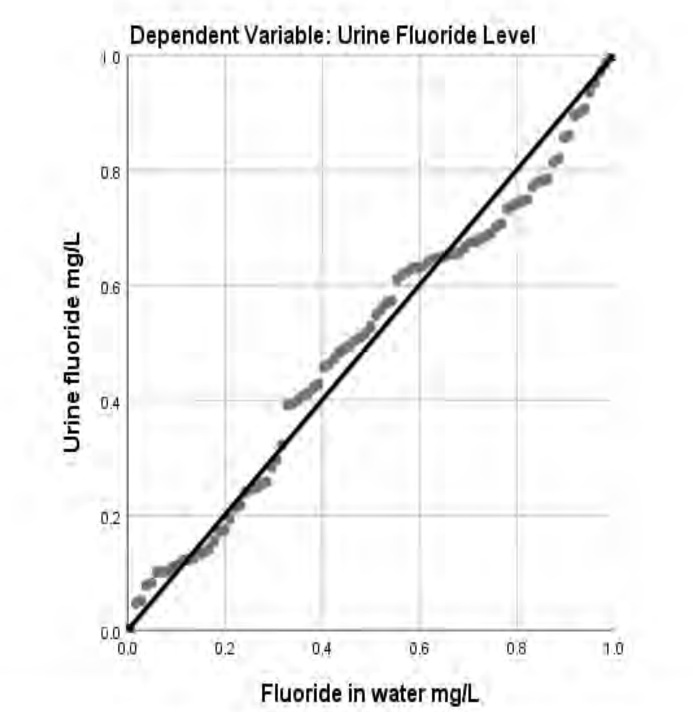


Figure 3.2 Simple linear regression analysis with urinary fluoride as a dependent variable and water fluoride as independent variable

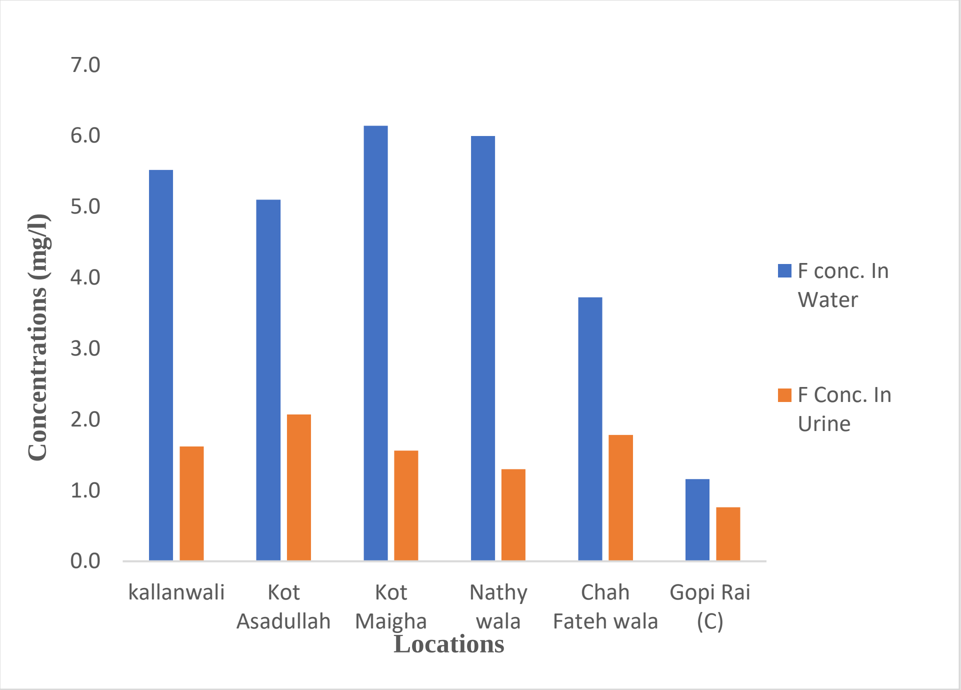


Figure 3.3 Fluoride concentration in Urine and Water

3.4. Dental Fluorosis

Dental fluorosis is a cosmetic condition. It is characterized by the presence of white or brownish spots on the tooth enamel(Das and Mondal, 2016). It arises from excessive fluoride during the developmental stage of tooth enamel. Numerous studies have established a positive correlation between fluoride level in drinking water and the presence and intensity of dental fluorosis(Saeed et al., 2021).

The findings of dental investigations revealed that out of the 116 adolescents examined, 35 exhibited very mild dental fluorosis, 58 displayed mild dental fluorosis, 19 had moderate dental fluorosis, and 4 exhibited severe dental fluorosis. The percentage of adolescents affected by dental fluorosis is presented in Annex 6 and graphically illustrated in Figure 3.1. The presence of dental fluorosis among the participants was 97.94%, ranging from mild to severe levels. This high prevalence can be attributed to the ingestion of elevated fluoride levels. The analysis of dental fluorosis cases indicated that 13 cases (15.23%) were classified as normal, while 30 cases (35.4%) were very mild, 26 cases (30.68%) were mild, 19 cases (22.42%) were moderate, and 5 cases (5.9%) were classified as severe. These findings clearly demonstrate the significant impact of fluoride exposure on the occurrence and severity of dental fluorosis. Table 3.4 explains an overview of the presence of dental fluorosis among the different groups. This high prevalence is primarily attributed to the ingestion of fluoride through drinking water, with concentrations ranging from 2.94±8.55.

Previous studies have consistently reported similar trends regarding the relationship between concentration of fluoride in drinking water and the presence of dental fluorosis. For instance, a study conducted in Lahore and Kasur districts, where fluoride concentrations exceeded the permissible limit, found that 81% of the participants (total of 118) exhibited varying degrees of dental fluorosis, ranging between very mild to severe (Saeed et al., 2021). Furthermore, study conducted in Bankura, where the fluoride concentration in water was 2.11 mg/L, yielded comparable results (Das and Mondal, 2016). In Lucknow, a study identified

188 cases of dental fluorosis (out of a sample size of 429), with concentration of fluoride in water was between 0.19 to 2.41 mg/L (khan et al., 2015). Another study conducted in the Mysore district reported 111 cases of dental fluorosis (out of a sample size of 270), with concentration of fluoride in water ranging between 0.4 to 2.0 mg/L (Sebastian & Sunitha, 2015). Additionally, a study conducted in Anyang and Neihuang, where fluoride levels exceeded the allowable limit, identified cases of dental fluorosis among children (Xiong et al., 2007). Similarly, in Mexico, a study found an overall prevalence of dental fluorosis of 72% among children (out of a

sample size of 734) (Irigoyen-Camacho et al., 2016). The results of the this study align with the existing literature. It provides further evidence that an increase in fluoride concentration in drinking water is a root cause of the presence of dental fluorosis in the community.

The community fluorosis index was measured using results of dental fluorosis examination. The mean CFI for all the four sites of the study was (3.02). Present results showed that dental fluorosis is a health problem among residents. The findings of this study are parallel with the previous study conducted in these areas. A previous study stated that this area is suffering from community fluorosis index(Saeed et al., 2021). The reason for such spread of dental fluorosis among the population is ground water of these areas. The CFI value for non-exposed areas was 3.02 which means that there was no public health problem regarding fluoride or dental fluorosis.

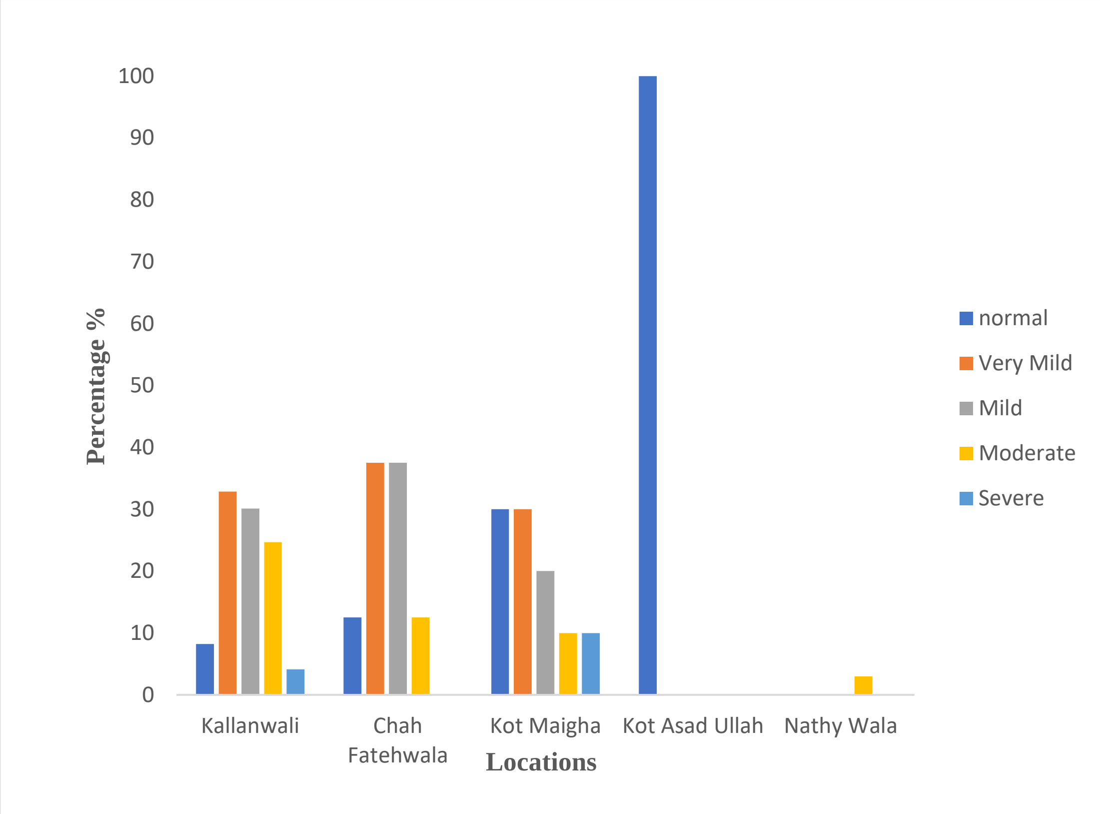


Figure 3.4 Percentage distribution of Dental fluorosis at sampling sites

3.4 Influence of Fluoride on Hepatic biomarkers

The liver in human body plays a vital role in maintaining several metabolic activities (Mohammed and Al.Okaily, 2017). Reactive oxygen species (ROS) have been implicated in the pathogenesis of various disorders in liver. Alanine aminotransferase (ALT) enzymes are predominantly found in the liver and cardiac tissues, while albumin maintains oncotic pressure in plasma. Serum albumin levels serve as indicators of liver and kidney function (J.Malin et al., 2019). The findings of the present study revealed increased activities of ALT and aspartate aminotransferase (AST) enzymes. In current study, significant differences in participants organs activities were observed among the groups residing in areas where fluoride level was in drinking water when compared to the control group (p=0.001, Table 3). When linear regression was applied on low group it was found that the relation between fluoride level in water as independent variable while ALT as dependent variable enzyme was significant (Fig. 3.5 R=0.20, R^2=.000, p=.000). while when it was applied on medium group a significant relation was found (fig.3.5 R=.378, R^2=.143, p=.003). A significant relation was also found in high group too (fig.3.5 R=.125, R^2=.016, p=.043). Furthermore, in very high group a non-significant but it explains the variation in the data (Fig. 3.5) R=.446, R^2=.199, p=.960). While in control a significant relation was found but does not explain the variation in data (fig.3.5 R=.147, R^2=.022, p=.000). When liner regression was applied on low group of AST SGOT (dependent variable) a significant relation was found but not with much variation (fig.3.5 R=.310, R2=.096, p=.000). while significant relation was found in medium and high group (Fig.3.5 R=.321, R^2=.103, p=.002, R=.089, R^2=.008, p=.033) respectively. While in the very high group a non-significant relation was found between AST SGOT and fluoride level in water (fig.3.5 R=.443, R^2=.197, p=.982). While figure 3.6 explains the regression analysis when urine was taken as independent variable and rest of the variables as dependent variables. However, when ANOVA was applied between the groups, Group 4 exhibited a significantly higher impact on hepatic biomarkers compared to low, medium, and high groups. In clinical practice, the functions of ALT and AST enzymes in the blood serve as important biomarkers for liver function. The elevation of fluoride levels can induce the generation of ROS and cytotoxicity, which leads to oxidative damage to cell membranes (Mohammed and Al.Okaily, 2017). This process can increase the rate of cellular necrosis, which results in the leakages of enzymes from liver cells (Mohammed and Al.Okaily, 2017). Furthermore, mitochondria releases cytochrome, which decreases the antioxidant phases of the liver and alters the liver functions (Sharma et al., 2023).

