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Vitamin D deficiency in patients with diabetes and its correlation with water fluoride levels

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ABSTRACT

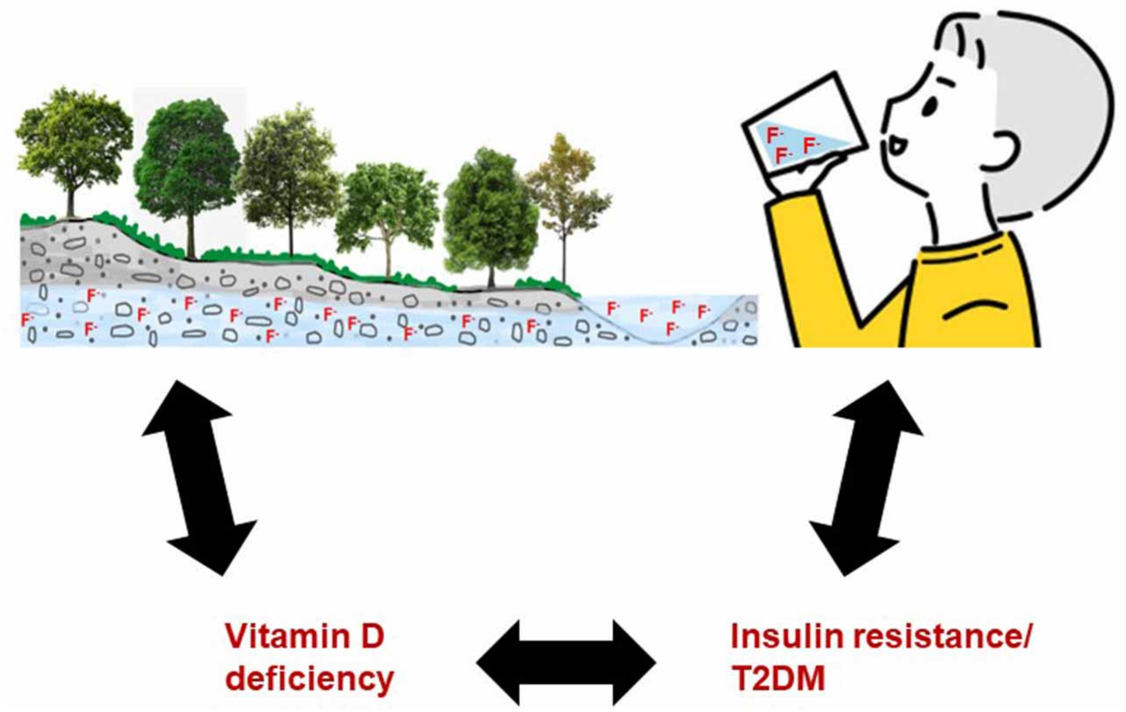
Chronic exposure to fluoride through drinking water has been linked to insulin resistance and resulting type 2 diabetes mellitus (T2DM). Here, we aim to study the impact of water fluoride levels on blood glucose and vitamin D levels. A hospital-based study was conducted on diabetic patients ($n = 303$) at All India Institute of Medical Sciences (AIIMS), Raebareli outstation patient department (OPD). The relationship between vitamin D or fasting blood glucose levels (BGLs) with water fluoride levels was estimated through Spearman's rank correlation. We found a significant negative correlation between water fluoride and vitamin D levels [$r_s = -0.777$, p-value < 0.001] and a positive correlation between water fluoride and fasting BGLs [$r_s = 0.178$, p-value < 0.05]. The participants residing in fluoride-endemic areas ($F > 1.5$ mg/L) had higher odds of severe vitamin D deficiency (odds ratio: 5.07, 95% CI: 1.9–13.2, p-value $= 0.0009$). The results demonstrate that vitamin D deficiency and fasting BGLs are significantly associated with water fluoride levels. This study signifies the role of fluoride toxicity in poor glycemic control and derived vitamin D deficiency. Vitamin D supplementation and the application of standard household water purification devices are recommended to tackle vitamin D deficiency in fluoride-endemic areas.

Key words: fasting blood glucose levels, fluoride, type 2 diabetes, insulin, vitamin D

HIGHLIGHTS

- Water fluoride levels had a negative correlation with vitamin D deficiency and a positive correlation with blood glucose levels.
- Toxic levels of fluoride in drinking water appeared as a risk factor for developing vitamin D deficiency.
- Severe vitamin D deficiency and high blood glucose levels were observed in patients from fluoride-endemic areas compared to non-endemic areas.