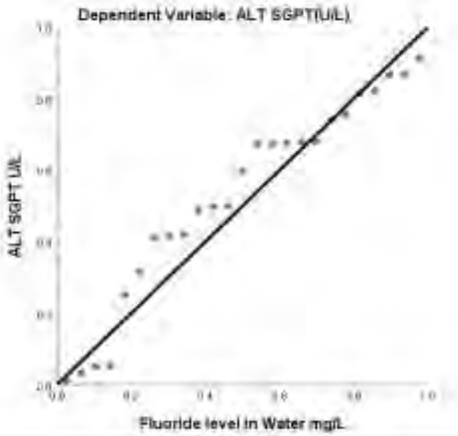
The observed elevation in ALT and AST levels may indicate damage to hepatocytes and myocardial cells. However, the potential impact of fluoride ingestion on human liver function remains a subject of controversy. Studies have suggested that fluoride intake from drinking water can affect protein synthesis and lead to increased ALT and AST activities (Michaeal et., 1996).

low

R = 0.20

R^2 = .000

P = .000

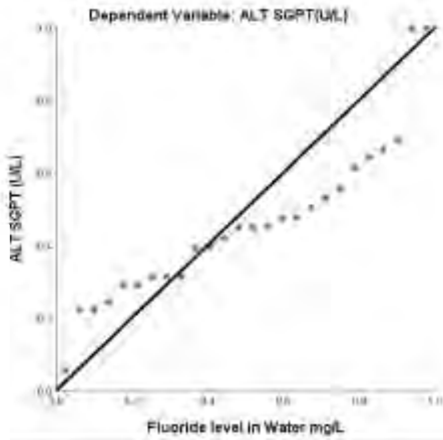


Medium

R = .378

R^2 = .143

P = .003

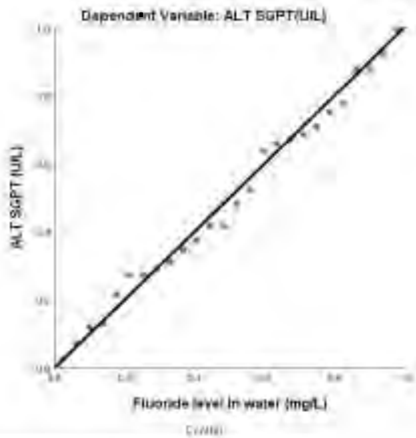


Contr

R = .147

R^2 =.022

p = .000

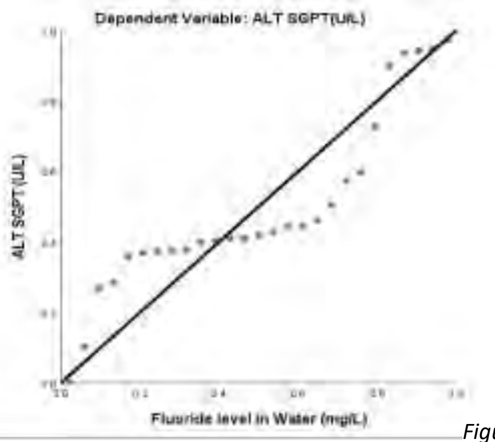


High

R =.125

R^2 =.016

P =.043



Very High

R =.446

R2=.199

P = .960

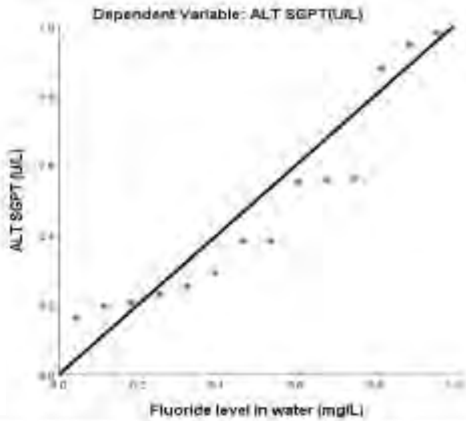


Figure 3.5 Simple linear regression analysis with ALT SGPT (Group base) as a dependent variable and water fluoride as independent variable

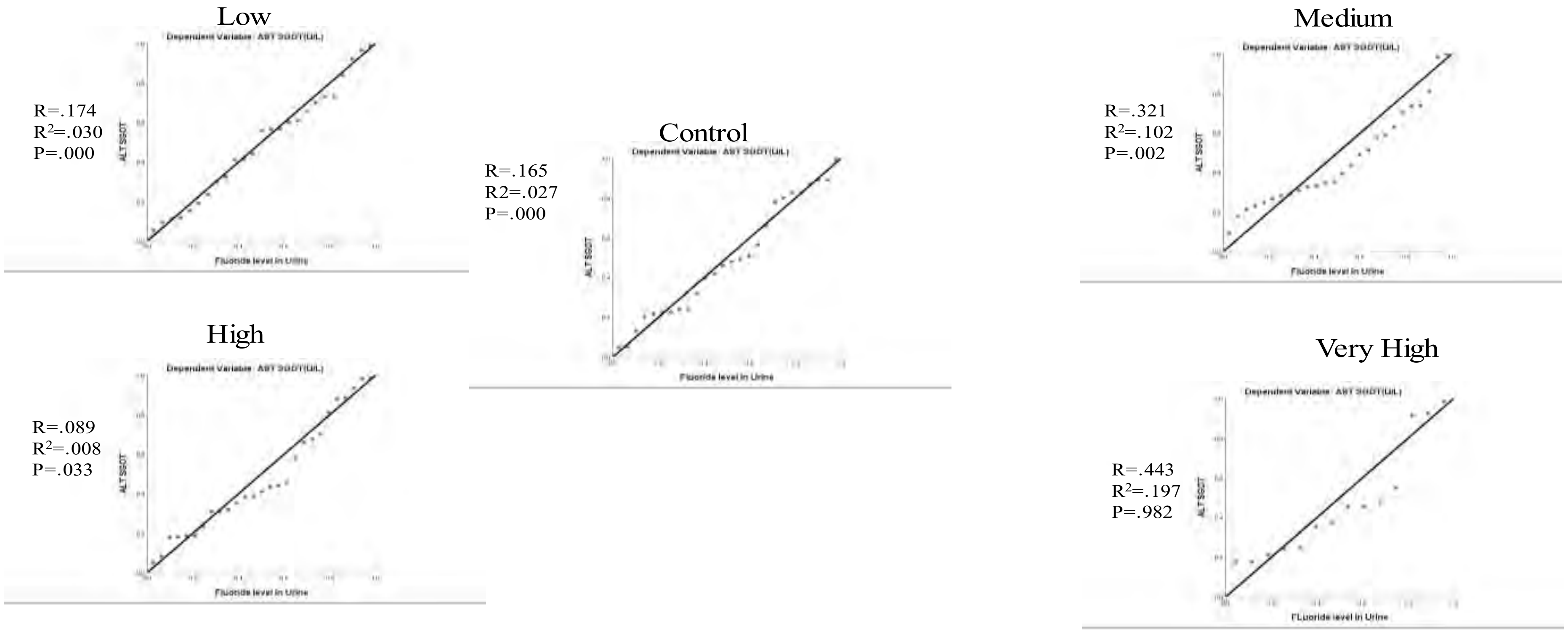


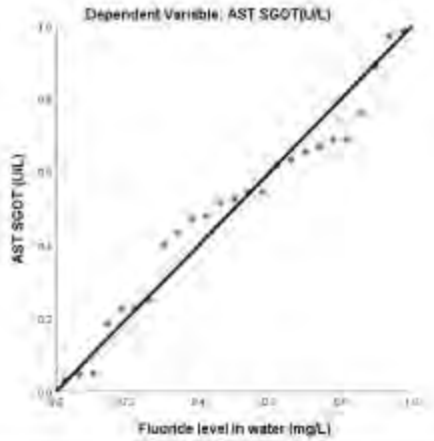
Figure 3.6 Simple linear regression analysis with AST SGOT (Group base) as a dependent variable and Urine fluoride as independent variable

Low

R=.310

R^2=.096

P=.000

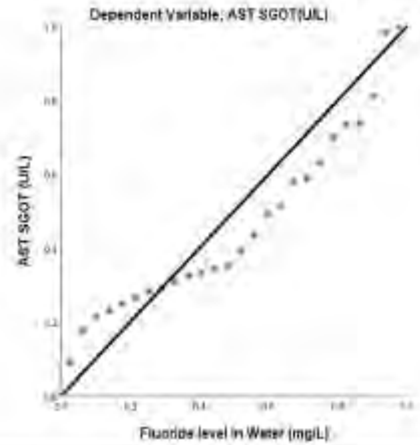


Medium

R=321

R^2=.103

P=.002

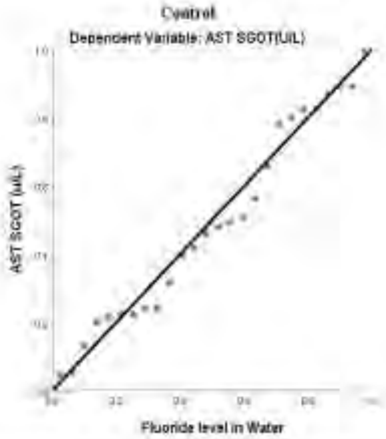


Control

R=.165

R^2=.027

P=.000

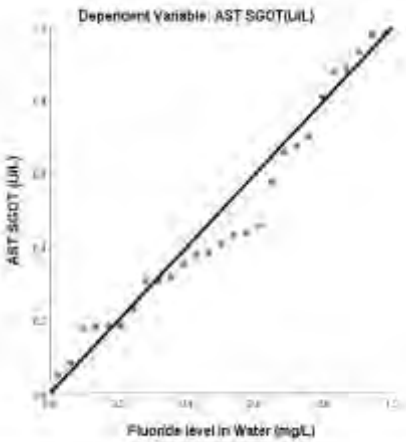


High

R=.089

R^2=.008

P=.033



Very High

R=.443

R^2=.197

P=.982

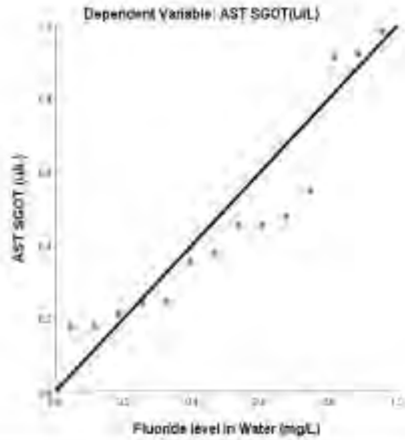


Figure 3.7 Simple linear regression analysis with AST SGOT (Group base) as a dependent variable and water fluoride as independent variable

Similar findings have been observed in a study conducted on rats (Mohammed and Al.Okaily, 2017). Abnormal behavior of several serum parameters related to liver function has been reported in children with skeletal fluorosis (Shivashankar et al., 2000). Nevertheless, conflicting results have been documented in some studies. Liang et al., (1999) reported no significant changes in serum indicators of liver function, including ALT, AST, and albumin. Even in those areas where levels of fluoride in drinking water ranged from 1.05 to 23 mg/L, no evidence of liver function damage was found.

In the present study, AST levels, measured by SGOT, exhibited significant differences among the groups. This observation suggests that variations in fluoride presence in water may be responsible for the observed significant differences. Notably, Group 4 displayed significantly different AST levels compared to all other groups, including the control group. This finding implies that high levels of fluoride may induce changes in liver function. The potential harm caused by elevated fluoride levels on liver function has been a topic of controversy. Several literatures have reported that elevated levels of drinking water fluoride do not adversely affect liver function in humans (Xiong et al., 2007).

Albumin and alkaline phosphatase are key biomarkers used to assess liver function. Albumin is involved in protein synthesis, while alkaline phosphatase is a hydrolytic enzyme widely distributed in the human body. Figure 4 illustrates a positive correlation between these two biomarkers. Additionally, total bilirubin demonstrated a positive correlation with both ALT and AST. Moreover, ALT and AST exhibited a positive correlation with each other.

3.5 Effects on purine metabolism associated products.

Uric acid, a byproduct of purine metabolism, is excreted via the urinary system. Deviations from the normal range of uric acid levels are considered indicative of potential kidney and metabolic disorders (J. Malin, 2019). In the present study, no statistically significant differences in uric acid levels were observed among the groups table 3. Conversely, urea levels displayed statistical significance (p-value=0.00 across all groups presented in table 3. This statistical disparity suggests variations in uric acid excretion among participants in each group, indicating the potential presence of kidney disorders. When linear regression was applied between urea (low group) was considered as a dependent variable while water was independent variable it was found that both have significant relation (fig. 3.9, R=.310, R^2=.096, p=.000). A significant relation was also found in medium, and it was non-significant for high and very high group between urea and water (fig.3.8 R=.321, R^2=.103, p=.002, R=.089, R^2=.008, p=.033) respectively. While figure 3.9 explains the linear regression analysis when urine was independent variable while urea was dependent variable. Furthermore, when linear regression was conducted between water as independent variable and Serum creatinine was dependent variable a non-significant relation (fig.3.10) was found between all four groups (low, medium, high and very high). While figure 3.12 explains the linear regression when urine was independent variable and S. creatinine was dependent variable. These findings imply that increasing fluoride levels may detrimentally affect various kidney parameters. However, it is worth noting that low fluoride levels do not appear to impact renal function. Similar outcomes have been reported in studies involving rats exposed to diverse fluoride doses over prolonged periods of time (Perera et al., 2018). Study also found that elevated level of fluoride may impact after a long time but the detail mechanism is still yet to be found (Danziger et al., 2022).

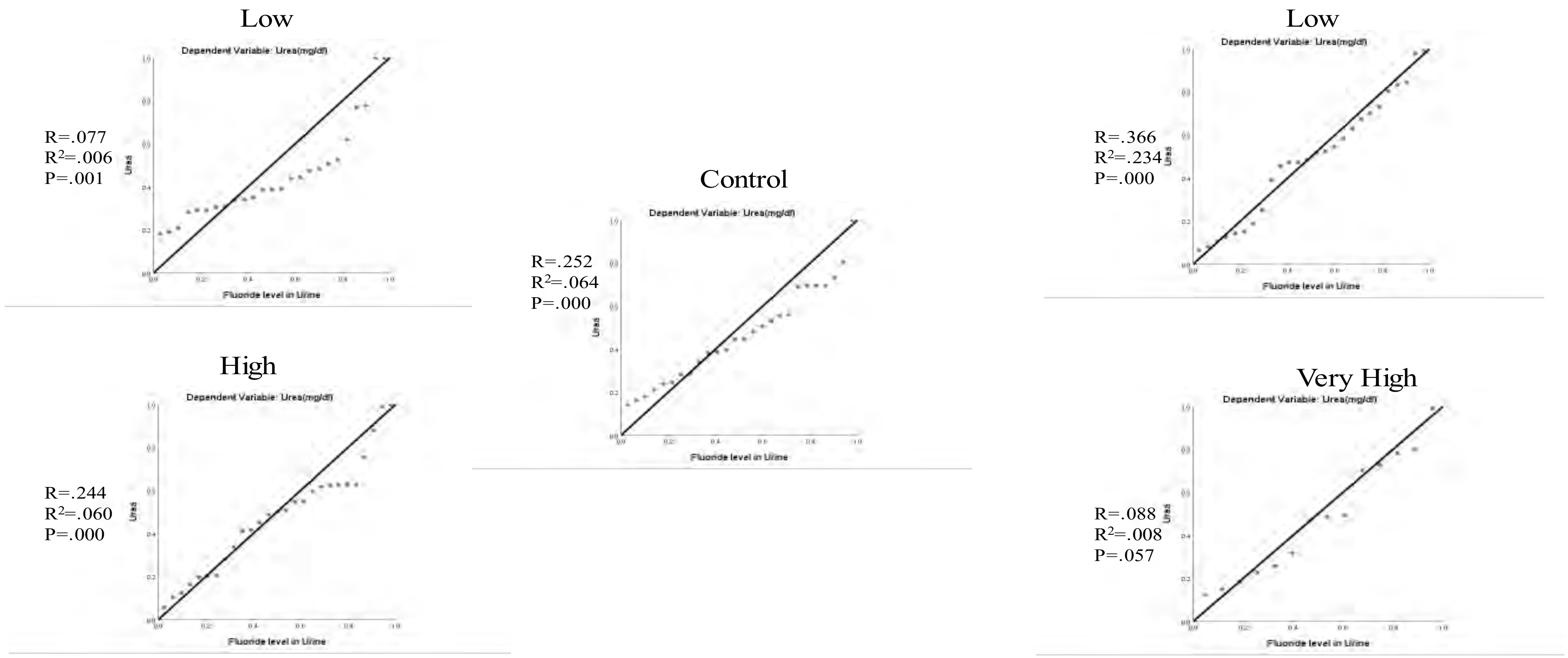


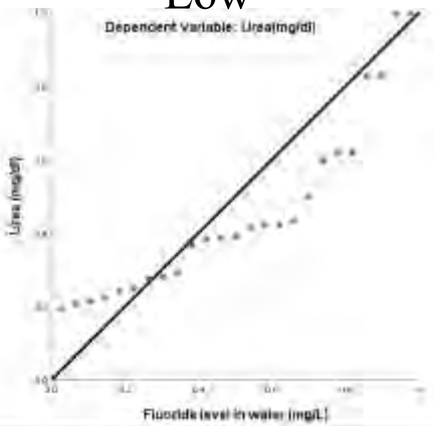
Figure 3.8 Simple linear regression analysis with Urea (Group base) as a dependent variable and Urine fluoride as independent variable

Low

R=.218

R^2=.048

P=.015

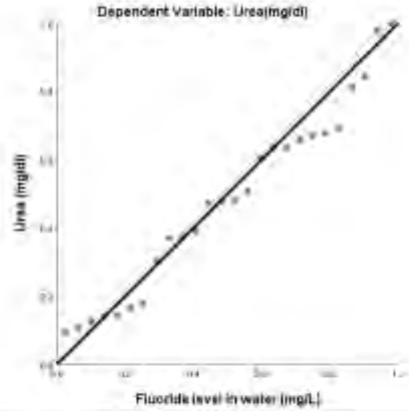


Medium

R=.181

R^2=.033

P=.000

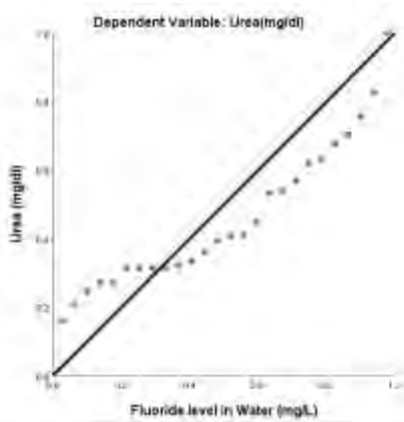


Control

R=.210

R^2=.040

P=.001

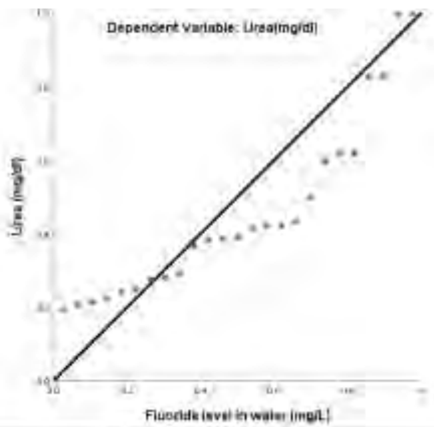


High

R=.338

R^2=.114

p=.017



Very High

R=.448

R^2=.201

P=.017

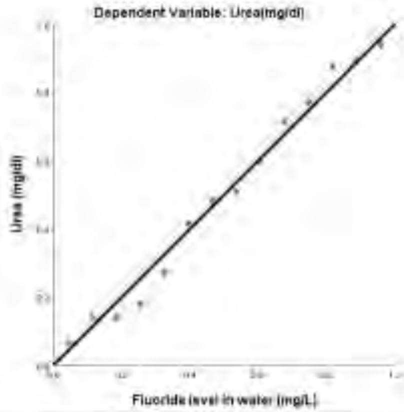


Figure 3.9 Simple linear regression analysis with Urea (Group base) as a dependent variable and water fluoride as independent variable