GRAPHICAL ABSTRACT



INTRODUCTION

Vitamin D is a fat-soluble vitamin derived from dietary intake and sunlight exposure (Mendes *et al.* 2018). Vitamin D level can be diagnosed with a blood test called 25-hydroxyvitamin D or 25(OH)D. Serum 25(OH)D concentration of less than 75 nmol/l (or 30 ng/ml) is considered vitamin D deficiency and less than 30 nmol/l (or 12 ng/ml) as severe vitamin D deficiency (Amrein *et al.* 2020). Vitamin D deficiency is a worldwide health problem influencing around 1 billion population (Bischoff-Ferrari *et al.* 2006; Nair & Maseeh 2012; Siddiqee *et al.* 2021). Its deficiency is more common in older persons (>60 years of age) and postmenopausal women (Lips 2001; Holick *et al.* 2005). A number of physiologic factors, e.g. 'age, sex, nutrition, location, and physical activity' influence vitamin D levels (Mithal *et al.* 2009). Low vitamin D levels are directly linked with the loss of bone density resulting into osteoporosis and fractures. In addition, vitamin D levels are also found to be associated with hypertension, cardiovascular diseases, diabetes, and chronic kidney disease (CKD) (Chonchol & Scragg 2007; Tanaka *et al.* 2009).

The onset and progression of diabetes mellitus (DM) have been linked with vitamin D deficiency through various mechanisms, e.g. 'low insulin secretion, pancreatic β-cell dysfunction and insulin resistance' (Chiu *et al.* 2004). Vitamin D supplementation is supposed to improve insulin resistance by promoting insulin secretion (Harris *et al.* 2012). However, a complete cure or prevention incidence of type 2 diabetes mellitus (T2DM) has not been reported upon supplementation with vitamin D (Pittas *et al.* 2019). T2DM is characterized by high blood sugar levels which may be caused by insulin resistance in which the utilization of insulin by cells is less efficient than normal. Due to the increasing demand of insulin to maintain a normal blood sugar level, pancreatic β-cells become less responsive to blood sugar levels and secrete less insulin leading to insulin resistance (Kasuga 2006). The persistent hyperglycemia in T2DM may arise due to increased glycogenolysis and reduced insulin secretion upon fluoride treatment in animal models (Varadacharyulu & Rao 1997).

The fluoride-contaminated water is a serious global health concern in developing countries, particularly in rural areas including India (Suthar *et al.* 2008; Abtahi *et al.* 2019; Del Bello 2020; Shanker *et al.* 2021). Fluoride content of more than 1.5 ppm or 1.5 mg/L in drinking water is endemic and critically impairs human health as per WHO (World Health Organization) and ICMR (Indian Council for Medical Research) guidelines (ICMR 1962; WHO 1963). Raebareli and nearby districts in eastern Uttar Pradesh have been reported for a number of diseases, e.g. 'macrocytic anemia, typhoid and CKD' (Gupta *et al.* 2020a, 2020b, 2020c). Fluoride was significantly and positively associated with the incidence of diabetes upon consumption of tap water in the United States of America (Fluegge 2016). Fluoride toxicity was found to be linked with increased insulin sensitivity, altered homeostasis, and insulin resistance based on animal studies (Lobo *et al.* 2015). Chronic exposure to fluoride imparts insulin resistance and results in T2DM (Trivedi *et al.* 1993; Chiba *et al.* 2012). In addition, water fluoride levels were also found to be significantly associated with the prevalence of hypertension (Amini *et al.* 2011; Ostovar *et al.* 2013).

The quality of groundwater is majorly compromised by the discharge of wastewater (e.g. brine) therefore, it cannot be used directly for potable and various industrial applications (Panagopoulos 2022a, 2022b; Panagopoulos & Giannika 2022). The major physiochemical pollutants of groundwater contamination are fluoride, nitrate, arsenic, iron, and salinity in Indo-Gangetic plains (Samal *et al.* 2020). The detailed water quality index of central Ganga plains including the Raebareli district demonstrated that fluoride and nitrate were majorly found above their permissible limits and made groundwater unsuitable for drinking (Shukla & Saxena 2020, 2021). A number of reports unanimously highlighted fluoride-contaminated groundwater and endemic fluorosis in the Raebareli district (Srivastava & Kumar 2010; Kanaujia *et al.* 2013; Sahu *et al.* 2018; Samal *et al.* 2020; Shukla & Saxena 2020). Recently, the maximum concentration of fluoride has been reported in the Raebareli district in Uttar Pradesh among all regions of Indo-Gangetic plains (Samal *et al.* 2020). Among all major pollutants, fluoride was only known to impose immediate health hazards such as diabetes, thyroid, and other metabolic disorders (Fluegge 2016; Kheradpisheh *et al.* 2018). Even, our recent finding indicated that CKD resulting from diabetic nephropathy (DN) was more prevalent in fluoride-endemic areas of the Raebareli district (Gupta *et al.* 2020c). Fluoride overexposure has been reported to increase the metabolic requirement of calcium and vitamin D resulting in vitamin D deficiency (Kragstrup *et al.* 1984). Recently, a significant association was reported between water fluoride levels and vitamin D deficiency in pregnant women and newborn infants; however, vitamin D deficiency during pregnancy and birth is a known confounding factor to conclude a direct association with water fluoride levels (Thippeswamy *et al.* 2021). Therefore, pregnant women and newborn infants have been excluded from our study to get a direct association between water fluoride levels and vitamin D levels.

The current understanding regarding the pathogenesis of endemic fluorosis is quite limited and needs to be investigated. Here, we have conducted a hospital-based observational study on vitamin D deficiency in patients with diabetes living in fluoride and non-fluoride-endemic areas. We have correlated the fluoride concentration in drinking water with vitamin D and fasting BGLs to develop early markers for detecting fluorosis in the current paucity of information regarding fluoride endemicity and associated health hazards.

MATERIALS AND METHODS

Study design and biochemical parameters

A hospital-based observational study was conducted on patients who visited at outstation department (OPD) during the period of July, 2019–March, 2020. A total of 11,962 patients appeared in General Medicine OPD and 305 patients were diagnosed for diabetes. Since a number of studies unanimously highlighted fluoride-contaminated ground water and fluoride endemicity in the Raebareli district, we solely focused on the fluoride content of drinking water as an environmental determinant for this study (Srivastava & Kumar 2010; Kanaujia *et al.* 2013; Sahu *et al.* 2018; Samal *et al.* 2020; Shukla & Saxena 2020). The water fluoride levels in the area as per patient's address were determined by fluoride ion selective electrode and SPADNS method (Srivastava & Kumar 2010; Kanaujia *et al.* 2013). A number of studies based on water quality parameters in Indo-Gangetic plain including the Raebareli district have reported no significant change in fluoride content during seasonal variation (Sankararamakrishnan *et al.* 2008; Sahu *et al.* 2018; Samal *et al.* 2020). The groundwater of Indo-Gangetic plain had a much lower *d*-excess value suggesting evaporation of rain/surface water before becoming a part of groundwater (Samal *et al.* 2020). The spatial variation of fluoride contamination in groundwater was negligible during the pre-, post- and during monsoon period (Shukla & Saxena 2020). For screening of excess fluoride in drinking water, 2–3 samples were analyzed from each block by excluding the possibility of seasonal variation in the fluoride content of water (Srivastava & Kumar 2010; Kanaujia *et al.* 2013).

Vitamin D reduces the risk of insulin resistance and is found associated with T2DM; therefore, fasting BGLs and HbA1c levels were checked (Martin & Campbell 2011). Both vitamin D deficiency and T2DM resulted into a number of complications such as anemia, dyslipidemia, hypothyroidism, nephropathy, and vitamin B_{12} deficiency. The blood samples for fasting blood sugar, HbA1C, lipid profile, vitamin B_{12}, vitamin D, and thyroid function were collected from the participants in a fasting condition. The serum 25(OH)D (vitamin D) levels below 30 nmol/L were treated as vitamin D deficiency (Holick 2007). The patients were diagnosed for diabetes based on fasting blood glucose levels (BGLs) >100 mg/dL (American Diabetes Association Professional Practice Committee 2022). The diabetic status of the participants was considered as controlled (HbA1C < 6.0), moderately controlled (HbA1C = 6.0–7.5), and severely uncontrolled (HbA1C > 7.5) after proper medication. Urine albumin creatinine ratio (ACR) below 30 was considered normal. LDL <100 mg/dL and HDL >40 mg/dL were considered normal levels for diabetic patients (Eldor & Raz 2009). TSH level > 5.0 mIU/L was considered for hypothyroidism. Vitamin B_{12} up to 200 pg/ml was considered a normal level (Hanna & Lachover 2009). Complete blood count (CBC) analysis was performed on a Sysmex XP-100 machine and fresh blood samples were collected in K2-EDTA vacutainers. Here, we considered hemoglobin (Hb) less than 13 g/dL (for males) and 12 g/dL (for females) for anemia. Mean corpuscular volume (MCV) values were 80–100 fl (for normocytic), less than 80 fl (for microcytic), and more than 100 fl (for macrocytic) (Maner & Moosavi 2020). The abbreviations and medical terms were described and well explained in Supplementary Material, Table S1.

Inclusion criteria

Diabetic patients, age >18 years, and of any gender were included.

Exclusion criteria

Unhealthy adults suffering from other degenerative and infectious diseases, pregnant women were excluded.

Statistical analysis

The dataset was analyzed and reported as mean ± standard error (SE) for a number of variables using IBM SPSS version 28.0 for windows. The normality and homogeneity of the data set was checked by Kolmogorov–Smirnov and Shapiro–Wilk test. Since the dataset exhibited a skewed nonnormal distribution, $\log_{10}$ transformation was done to approximately conform to normality. Spearman's rank correlation was used to determine the association between two variables with greater confidence. The odds ratio (OR), SE, 95% confidence interval (95% CI), and *p*-values were calculated by using the following formulas (Walter & Altman 1992; Sheskin 2004):

$$\mathrm{OR} = \frac{a/b}{c/d} = \frac{a \times c}{b \times d}$$

$$\mathrm{SE}\ \{\ln\ (\mathrm{OR})\} = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$

$95\%\ CI = \exp\ (\ln\ (OR) – 1.96 \times \mathrm{SE}\ \{\ln\ (OR)\})$ to $(\ln\ (OR) + 1.96 \times \mathrm{SE}\ \{\ln\ (OR)\})$.