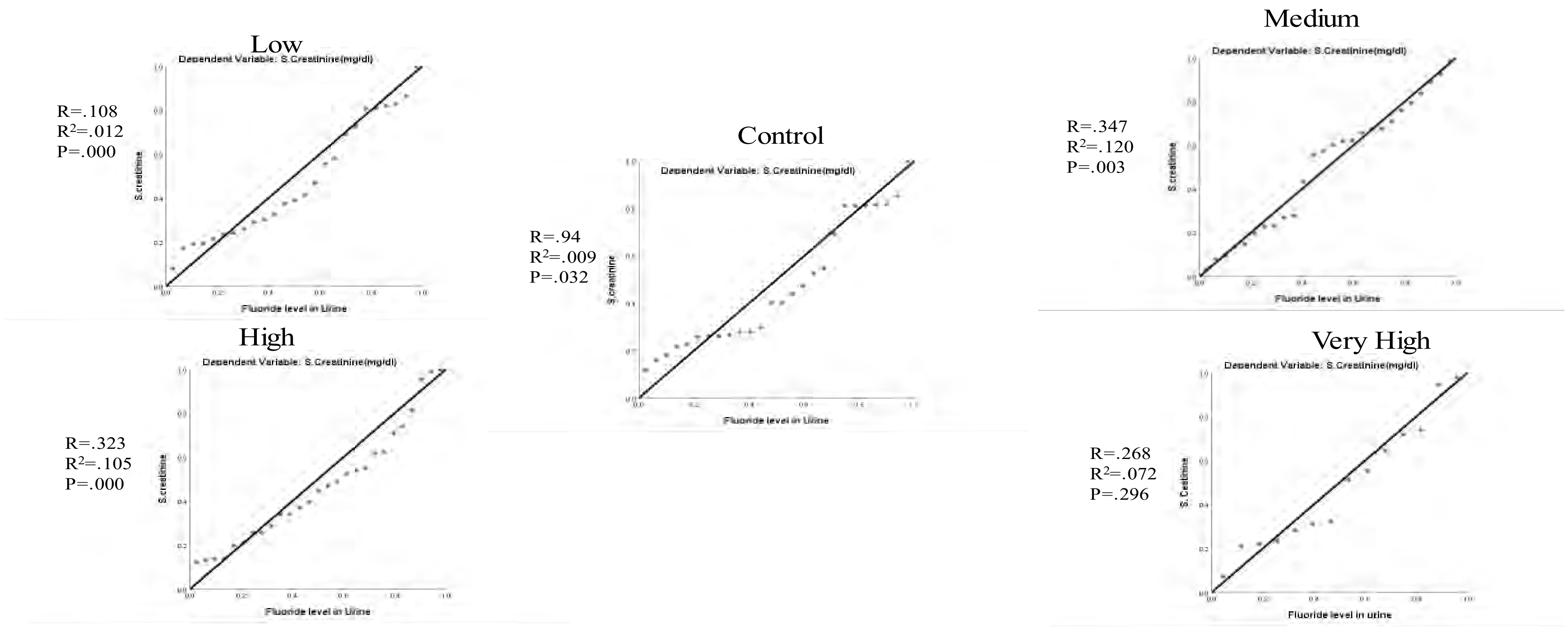
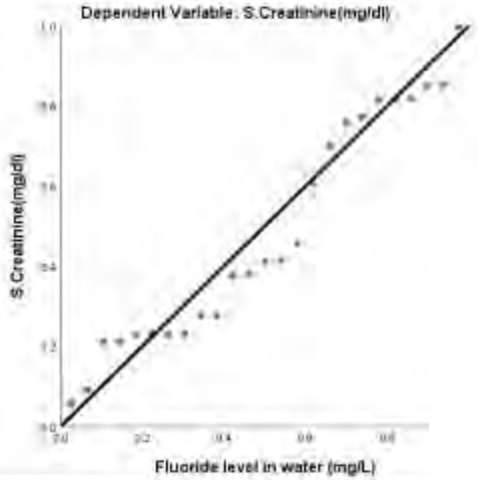


Figure 3.10 Simple linear regression analysis with Serum creatinine (Group base) as a dependent variable and Urine fluoride as independent variable

Low

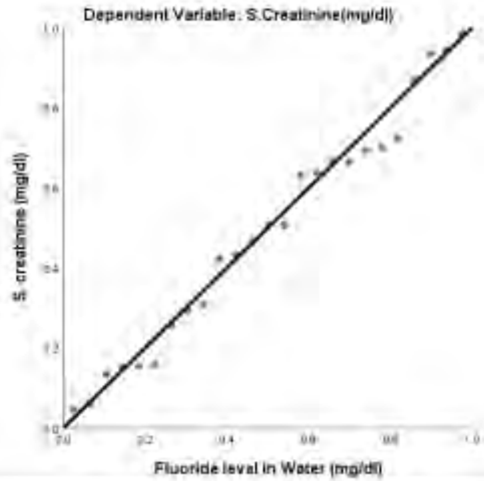


P=.339

R^2=.115

p=.119

Medium

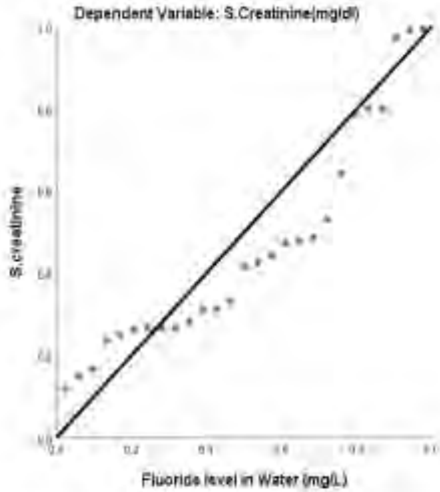


R=.089

R^2=.008

p=.159

Control

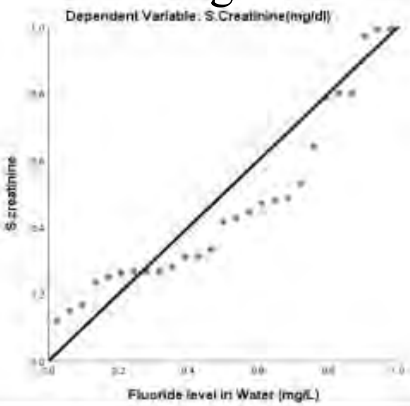


R=.099

R=.010

P=.002

High

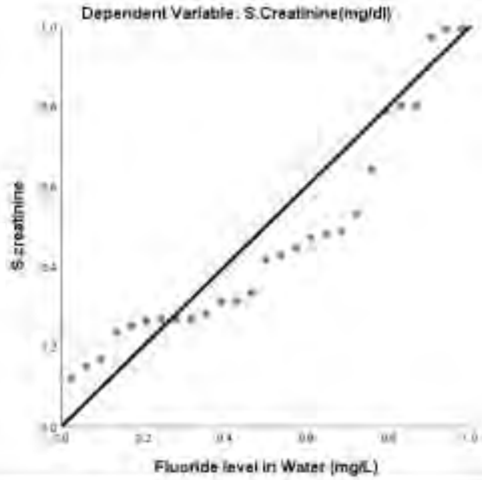


R=.206

R^2=.042

P=.120

Very High



R=.381

R^2=.145

P=.564

Figure 3.11 Simple linear regression analysis with Serum creatinine (Group base) as a dependent variable and water fluoride as independent variable

3.7 Manifestation of nephrotoxicity

Estimated glomerular filtration rate (eGFR) is a gold standard measurement of the filtration rate of functioning nephrons in the kidneys. It is commonly assessed through the urine-to-albumin creatinine ratio and serves as an important indicator for evaluating chronic kidney disease(Project, n.d.). Medical reports have highlighted the clinical significance of eGFR in detecting and categorizing chronic kidney disease, as well as determining its severity and guiding treatment decisions. eGFR is widely regarded as the gold standard parameter for assessing functions of kidney (Levey and inker, 2016). In current study, significant variations in eGFR were observed among the groups, with Group 5 exhibiting the most notable distinction compared to all other groups (table 3). The minimum eGFR value recorded in the exposed group was 45, while the maximum was 106, as shown in Table 3. Notably, Group 3, characterized by concentration of fluoride in water of ≥6 mg/L, displayed the lowest eGFR value. Furthermore, no significant correlations were found between variations in eGFR and gender or age, as presented in Tables 3. When the linear regression was performed between urine as independent variable and eGFR as dependent variable a significant relation was found among Low, medium and high while it was non-significant for very high group with variation in data. respectively (figure 3.12 R=.126, R^2=.016, p=.000, R=.230, R^2=.053, p=.000, R=.134, R^2=.018, p=.000, R=.416, R^2=.173, p=.000). When the simple linear regression was performed with eGFR as dependent variable and water as independent variable, a significant relation was found among low, medium, high and very high groups respectively (fig. 3.13 R=.058, R^2=.003, p=.000, R=.244 R^2=.059, p=.000, R=.067, R^2=.005, p=.041, R=.417 R^2=.174, p=.005).

Studies conducted in countries such as India and China have demonstrated a relation between high level of fluoride in urine and lower eGFR in young individuals (Ando et al., 2001; Khandare et al., 2017). Conversely, a study involving 374 Mexican children revealed higher urinary fluoride levels and elevated eGFR (Jimenez-Cordova et al., 2017 It is evident that chronic fluoride exposure may have long-term detrimental effects on kidney function (Jimenez-Cordova et al., 2017). In the present study, fluoride exposure was found to potentially impair kidney function, particularly in groups where the fluoride concentration in water exceeded 8 mg/L, leading to eGFR values outside the normal range. These findings suggest that chronic fluoride exposure may impair kidney function. Regarding serum creatinine, Group 2 displayed a statistically significant difference compared to the other groups. The question of whether elevated fluoride levels harm kidney function in humans remains controversial. Several studies have reported no significant evidence supporting the impact of fluoride on renal function in humans (Saylor et al., 2022). Another study conducted in China also highlights the ongoing controversy surrounding this claim, although there is substantial evidence supporting it(Xiong et al., 2007). Furthermore, a study involving 1985 adolescents in the United States concluded that a 1-µmol/L increase in plasma fluoride was associated with a 10.36 mL/min decrease in eGFR and higher level of serum uric acid (Saylor et al., 2022). Our findings, which demonstrate a significant association between fluoride exposure and renal function biomarkers, further enhance the notion of cumulative impacts of fluoride. Another study conducted in China revealed that exposure to fluoride due to coal pollution also resulted in reduced eGFR. Additionally, hyperfiltration has been visible in youth exposed to certain metals (Jiménez-Córdova et al., 2019).

Fluoride accumulate in bones(Meyer et al., 2022). which may contribute to chronic exposure and subsequent effects on kidney function over a prolonged period. During adolescence, when significant changes in bone structure and density occur, the body becomes more exposed to the adverse impacts of fluoride on the kidneys. In our study, hyperfiltration could be attributed to fluoride metabolism, leading to a higher excretion rate of fluoride. However, at this moment, there are insufficient evidences to support this assumption. Further studies are warranted to explore the potential mechanisms involved soon.