A *p*-value less than 0.05 was considered statistically significant. The statistical analyses were done by using Mann–Whiney U and Kruskal–Wallis test. The bar diagrams and plots were created by IBM SPSS version 28.0 and SigmaPlot10.

RESULTS

Characteristics of demographic and health profile of participants

A total of 303 responses from T2DM patients were obtained in this duration and assessed for the study (Table 1). The male participants (56.43%) were predominantly diabetic over females (43.56%) and majorly belonged to the age group of 51–60 years (33.99%). The mean age was ~55 years. The majority of participants were found to belong to rural areas (55.77%). The participants were presented with several comorbidities but the most common and highly prevalent comorbidity was vitamin D deficiency. Vitamin D deficiency was diagnosed in 87.09% of participants. Diabetic dyslipidemia was diagnosed in 46.28% and 20.67 of participants with low HDL and high LDL cholesterol, respectively, with a high LDL:HDL ratio (reference range: 3.5:1) in 6.8% of the participants. The imbalance of body lipids such as HDL (good cholesterol) and LDL (bad cholesterol) resulted into dyslipidemia during T2DM. Low HDL and high LDL resulted into an increased LDL:HDL ratio. These changes in lipid composition represented the major link between T2DM and the increased risk of developing cardiovascular diseases. Vitamin D levels are associated with the risk of developing anemia which was tested through CBC. Anemia and thrombocytopenia were diagnosed in 34.36% and 35.24% of participants. The anemia observed in our study was typically normocytic.

Table 1 | Socio-demographic and biochemical profile of study participants

Patient characteristics		
Gender ($n = 303$)	***n***	**%**
Male	171	56.435644
Female	132	43.564356
Age (years, $n = 303$)		
18–30	6	1.980198
31–40	20	6.6006601
41–50	82	27.062706
51–60	103	33.993399
61–70	77	25.412541
> 70	15	4.950495
Town ($n = 303$)		
Urban	134	44.224422
Rural	169	55.775578
Blood parameters		
Anaemia ($n = 251$)	86	34.36
Thrombocytopenia ($n = 244$)	86	35.24
Lipid profile		
Low HDL cholesterol ($n = 175$)	81	46.28
High LDL cholesterol ($n = 179$)	37	20.67
LDL:HDL ratio ($n = 175$)	12	6.8
Diabetic status based on HbA1c ($n = 249$)		
Controlled (up to 7.0)	64	25.7
Moderately uncontrolled (7.1–9.0)	83	33.33
Severely uncontrolled (>9.0)	102	40.96
Other parameters		
Vitamin B_{12} deficiency ($n = 194$)	28	19.07
Vitamin D deficiency ($n = 186$)	162	87.09
Hypothyroidism ($n = 183$)	42	22.95
Albuminuria ($n = 158$)	58	36.7

The HBA1c levels were found to be controlled only in 25.7% of diabetic patients running on proper medication. However, the majority of the participants were presented with elevated HBA1c levels indicating worse glycemic control even after running on adequate medication (Table 2). The diabetes was found to be moderately and severely uncontrolled in 33.33% and 40.96% of participants, respectively. Vitamin B_{12} deficiency was reported in 19.07% participants which is a potential comorbidity associated with type 2 diabetes. In addition, 22.95% of the participants were diagnosed for hypothyroidism. A progressive renal function loss was reflected by increased albuminuria in 36.7% of participants resulting from DN.

An inverse correlation between vitamin D and water fluoride levels

Vitamin D deficiency was found highly prevalent (87.09%) in the participants; therefore, we correlated vitamin D levels of the participants with water fluoride levels as per their residential location (Figure 1(a)) (Srivastava & Kumar 2010; Kanaujia *et al.* 2013). A statistically significant and inverse relationship was seen in between vitamin D and water fluoride levels on a logarithmic scale with Spearman's rank correlation coefficient $(r_s) = -0.777$, $p < 0.001$ (Figure 1(a)). The OR for vitamin D deficiency was 5.07 (95% CI: 1.94–13.27, $p = 0.0009$) in fluoride-endemic areas compared to control population. The vitamin D levels were significantly low (17.85 ± 8.93 nmol/L) in the participants belonging to fluoride-endemic areas ($F > 1.5$ mg/L)

Table 2 | List of medicines and doses prescribed to the patients with diabetes

Medicines	Dose per day (mg)
Metformin	1–2
Teneligliptin	20–40
Dapagliflozin	5–10
Glimepiride	4–8

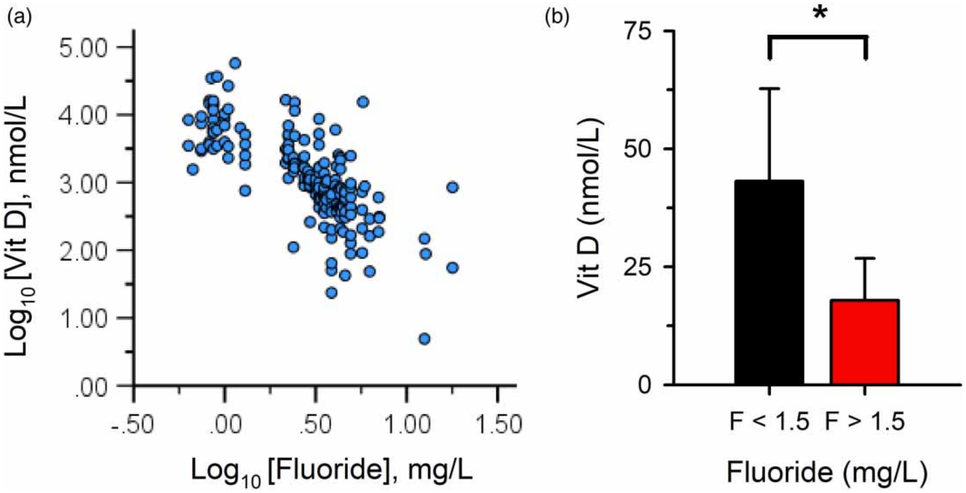


Figure 1 | Vitamin D levels are inversely correlated with water fluoride levels. (a) Association of Log_{10} vitamin D (nmol/L) with Log_{10} water fluoride levels (mg/L). (b) Mean ± SD (standard deviation) values of vitamin D levels in the participants from fluoride non-endemic (black bar, $F < 1.5$ mg/L) and endemic areas (red bar, $F > 1.5$ mg/L) were compared by using the Mann–Whitney *U* test. *$p < 0.05$. Please refer to the online version of this paper to see this figure in color: http://dx.doi.org/10.2166/wh.2022.254.

than from areas with normal fluoride levels ($F < 1.5$ mg/L) (Figure 1(b)). The result demonstrated that participants belonging to fluoride-endemic areas ($F > 1.5$ mg/L) were severely vitamin D deficient (levels below 30 nmol/L); whereas, normal vitamin D levels were found in control population from the areas with water fluoride levels within the permissible limit ($F <$ 1.5 mg/L) (Holick 2007).

A positive correlation between water fluoride and fasting BGLs

We obtained a weak but statistically significant positive correlation between fasting BGLs and fluoride levels in drinking water ($r_s = 0.178$, $p = 0.019$, $n = 174$) (Figure 2(a)). The participants living in the areas with fluoridation had a lower OR (OR = 0.5952, 95% CI: 0.20–1.72) for fasting BGLs compared to control population. The fasting BGLs were 150.36 ± 62 mg/dL and 178.57 ± 86.73 mg/dL in the participants from normal ($F < 1.5$ mg/L) and fluoride-endemic ($F > 1.5$ mg/L) areas, respectively (Figure 2(b)).

Deficiency of vitamin D in patients with diabetes

We found a weak but statistically significant negative correlation between vitamin D levels and fasting BGLs ($r_s = -0.218$, $p = 0.005$, $n = 173$) (Figure 3(a)). In addition, the patients with uncontrolled diabetes (elevated fasting BGLs > 99 mg/dL) had a higher OR (OR = 1.36, 95% CI: 0.51–3.65) for vitamin D deficiency compared to control population. We also looked for vitamin D levels in diabetic patients under treatment based on HbA1c values. The fasting BGLs and vitamin D levels were 81.1 ± 26.28 mg/dL and 33.98 ± 27.33 nmol/L in controlled (HBA1c < 7.0), 110.13 ± 8.03 mg/dL and 27.82 ± 15.58 nmol/L in moderately controlled (HBA1c = 7.0–9.0), 209.70 ± 76.82 mg/dL and 22.46 ± 13.38 nmol/L in severely uncontrolled (HBA1c > 9.0) diabetic participants (Figure 3(b)). Interestingly, we found that vitamin D levels were significantly low ($p < 0.05$) in severely uncontrolled diabetic participants as compared to controlled ones (Figure 3(b)).