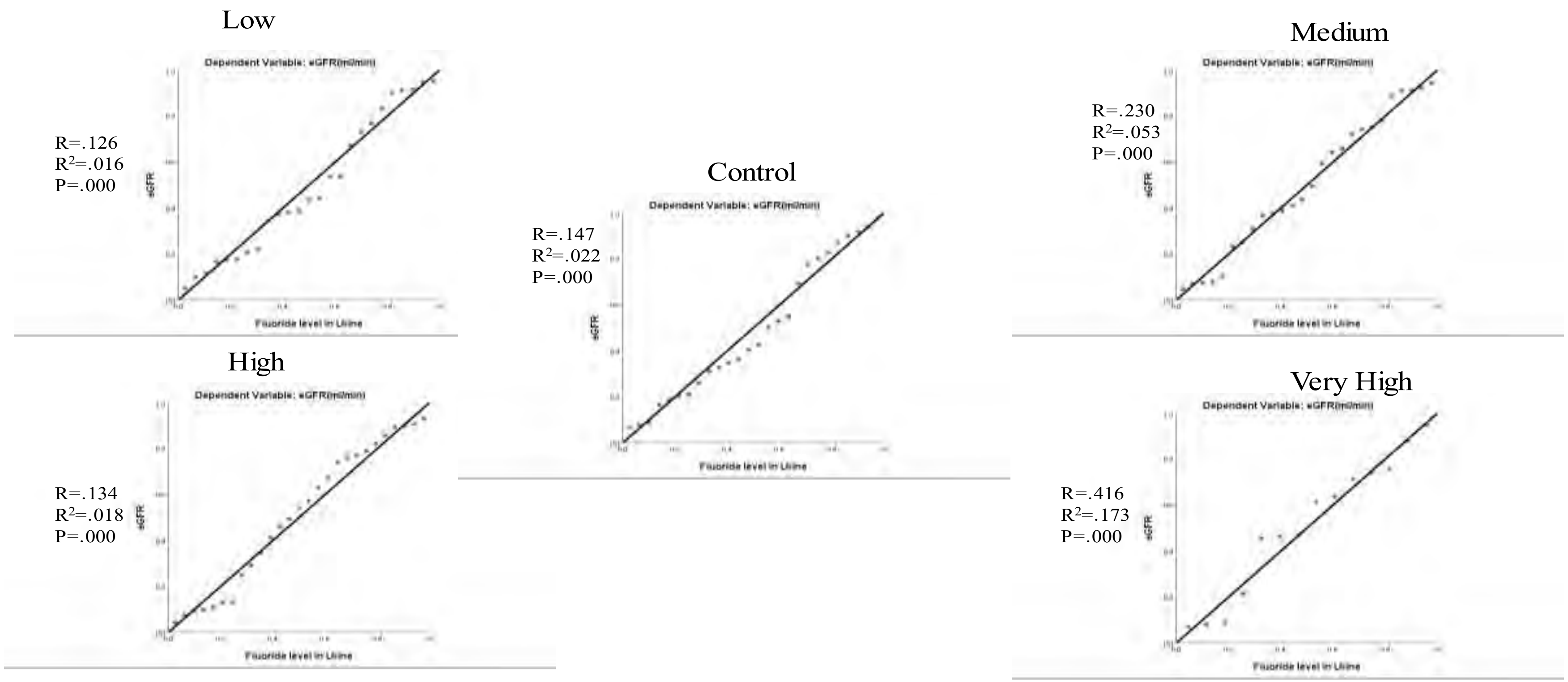
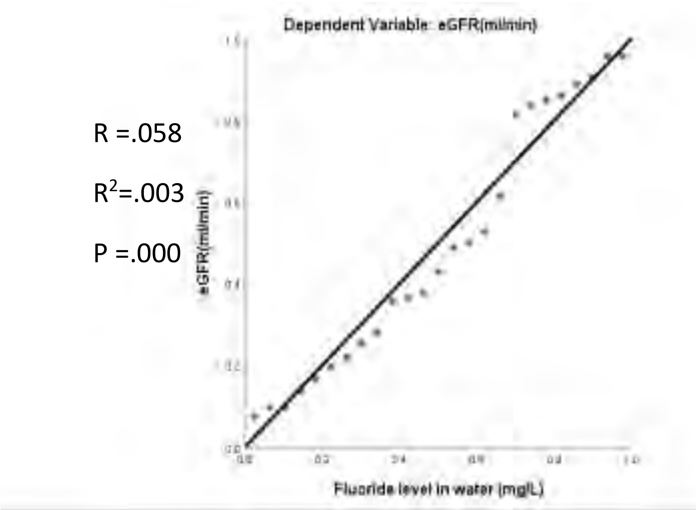


Figure 3.12 Simple linear regression analysis with eGFR (Group base) as a dependent variable and Urine fluoride as independent variable

Low

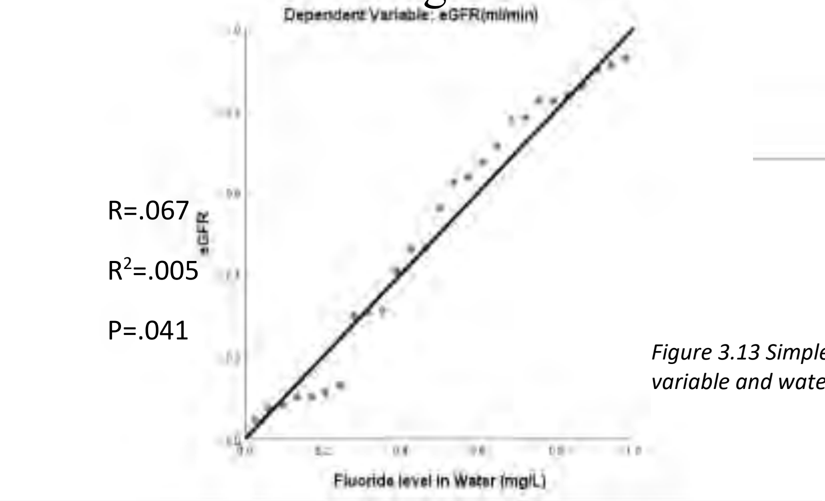


R =.058

R^2=.003

P =.000

High

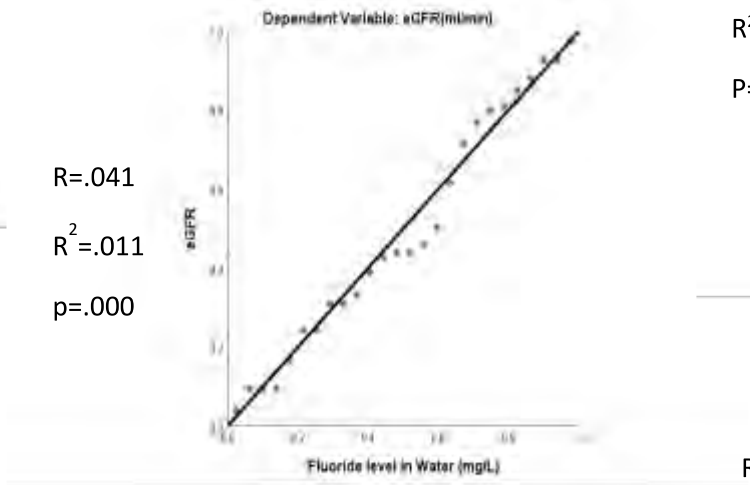


R=.067

R^2=.005

P=.041

Control

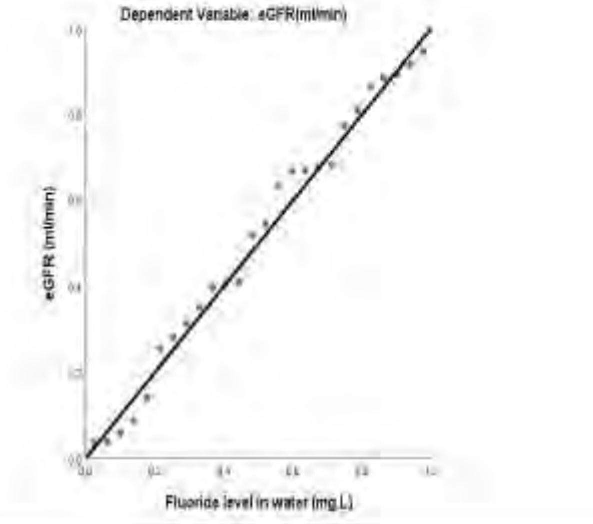


R=.041

R^2=.011

p=.000

Medium

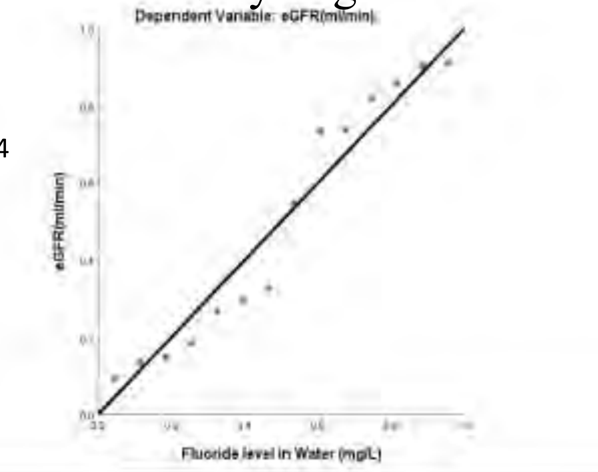


R=.244

R^2=.059

P=.000

Very High



R=.417

R^2=.174

P=.005

Figure 3.13 Simple linear regression analysis with eGFR (Group base) as a dependent variable and water fluoride as independent variable

Table 3-2 Group based variation in group parameters descriptive summary and t-test p-value.

Group	Statistics	F level in water	Tea Intake	Dental Fluorosis	RBS (mg/dl)	T.Bilirubin (mg/dl)	ALT SGPT (U/L)	AST SGOT (U/L)	Alk.Phosphatase (U/L)	Albumin(g/dl)	S.Creatinine (mg/dl)	Uric Acid (mg/dl)	Urine Fluoride Level(ppm)	eGFR (mg/dl)	Urea(ml/min)
1	Mean ±SD (Min-Max)	2.94± 0.71 (1.60-4.30)	1.56± 1.15 (0-3)	1.32± 0.94 (0-3)	111.8± 14.40 (87-145)	0.68± 0.19 (0.3-0.9)	29.04± 4.25 (19-3)	25.6 ± 4.5 (19-36)	190.32± 28.74 (122-240)	4.276± 0.35 (3.91-5.1)	0.73± 0.26 (0.4-1.6)	6.52 ± 9.50 (3.10-5.20)	1.84 ± 0.38 (.87-2.78)	74.00± 13.96 (52.75-98.58)	32.08 ±10.17 (22-63)
2	Mean ±SD (Min-Max)	4.8± 0.56(2.50-5.80)	1.12±1.39 (0-4)	1.62±0.98 (0-3)	111± 13.33 (90-131)	0.673± 0.40 (0.3-1.9)	33± 15.39 (16.88)	25.19± 8.52 (17-56)	203.15± 33.99 (149-301)	4.188± 0.39 (3.5-5.3)	0.82± 0.18 (0.3-1.0)	5± 0.72 (3.40-6.40)	1.72 ± 0.51 (1.01-2.89)	81.08 ±15.96 (54.41-106.92)	28.61±4.23 (23-49)