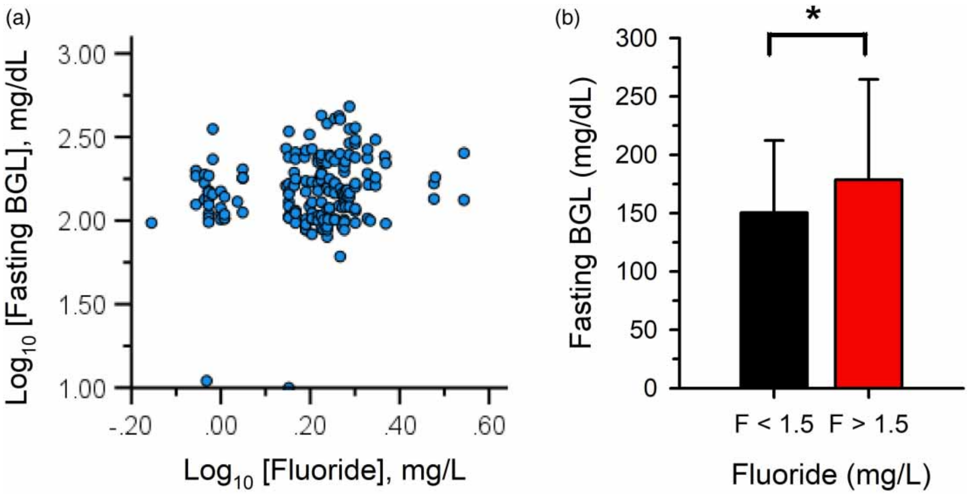


Figure 2 | Impaired glucose tolerance is positively correlated with water fluoride levels. (a) Association of Log_{10} fasting BGLs (mg/dL) with Log_{10} water fluoride levels (mg/L). (b) Mean ± SD values of fasting BGLs in the participants from fluoride non-endemic (black bar, $F < 1.5$ mg/L) and endemic areas (red bar, $F < 1.5$ mg/L) were compared by the Mann–Whitney *U* test. $^{*}p < 0.05$. Please refer to the online version of this paper to see this figure in color: http://dx.doi.org/10.2166/wh.2022.254.

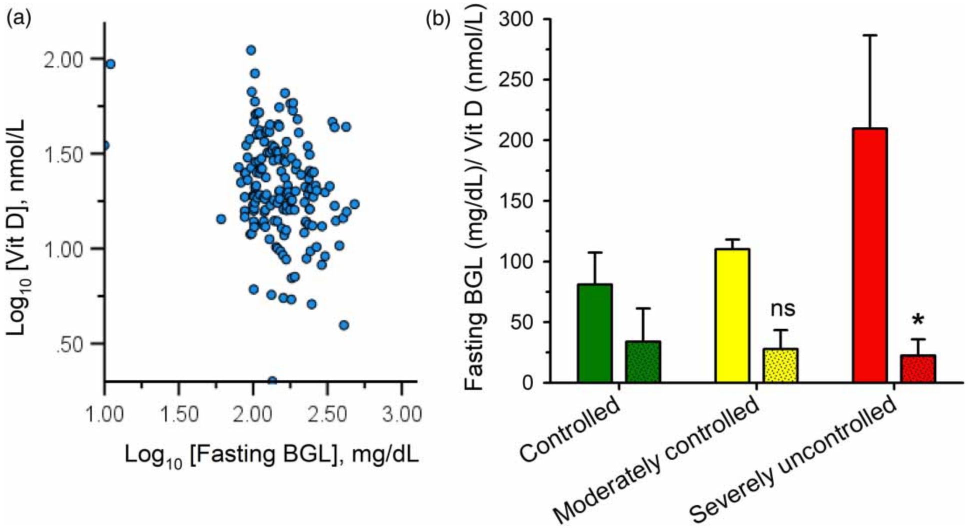


Figure 3 | Vitamin D levels are inversely correlated with fasting BGLs. (a) Association of Log_{10} vitamin D with Log_{10} fasting BGLs of the participants. (b) Mean ± SD values of fasting BGLs (simple bars) and vitamin D levels (bars with dots) were compared in participants with controlled (green, $\text{HBA1c} < 7.0$), moderately controlled (yellow, $\text{HBA1c} = 7.0\text{–}9.0$) and severely uncontrolled (red, $\text{HBA1c} > 9.0$) diabetic groups. The vitamin D levels of moderately and severely controlled diabetic patients were compared to the levels of controlled diabetic patients by independent-samples Kruskal–Wallis test ($\text{df} = 165$). $^{*}p < 0.05$ and ns represents non-significant values with $p > 0.05$. Please refer to the online version of this paper to see this figure in color: http://dx.doi.org/10.2166/wh.2022.254.

DISCUSSION

Vitamin D plays a key role in calcium and bone metabolism. Its deficiency has been associated with rickets in children and osteomalacia in adults. The main source of vitamin D is sun exposure; however, dietary intake also contributes minimally up to some extent. Mostly infants, children below the age of 5 years, and pregnant women are considered at risk of vitamin D deficiency and recommended for supplementation through a vitamin D-rich diet. The study included adult population supposed to be mostly vegetarian and consuming dairy products on a daily basis. Vitamin D deficiency has been found to be associated with poor glycemic control and a greater risk of developing T2DM (Mitri *et al.* 2011; Pittas *et al.* 2012). Repletion of vitamin D has been reported to improve insulin secretion and its sensitivity by a number of *in vitro* studies (Bourlon *et al.* 1999; Maestro *et al.* 2000). Insulin resistance is also a major pathophysiological event contributing to developing T2DM besides pancreatic β-cell dysfunction (Saltiel 2001; Saini 2010). An inverse relationship between circulating vitamin D levels and insulin resistance has been reported in Chinese population (Zhang *et al.* 2016). However, a number of other risk factors and pathophysiological events leading to insulin resistance and related vitamin D deficiency are still unknown and debatable those need to be investigated.