3	Mean ±SD (Min-Max)	6.78± 0.57(5.20-7.90)	2.11± 1.28 (0-5)	2.07± 1.15 (0-4)	110.74± 17.82 (78-137)	0.67± 0.22 (0.3-1.1)	30.85 ± 8.66 (4.1-47)	25.3 ± 5.88 (16-38)	204.7± 31.77 (156-300)	4.32± 0.51 (3.6-5.5)	1.03 ± 0.78 (0.3-3.1)	5.47± 1.53 (3.30-8.50)	1.56 ± 0.66 (.03-3.80)	75.48± 17.47 (45.58-101.16)	26.01±15.29 (5.3-78.0)
4	Mean ±SD (Min-Max)	8.55± 0.83(5.20-10.40)	1.50± 1.01 (0-3)	1.79± 1.18 (0-4)	106.07± 18.74 (89-134)	0.60± 0.16 (0.3-0.8)	35.14 ± 7.90 (28-50)	28.14 ± 6.08 (22-40)	219.5± 33.30 (178-320)	4.55± 0.84 (3.5-6.0)	0.82± 0.35 (0.3-1.5)	6.88 ± 1.25 (5.40-8.10)	1.70 ± 0.43 .74-2.29)	72.13± 14.13 (47-90.40)	15.37±5.48 (9-30)
Control	Mean ±SD (Min-Max)	1.08± .30(0.50-1.40)	1.00± 0.76 (0-2)	0.04± 0.19 (0-1)	110.88± 23.25 (79-168)	0.54± 0.18 (0.3-0.9)	28.61± 5.70 (18-44)	20.58± 4.35 14-32)	199.38± 22.20 (129-228)	4.29± 0.50 (3.2-5.2)	0.75± 0.25 (0.4-1.6)	5.72± 0.90 (3.40-6.70)	1.52± 0.58 .03-3.80)	80.69± 16.92	28.50± 7.88 (5.30-78)

														(80.54-117)	
	P-value	**.000**	**.007**	**.000**	**.990**	**.276**	**.002**	**.001**	**.078**	**.379**	**.026**	**.586**	**.000**	**.000**	**.000**
Gender	**Male**	5.33±2.13 (1.6-10.4)	1.71±1.26 (0-5	1.76±1.03 (0-4)	110.68±15.53 (78-145)	0.65±0.24 (0.3-1.9)	31.08±8.74 (4.1-78)	25.71±5.77 (16-46)	201.67±34.46 (122-320)	4.27±0.50 (3.5-5.9)	0.80±0.50 (0.3-3.1)	5.24±1.32 (3.1-8.5	1.73±0.52 (0.03-3.8)	76.24±15.98 (47-106.925)	26.90±12.27 (5.3-78)

	Female	5.638 ± 1.8777 (3.3-9.3	0.85± 1.214 (0-3	1.31± 1.316 (0-4	110.92± 14.958 (85-129	0.738 ± 0.4093 (0.3-1.9	35.62± 16.495 (28-88	27.23± 9.619 (20-56	200.31± 22.911 (157-245	4.469± 0.5851 (3.8-6	0.715± 0.3184 (0.3-1.6	5.82± 1.14 (4.4-8.05	1.55± 0.48 (1.01 - 2.51	74.71± 14.16 (45.58-91.41	27.69± 7.20 (12-40
	P-Value	**.432**	**.987**	**.173**	**.714**	**.217**	**.057**	**.228**	**.206**	**.577**	**.230**	**.532**	**.707**	**.208**	**.379**
Age	**11-14 Years**	5.14± 2.21	1.59± 1.27	1.57± 1.04	110.50± 15.28	.659±.25	31.39± 10.42	26.05 ±6.96	201.67±33.19	4.309±.57	.715±.28	6.09± 7.02	1.76±.41	64.65± 11.34	27.15± 8.85

	15-19 Years	5.66± 1.96	1.63± 1.34	1.86± 1.10	111.98± 15.37	.686±. 28	32.25± 10.44	25.29 ±6.21	202.07±3 4.17	4.302±. 46	.856±. 65	5.56± 1.41	1.62±. 63	87.28± 10.59	27.20± 14.43
	P-value	**.281**	**.568**	**.867**	**.573**	**.339**	**.656**	**.580**	**.460**	**.781**	**.007**	**.259**	**.063**	**.562**	**.060**
Exp Vs Control	**P-value**	**.000**	**.000**	**.000**	**.267**	**.007**	**.267**	**.478**	**.213**	**.114**	**.104**	**.262**	**.82**	**.001**	**.746**

Group 1= low level of fluoride in water (1.5-2 mg/L)

Group 2= Medium level of fluoride (2.1-6 mg/L)

Group 3= High level of fluoride (6.1-8 mg/L)

Group 4= Very high level of fluoride(>8mg/L)

Control= (<1.5 mg/L)

Significance at P<0.05

3.8 Effects of Passive Smoking Exposure on Biomarkers

It is widely known that smoking has detrimental impacts on the human health. Several research studies concluded that, smoking can lead to impaired renal function, and it increases the potential risks of chronic kidney diseases. The present study did not include any active smokers, but some participants were exposed to secondhand smoke. To assess the potential adverse impacts of secondhand smoke exposure, participants were distributed into two groups based on the presence or absence of such exposure. A t-test was applied to determine the statistical significance of differences between the groups. Results revealed a significant difference in the levels of urea between the two groups. This can be asserted that smoke exposure does impact the renal function.

A cross sectional study conducted on 280 participants (140 smokers, 70 active smokers, 70 passive smokers, 140 non-smokers) concluded that smoking exposure does impact the renal function of humans(Eid et al., 2022). Another study concluded that both active and passive smoking is toxic for renal function(Cooper, 2006).

Moreover, passive smoking exposure has been associated with different types of cardiovascular diseases and malignancies. The side stream smoke, a main component to passive smoking was a source of more toxic substances than those which are found in main stream smoke (Cooper, 2006).

Table 3-3 P-value of t-test of biomarkers between participants having smoking exposure and no-smoking exposure.

Biomarkers	P-Value
RBS	0.5165
T.Bilirubin	0.435
ALT SGPT	0.342
AST SGOT	0.716
Alk.Phosphatase	0.403
Albumin	0.634
Urea	0.032
Creatinine	0.089
Urine Fluoride Level	0.093
eGFR	0.945

3.9 Effects of gender/age variability on fluoride toxicity

As presented in table 3.1 urinary fluoride concentration did not manifest any significant difference when urinary fluoride values of boys and girls were compared. This result was in accordance with the previous findings of (Saeed et al., 2021). In that study there was no variation based on gender were found. Similar findings were observed in prevalence of dental fluorosis. The dental fluorosis findings were also accordance with a previous study conducted in the same area (Saeed et al., 2021). In this study it was also founded that there has been no significant variation of urinary fluoride based on age. This finding of present study was similar with the previous study (Das and Mondal, 2016). Table 3.1 also presented that age does not have impact on prevalence of dental fluorosis among participants. We found this result in accordance with a previous study conducted in this area (Saeed et al., 2021).

To assess the association of biomarkers the results of the participants were compared on basis of gender (male, female). Moreover, the results of the participants were divided into two age groups (11-14, 15-19 Years). Table 3 presents the variation of hepatic biomarkers based on gender and age.

Table 3.1 also explains that there has been no variation in fluoride toxicity on hepatic biomarkers based on gender and age of the participant. The result of this study was accordance with several previous studies conducted globally(Perera et al., 2018). Statistical results show that there has been no significant difference at significance level 0.005 between biomarkers when compared with each other based on gender and age. Previous studies conducted on assessment of drinking water fluoride impact on hepatic biomarkers concluded different results. It has been reported that Factors like gender does not show any influence on hepatic biomarkers. However, there are mixed findings about another factor like. It also been concluded that fluoride exposure for long period may exert chronic symptoms (Sanders et al., 2018).

The table also presents that there has been no variation found in fluoride toxicity on kidney parameters. The fluoride toxicity was independent from gender and age parameters. It was due to the accumulation of fluoride in organs.

eGFR with urinary and water fluoride showed a negative correlation in Pearson correlation analysis as shown in fig (4). It explains that elevated levels of fluoride do impact the eGFR which has been reported in previous studies too. School children with mean urinary concentration of 3µg/mL have showed a decreased eGFR (khandare et al., 2017). More than 2 mg/L concentrations of drinking water fluoride were linked with kidney damage in children (Xiong, et al., 2007). Our results confirm the negative association between urinary and water fluoride concentration. Urea, another biomarker of nephrotoxicity, had a negative correlation with water. Thus, more studies which include other renal biomarkers are required to assess the potential nephrotoxic impacts of fluoride.

3.10 Principal Component Analysis

This Euclidean biplot got by the help of two axes of PCA to examine the variation between exposed and control group (Fig.3.10). Components of the plot summed 56.34% variation in data. First component showed 41.09% of the total variance while the other component accounted for 15.25% of the total variation in data. It is important to know that, first component was consisted of Uric acid, S.Creatinine, ALT SGPT, T.Bilirubin, RBS, T.bilirubin, F¯ level in urine and water, Albumin, and alk.phosphatese,. The cluster of exposed groups showed positive correlation with F¯ level in water and urine. There was a clear distinction between the cluster of control and exposed group. The overall PCA showed that exposed group had high level of F¯ in water and urine. While other health parameters also showed a distinct cluster than control group.

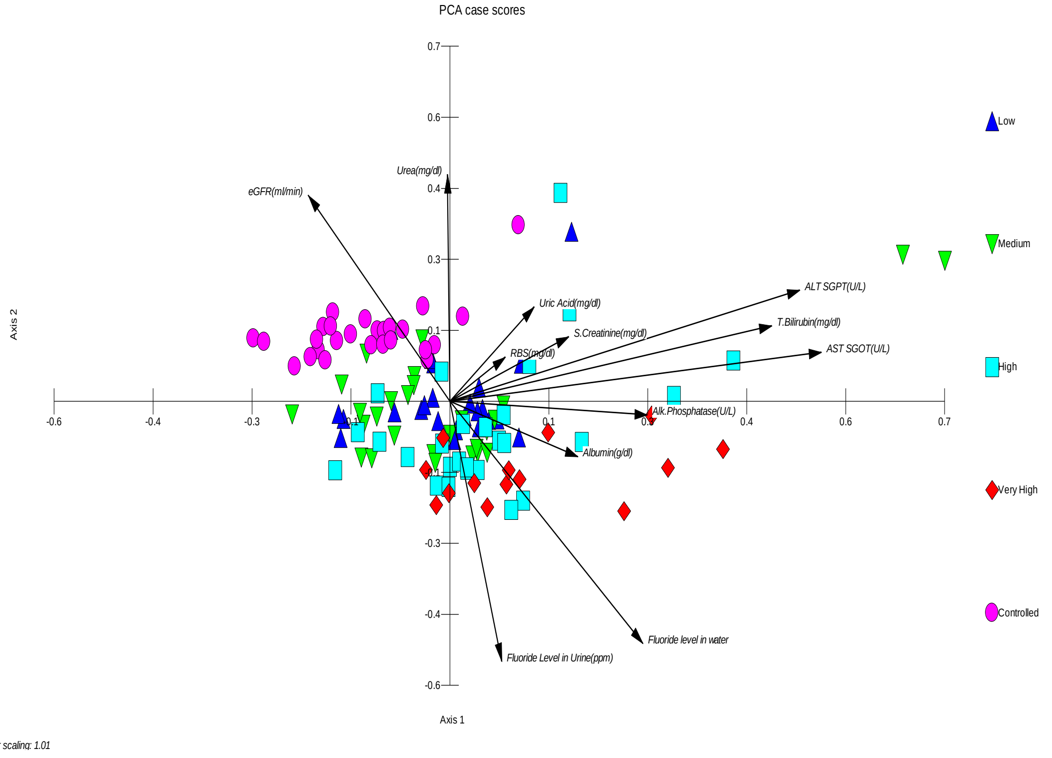


Figure 3.14 Euclidean biplot of the first two components (56.34%) shows association between health parameters of exposed and control.