Prevalence of diabetes has been found to be positively associated with a higher concentration of fluoride in drinking water (Fluegge 2016). The daily intake of toxic levels of fluoride has been reported from the consumption of various sources, e.g. 'fruit juices, dairy milk, fish, and drinking water' (Dobaradaran *et al.* 2011; Hajiouni *et al.* 2019; Jamali *et al.* 2020). Intake of toxic levels of fluoride through groundwater has been documented worldwide including India (Kataria *et al.* 2008; Gopalakrishnan *et al.* 2012; Akhavan *et al.* 2016; Dobaradaran *et al.* 2018). Fluoride-endemic areas have been mapped in Uttar Pradesh, India (Srivastava & Kumar 2010; Kanaujia *et al.* 2013). Recently, fluoride endemicity was found to be linked with the prevalence of CKD in prolonged uncontrolled diabetic patients in Raebareli district (Gupta *et al.* 2020c). Excess fluoride has been found to be toxic to kidneys due to its adverse effect on the tubular area leading to impaired tubular resorption, changes in urinary ion excretion, and inhibition of kidney enzymes (Dharmaratne 2019).

The majority of participants were males in the age group of 51–60 years and belonged to rural areas (Table 1). Intake of excess fluoride resulted into low appetite and reduced hemoglobin synthesis leading to anemia in one third of the participants (Bober *et al.* 2001; Bouaziz *et al.* 2006). In addition, low platelets count (thrombocytopenia) was diagnosed in one-third of the participants which may be resulted from fluoride toxicity (Mittal & Flora 2007). Insulin resistance is known to increase the release of free-fatty acids from adipose tissue and the uptake of free-fatty acids by liver resulting into an increased synthesis of triglycerides and cholesterol. Diabetes and fluoride-induced oxidative stress has been reported to cause an imbalance in lipid composition (dyslipidemia) which was found in our study (Mooradian 2009; Afolabi *et al.* 2013). Metformin is the main first-line medication for the treatment of T2DM patients which lowers BGLs by helping to restore the body's response to insulin. The diabetes was found to be controlled in ~25% of participants running on adequate medication which can be attributed to the younger age group (18–40 years) with normal insulin levels. Whereas, ~75% of participants had uncontrolled diabetes even after proper medication probably due to low insulin secretion during old age (41–70 years or more) or fluoride-induced insulin resistance (Lima Leite *et al.* 2014).

The most common side effect of metformin usage is vitamin B_{12} deficiency, which was seen in 28% of participants (de Jager *et al.* 2010). Thyroid dysfunction and T2DM have been reported to be closely linked which was reflected in our results; where, 42% of participants with T2DM were diagnosed for hypothyroidism (Biondi *et al.* 2019). Uncontrolled diabetes may result into life-threatening complications of the heart (cardiovascular diseases), eye (retinopathy), nervous system (neuropathy), kidney (nephropathy), etc. Uncontrolled diabetes for a longer period of time may result into impaired kidney function; where albumin is lost through urine (albuminuria). Our results demonstrated albuminuria in 58% of participants with uncontrolled diabetes (Gupta *et al.* 2020c). Since the study was conducted during the COVID-19 pandemic; therefore, various other factors may also contribute to uncontrolled diabetes such as lack of physical activity, improper diet, financial constraints, infrequent visits to clinics, unavailability of medicines, and limited diagnostic services (Khader *et al.* 2020).

We found an inverse relationship between vitamin D and water fluoride levels (Figure 1(a)). Interestingly, vitamin D levels were ~2.5 times lower in the participants from fluoride-endemic areas (Figure 1(b)). Additionally, high OR for vitamin D deficiency in fluoride-endemic areas indicated toward fluoride overexposure as a significant risk factor for vitamin D deficiency (Verma *et al.* 2019). Fluoride stimulates bone formation to a greater extent where the requirement for vitamin D and calcium is increased which eventually leads to vitamin D deficiency (Kragstrup *et al.* 1984). The impaired glucose tolerance (reflected in terms of increased fasting BGLs) was positively correlated with water fluoride levels and increased significantly in fluoride-endemic areas (Figure 2). However, a lower OR (OR = 0.59) for elevated fasting BGLs in fluoride-endemic areas suggested that there may be other risk factors contributing to it. Excess fluoride has been shown to disrupt glucose homeostasis by stimulating insulin resistance and thus resulting into T2DM (Lima Leite *et al.* 2014). Vitamin D maintains glucose homeostasis by stimulating insulin release from pancreatic β-cells and increasing insulin sensitivity (Borissova *et al.* 2003; Alvarez & Ashraf 2010). Our results demonstrated that vitamin D levels were significantly low in the participants with impaired glucose tolerance and uncontrolled T2DM (Figure 3). High odds score (OR = 1.36) for vitamin D deficiency in patients with impaired glucose tolerance indicated that T2DM increases the risk of developing vitamin D deficiency (Mezza *et al.* 2012).

The quality of groundwater is determined by several inorganic constituents such as pH, noncarbonate hardness, carbonate hardness, total hardness, alkalinity, F^-, Cl^-, SO_4^{2-}, NO_3^-, Ca^{2+}, and Mg^{2+} (Dobaradaran *et al.* 2009). A number of groundwater pollutants, e.g. 'fluoride, nitrate, arsenic, iron, and salinity' have been reported in Indo-Gangetic plains (Samal *et al.* 2020). Out of them, fluoride and nitrate are majorly reported above permissible limits in groundwater of Raebareli and adjacent regions (Shukla & Saxena 2020). A number of studies based on the water quality index of the Raebareli district solely highlighted fluoride endemicity that imposes immediate health hazards (Srivastava & Kumar 2010; Kanaujia *et al.* 2013; Samal *et al.* 2020; Shukla & Saxena 2020). Nitrate toxicity has been reported to cause methemoglobinemia and cancer, but no experimental evidence supported its role or association with vitamin D deficiency (Gupta *et al.* 2017). In spite of this, we did not overrule the association of nitrate or water contaminants other than fluoride with vitamin D deficiency, which further needs to be investigated. Here, we solely focused on water fluoride levels and did not look for other water quality measures or parameters; therefore, the possibility of the impact of other parameters on vitamin D deficiency independently or via interacting with fluoride cannot be excluded. This study highlighted immediate health hazards of fluoride overexposure in Raebareli district and adjacent regions which demands to conquer this problem by making strategies for the management of fluoride contamination of ground water and vitamin D supplementation in affected areas. The government is already working on strategic plans for surface water based piped water supply schemes to provide safe and purified drinking water especially in rural areas. However, the relationship between water fluoride levels and vitamin D deficiency draws public attention toward the supply of safe water which is a matter of utmost importance in fluoride-endemic areas of the country. A number of biological processes involving microalga and oxidizing bacteria are known for wastewater treatment and removal of contaminants from ground water (Sepehri & Sarrafzadeh 2018; Sepehri & Sarrafzadeh 2019; Sepehri *et al.* 2020). The application of the point-of-use water treatment (POU-WT) systems based on reverse osmosis has been reported successfully to enrich drinking water quality and decrease health-related problems in Iran (Rezaeinia *et al.* 2018). Therefore, standard domestic water purification devices (using methods such as reverse osmosis, activated alumina or carbon filter, electrodialysis and other adsorption/ion-exchange) are strongly recommended for defluorination purposes in the affected areas (Karbasdehi *et al.* 2016). The use of bottled drinking water, desalinator plants, and domestic water purification systems also reduce the excess fluoride consumption and risk of fluoride-induced health hazards including vitamin D deficiency in fluoride-endemic areas (Amanlou *et al.* 2010; Shams *et al.* 2012; Nabipour & Dobaradaran 2013; Soleimani *et al.* 2016).

Diabetes and vitamin D deficiency have a multifactorial etiology including ageing, stress, diet, epigenetic processes, etc. Here, we also did not overrule the impact of COVID-19 pandemic restrictions on severe disruption of glycemic control in the participants. Despite several limitations, this study suggested that fluoride toxicity has a relationship with vitamin D deficiency in diabetic patients.

LIMITATIONS

We lacked the data for seasonal dynamics of vitamin D deficiency and water fluoride levels, serum fluoride levels, and health of non-diabetic participants for comparison. This is a hospital-based study that includes the participants who voluntarily visited OPD; therefore, it does not represent the whole population. We were unable to get proper information regarding BMI, duration of sun exposure, alcohol and tobacco consumption, smoking, etc. Genetic polymorphisms in vitamin D and insulin receptors are also associated with the risk of T2DM and vitamin D deficiency which need to be investigated (Bid *et al.* 2009).

CONCLUSIONS

This study suggested a significant association between water fluoride levels and vitamin D deficiency or fasting BGLs. Furthermore, increased levels of fluoride appeared as a key risk factor for vitamin D deficiency and T2DM. Vitamin D levels were found significantly low in individuals with uncontrolled T2DM. Both vitamin D deficiency and fluoride-induced insulin resistance are known to disrupt glucose homeostasis leading to uncontrolled diabetes in fluoride-endemic areas. Therefore, vitamin D supplementation and the use of purified defluorinated water may reduce the risk of fluoride-induced uncontrolled diabetes. The findings attract public interest toward the usage of safe water and further research in the area of gene–environment relationship in the context to epidemiology of T2DM in fluoride-endemic areas.

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AUTHOR CONTRIBUTION

P.K. collected the data. All three authors (P.K., R.G., A.G.) analyzed the data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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