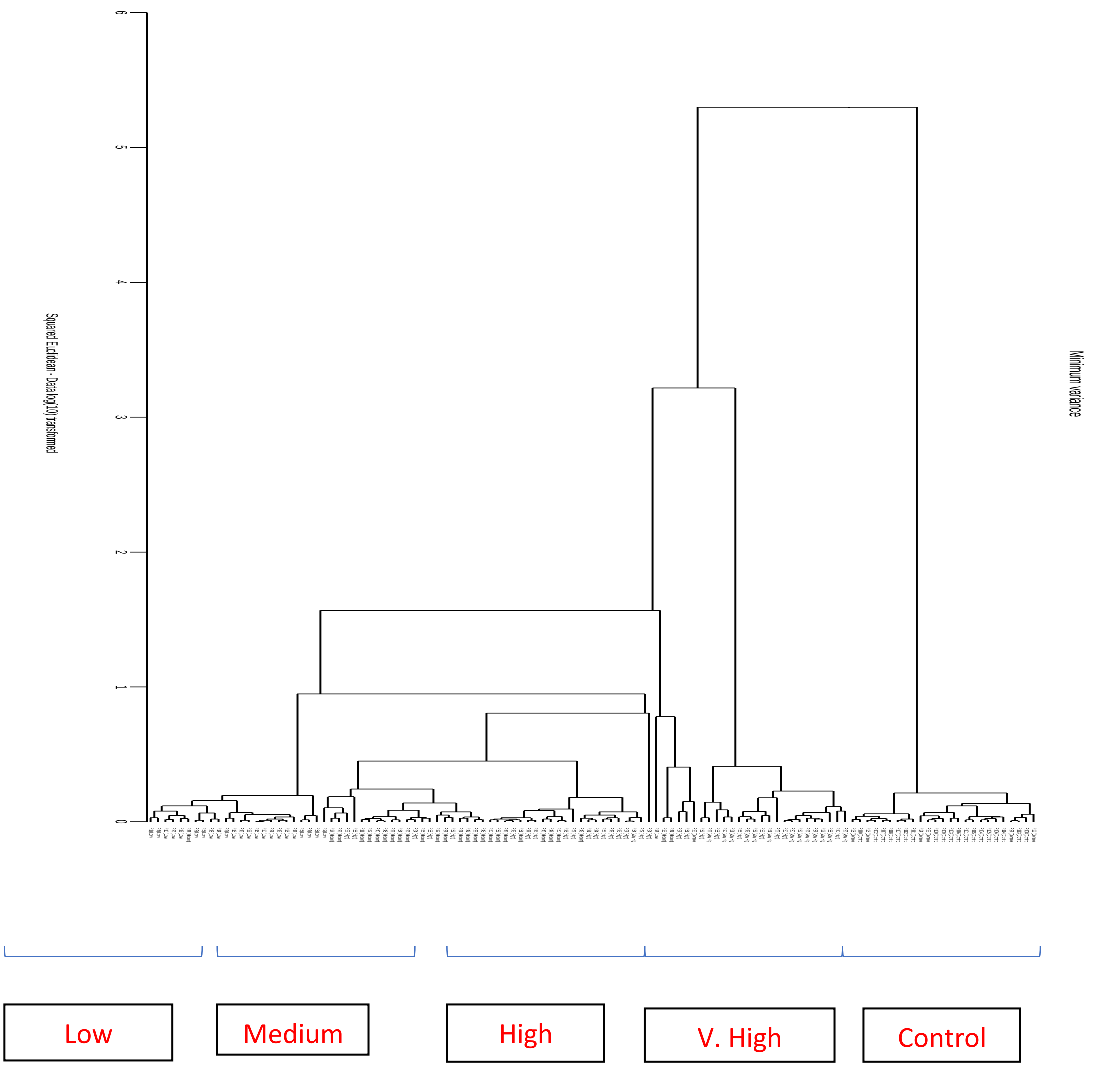


Figure 3.15 Dendrogram presenting hierarchical relationship between groups.

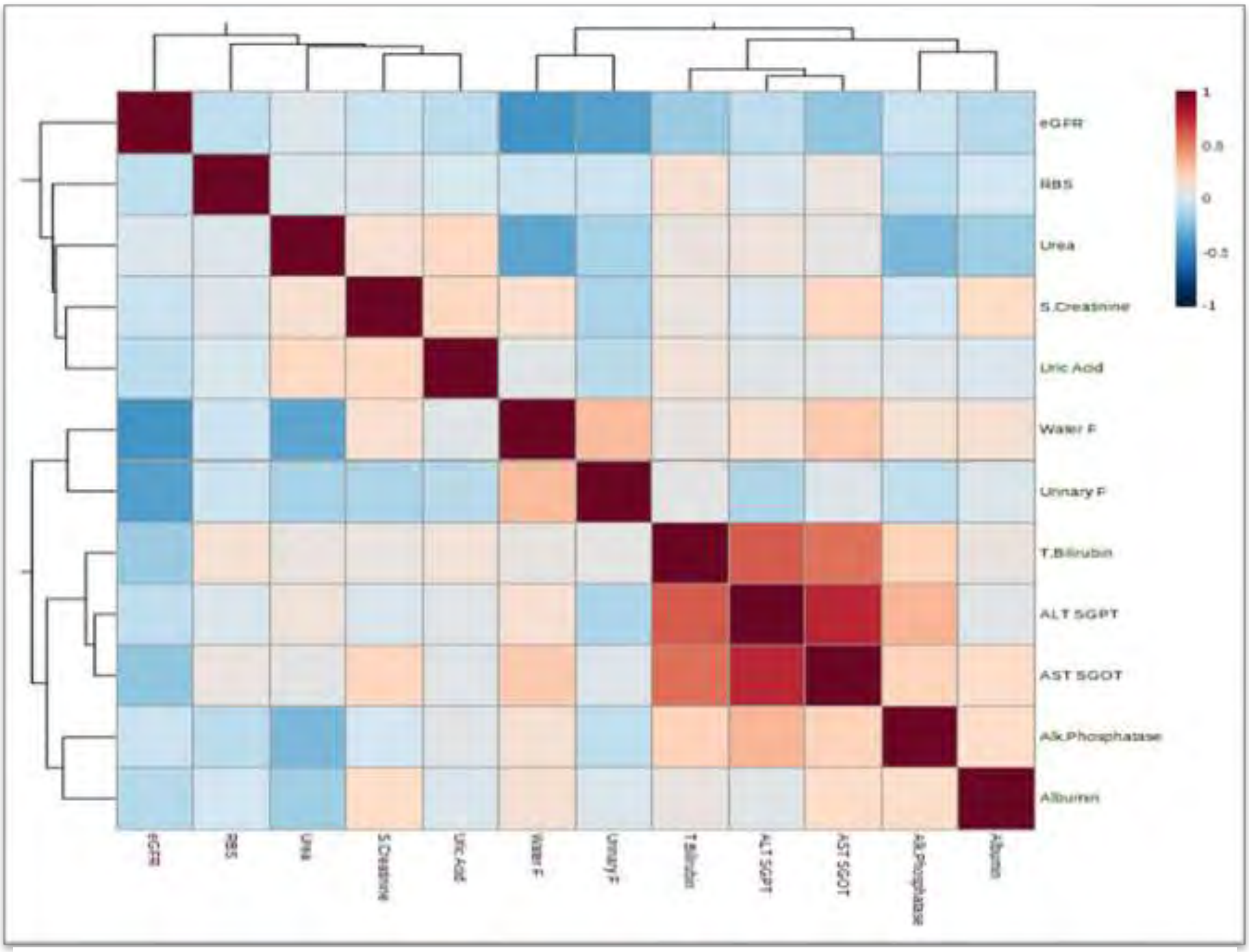


Figure 3.16 Heat Map representing correlation between biomarkers.

CHAPTER 4

CONCLUSION

Objective of this current study was to assess the chronic effects of consuming fluoride-contaminated drinking water on the health of adolescents residing in the areas of district Lahore and district Kasur. Various rural regions belonging to these districts were found to fluoride level in drinking water that exceeded allowable limit set by WHO. The study confirmed the presence of elevated fluoride levels in water, which was causing the presence of dental fluorosis in areas such as Kallanwala, Kot Maigha, Chah Fateh Wala, and Kot Asadullah. Additionally, the community fluorosis index results indicated that dental fluorosis was prevalent in community. Furthermore, fluoride level in urine was also found more than the allowable limit of WHO, indicating the existence of fluoride toxicity in community. The results of dental examination showed that 35 of the adolescents had a very mild level of dental fluorosis, 58 had a mild level, 19 had dental fluorosis, and 4 had a severe level. The study also concluded that there has been no association of dental fluorosis with age and gender. A statistically significant difference among the exposed and controlled groups in this study was found. The mean urinary concentration of the exposed groups was low, medium, high, and very high, with values of 1.84 µg/L, 1.72 µg/L, 1.56 µg/L, and 1.70 µg/L, respectively, compared to 0.88 µg/L for the control group. Additionally, the results of different liver and kidney biomarkers of the participants showed evidence of fluoride toxicity. The present study concluded that exposure to fluoride in drinking water showed a significant impact on the hepatic biomarkers of the study gorup. Specifically, the results showed a statistically significant difference in the AST levels among exposed and controlled groups. The findings suggested that the variation in fluoride levels in drinking water might be responsible for the observed difference in AST levels. When linear regression was applied a significant relation was found among water, urine and AST in all groups except very high group. Moreover, the present study also concluded that the impact of drinking water fluoride on hepatic biomarkers was independent from demographic parameters such as age and gender. Furthermore, the study found that the purine products of the kidney were affected by fluoride toxicity. The observed statistical difference in value of urea between the participants of each group may indicate the presence of kidney disorder. However, Serum creatinine levels were significantly different between the groups. The eGFR was also found to be significantly different between all the groups, with control showing the most significant difference compared to all other groups. The lowest value of eGFR was found in the exposed group, indicating impaired kidney function. This study also summed that there has been no correlation between the kidney parameters and demographic parameters (age, gender). During the study, it was also observed that the residents of the affected areas were poor, with most being farmers or daily wage laborers. The residents had limited knowledge about the severity of the fluoride contamination issue, and the hygienic conditions in the affected areas were poor. As such, the residents of these rural settlements are in dire need of a water treatment plant to control the chronic toxicity of fluoride in their drinking water. These findings provide crucial evidence for the need to address the issue of fluoride contamination in rural areas to prevent the negative health impacts associated with chronic exposure. There is dire need of research to investigate the long-term adverse impacts of fluoride toxicity on the health of affected populations, and to develop effective interventions to mitigate the risks associated with fluoride contamination in drinking water.

4.1 Recommendations:

Monitoring and Regulation: Implement a strict monitoring system to regularly test the level of fluoride in drinking water sources. It is crucial to ensure that fluoride concentrations are consistently below the recommended levels to prevent adverse health effects.

Water Treatment: Invest in water treatment facilities, such as defluoridation units or alternative water sources, to decrease the level of fluoride in drinking water. This approach can help bring the fluoride levels within the acceptable range and minimize the potential health risks associated with excessive fluoride consumption.

Awareness to public and Education: There should be public awareness campaigns to inform the residents about the adverse effects of fluoride toxicity on liver and kidney health. Emphasize the importance of consuming water with safe fluoride levels and promote healthy alternatives, such as filtered water or bottled water, if necessary.

Regular Health Check-ups: Encourage regular health check-ups, particularly focused on liver and kidney function, for individuals residing in those areas where fluoride concentrations in drinking water are more than the permissible limit. Early detection of any adverse effects can prompt timely interventions and appropriate medical treatments to mitigate potential health risks.

Collaboration with Health Authorities: Foster collaboration between local health authorities, water management departments, and relevant research institutions to address the issue comprehensively. This collaboration can facilitate the exchange of knowledge, resources, and expertise to develop and implement effective strategies to mitigate the toxicity caused by fluoride accumulation on hepatology and nephrology of humans.

REFERENCES

Ali, S., Thakur, S.K., Sarkar, A., Shekhar, S., 2016. Worldwide contamination of water by fluoride. Environ. Chem. Lett. 14, 291–315. https://doi.org/10.1007/s10311-016-0563-5

Ali, W., Aslam, M.W., Junaid, M., Ali, K., Guo, Y., Rasool, A., Zhang, H., 2019. Elucidating various geochemical mechanisms drive fluoride contamination in unconfined aquifers along the major rivers in Sindh and Punjab, Pakistan. Environ. Pollut. 249, 535–549. https://doi.org/10.1016/j.envpol.2019.03.043

Amini, H., Taghavi Shahri, S.M., Amini, M., Mehrian, M.R., Mokhayeri, Y., Yunesian, M., 2011. Drinking water fluoride and blood pressure? An environmental study. Biol. Trace Elem. Res. 144, 157–163. https://doi.org/10.1007/s12011-011-9054-5

Choubisa, S.L., Choubisa, D., 2016. Status of industrial fluoride pollution and its diverse adverse health effects in man and domestic animals in India. Environ. Sci. Pollut. Res. 23, 7244–7254. https://doi.org/10.1007/s11356-016-6319-8

Cooper, R.G., 2006. Effect of tobacco smoking on renal function. Indian J. Med. Res. 124, 261–268.

Danziger, J., Dodge, L.E., Hu, H., 2022. Role of renal function in the association of drinking water fluoride and plasma fluoride among adolescents in the United States: NHANES, 2013–2016. Environ. Res. 213, 113603. https://doi.org/10.1016/j.envres.2022.113603

Das, K., Mondal, N.K., 2016. Dental fluorosis and urinary fluoride concentration as a reflection of fluoride exposure and its impact on IQ level and BMI of children of Laxmisagar, Simlapal Block of Bankura District, W.B., India. Environ. Monit. Assess. 188. https://doi.org/10.1007/s10661-016-5219-1

Eid, H.A., Moazen, E.M., Elhussini, M., Shoman, H., Hassan, A., Elsheikh, A., Rezk, A., Moursi, A., Atef, M., Kabil, A., 2022. The Influence of Smoking on Renal Functions Among Apparently Healthy Smokers. J. Multidiscip. Healthc. Volume 15, 2969–2978. https://doi.org/10.2147/jmdh.s392848

Green, R., Till, C., Cantoral, A., Lanphear, B., Angeles Martinez-Mier, E., Ayotte, P., Wright, R.O., Tellez-Rojo, M.M., Malin, A.J., 2020. Associations between urinary, dietary, and water fluoride concentrations among children in Mexico and Canada. Toxics 8, 1–11. https://doi.org/10.3390/toxics8040110

Irigoyen-Camacho, M.E., García Pérez, A., Mejía González, A., Huizar Alvarez, R., 2016. Nutritional status and dental fluorosis among schoolchildren in communities with different drinking water fluoride concentrations in a central region in Mexico. Sci. Total Environ. 541, 512–519. https://doi.org/10.1016/j.scitotenv.2015.09.085

Jiménez-Córdova, M.I., González-Horta, C., Ayllón-Vergara, J.C., Arreola-Mendoza, L., Aguilar-Madrid, G., Villareal-Vega, E.E., Barrera-Hernández, Á., Barbier, O.C., Del Razo, L.M., 2019. Evaluation of vascular and kidney injury biomarkers in Mexican children exposed to inorganic fluoride. Environ. Res. 169, 220–228. https://doi.org/10.1016/j.envres.2018.10.028

Kabir, H., Gupta, A.K., Tripathy, S., 2020. Fluoride and human health: Systematic appraisal of sources, exposures, metabolism, and toxicity. Crit. Rev. Environ. Sci.

Technol. 50, 1116–1193. https://doi.org/10.1080/10643389.2019.1647028

Khattak, J.A., Farooqi, A., Hussain, I., Kumar, A., Singh, C.K., Mailloux, B.J., Bostick, B., Ellis, T., van Geen, A., 2022. Groundwater fluoride across the Punjab plains of Pakistan and India: Distribution and underlying mechanisms. Sci. Total Environ. 806, 151353. https://doi.org/10.1016/j.scitotenv.2021.151353

Maria, A., Version, R., Technologies, W., 2003. The costs of water pollution in India .

Meyer, J., Margaritis, V., Jacob, M., 2022. The Impact of Water Fluoridation on Medicaid-Eligible Children and Adolescents in Alaska. J. Prim. Prev. https://doi.org/10.1007/s10935-021-00656-x

Mohammed, I.A., Al.Okaily, B.N., 2017. Effect of sodium fluoride on liver functions of rats and amelioration by CoQ10. J. Entomol. Zool. Stud. 5, 887–893.

Perera, T., Ranasinghe, S., Alles, N., Waduge, R., 2018. Effect of fluoride on major organs with the different time of exposure in rats. Environ. Health Prev. Med. 23, 1–9. https://doi.org/10.1186/s12199-018-0707-2

Project, J., n.d. F R E Q U E N T Ly a S K E D Q U E S T I O N S a B O U T.

Rezaei, M., Nikbakht, M., Shakeri, A., 2017. Geochemistry and sources of fluoride and nitrate contamination of groundwater in Lar area, south Iran. Environ. Sci. Pollut. Res. 24, 15471–15487. https://doi.org/10.1007/s11356-017-9108-0

Rives, L.H., 2020. K i n e t i c method 02, 1–2.

Saatkamp, C.J., Silveira, L., 2023. Quantifying creatinine and urea in human urine through Raman spectroscopy aiming at diagnosis of kidney disease Quantifying creatinine and urea in human urine diagnosis of kidney disease. https://doi.org/10.1117/1.JBO.21.3.037001

Saeed, M., Rehman, M.Y.A., Farooqi, A., Malik, R.N., 2021. Arsenic and fluoride co-exposure through drinking water and their impacts on intelligence and oxidative stress among rural school-aged children of Lahore and Kasur districts, Pakistan. Environ. Geochem. Health 4. https://doi.org/10.1007/s10653-021-01141-4

Saha, J.C., 2000. Comparative Studies for Selection of Technologies for Arsenic Removal from Drinking Water. Proc. BUET Int. Conf. 76–84.

Sanders, A.P., Svensson, K., Gennings, C., Burris, H.H., Oken, E., Amarasiriwardena, C., Basnet, P., Pizano-Zarate, M.L., Schnaas, L., Tamayo-Ortiz, M., Baccarelli, A.A., Satlin, L.M., Wright, R.O., Tellez-Rojo, M.M., 2018. Prenatal lead exposure modifies the effect of shorter gestation on increased blood pressure in children. Environ. Int. 120, 464–471. https://doi.org/10.1016/j.envint.2018.08.038

Saylor, C., Malin, A.J., Tamayo-Ortiz, M., Cantoral, A., Amarasiriwardena, C., Estrada-Gutierrez, G., Tolentino, M.C., Pantic, I., Wright, R.O., Tellez-Rojo, M.M., Sanders, A.P., 2022. Early childhood fluoride exposure and preadolescent kidney function. Environ. Res. 204, 112014. https://doi.org/10.1016/j.envres.2021.112014

Sharma, P., Verma, P.K., Sood, S., Singh, M., Verma, D., 2023. Impact of Chronic Sodium Fluoride Toxicity on Antioxidant Capacity, Biochemical Parameters, and Histomorphology in Cardiac, Hepatic, and Renal Tissues of Wistar Rats. Biol.

Trace Elem. Res. 201, 229–241. https://doi.org/10.1007/s12011-022-03113-w

Singh, B., Gaur, S., Garg, V.K., 2007. Fluoride in drinking water and human urine in Southern Haryana, India. J. Hazard. Mater. 144, 147–151. https://doi.org/10.1016/j.jhazmat.2006.10.010

Tahir, M.A., Rasheed, H., 2013. Fluoride in the drinking water of Pakistan and the possible risk of crippling fluorosis. Drink. Water Eng. Sci. 6, 17–23. https://doi.org/10.5194/dwes-6-17-2013

Ugran, V., Desai, N.N., Chakraborti, D., Masali, K.A., Mantur, P., Kulkarni, S., Deshmukh, N., Chadchan, K.S., Das, S.N., Tanksali, A.S., Arwikar, A.S., Guggarigoudar, S.P., Vallabha, T., Patil, S.S., Das, K.K., 2017. Groundwater fluoride contamination and its possible health implications in Indi taluk of Vijayapura District (Karnataka State), India. Environ. Geochem. Health 39, 1017–1029. https://doi.org/10.1007/s10653-016-9869-2

Vithanage, M., Bhattacharya, P., 2015. Fluoride in the environment: sources, distribution and defluoridation. Environ. Chem. Lett. 13, 131–147. https://doi.org/10.1007/s10311-015-0496-4

Xiong, X.Z., Liu, J.L., He, W.H., Xia, T., He, P., Chen, X.M., Yang, K. Di, Wang, A.G., 2007. Dose-effect relationship between drinking water fluoride levels and damage to liver and kidney functions in children. Environ. Res. 103, 112–116. https://doi.org/10.1016/j.envres.2006.05.008

Younas, A., Mushtaq, N., Khattak, J.A., Javed, T., Rehman, H.U., Farooqi, A., 2019. High levels of fluoride contamination in groundwater of the semi-arid alluvial aquifers, Pakistan: evaluating the recharge sources and geochemical identification via stable isotopes and other major elemental data. Environ. Sci. Pollut. Res. 26, 35728–35741. https://doi.org/10.1007/s11356-019-06610-z