

Estimation of Serum Fluoride and Renal Parameters in Diabetic Nephropathy- A Facility Based Observational Case Control Study

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Diabetes mellitus (DM) and its microvascular complication; diabetic nephropathy (DN) is documented as a more prevalent disorder in fluoride endemic areas across the world. Kidneys are the major route of excretion of fluoride and are thus the primary organ affected. Fluoride in minor quantities may also be excreted through sweat, saliva and faeces. Though Fluoride in minor quantities (<1.5 ppm) is considered to be essential for dental and bone enamel mineralization which form fluoroapatite. Chronic exposure (> 2ppm) is considered to be toxic leading to fluorosis. Aim of this study is to estimate and compare Blood Glucose and Fluoride levels within study groups and to correlate serum renal parameters with fluoride among study subjects. This study was conducted at Sri R.L. Jalappa Hospital attached to Sri Devaraj Urs Medical College, Constituent of Sri Devaraj Urs Academy of Higher Education and Research, total 90 subjects with the age group 45-75 years of either gender were included. Our results showed that Fasting, post prandial blood glucose values and serum Fluoride were significantly higher in T2DM without CKD group as compared to the controls and T2DM with CKD. Renal profile when compared between three groups Urea, Creatinine and Potassium were significantly higher in T2DM with CKD as compared to controls and T2DM without CKD. We conclude that analysis of serum/ urine fluoride can be preferred for the subjects attending medicine OPD who are at risk, Installing water defluoridation plants at every village as they and livestock are mainly exposed to fluoride, Creating awareness among residents by conducting regular camps and routine medical checkups and Estimate fluoride levels of potable water at regular intervals, which would help decrease its effect among living system. Evaluation of these parameters may help in early diagnosis and management and may help better patient care and nation as well.

Keywords: Diabetic Nephropathy; Fluorosis.

Type 2 diabetes mellitus (T2DM) consists of an array of dysfunctions characterized by hyperglycemia due to the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion. Diabetes of long duration leads to various multi-organ dysfunctions such as atherosclerosis,

peripheral neuropathy, retinopathy, nephropathy and heart disease¹. The immense scale of the Diabetes problem is summarized by the World Health Organization². It has been documented that the number of diabetics in the world is expected to increase from 194 million in 2003 to 330 million in 2030 with three of four affected individuals

living in developing countries². The global health expenditure on diabetes alone is expected to rise to US\$ 490 billion in 2030 – 12% of all per capita health-care expenditures³. The burden of premature death from diabetes in developing countries is similar to that of HIV/AIDS, yet the problem is largely unrecognised in these areas³.

Fluorosis has a worldwide occurrence posing a serious health problem. Approximately 25 to 30 million people are exposed to water polluted with fluoride. Half to one million people are suffering from severe forms of fluoride intoxication in our country⁴. Type 2 Diabetes Mellitus is due to the body's cells less responsive to insulin. Fluoride is a low dose endocrine disruptor which effects insulin production and sensitivity resulting in Diabetes.

Study conducted by Prystupa and Marier *et al* have emphasized the adverse impact of Fluoride on diabetic patients because they typically consume much larger quantities of water than average humans and have impaired kidney function leading to higher risk from the diverse toxic effects of Fluoride [5, 6]. It has also been shown that Fluoride toxicity is greater in diabetics⁷. Study conducted by Hanhijarvi *et al*, proposed that Diabetics have a higher incidence of chronic kidney disease⁸.

Studies have established that individuals with kidney disease especially chronic renal insufficiency is susceptible to bone damage due to

increased accumulation of fluoride in skeleton causing elevated plasma fluoride concentrations compared to healthy individuals. The other ill effects of fluoride exposure are reduced glomerular filtration rate and decreased ability in excretion of fluoride in the urine. Thus are at higher risk of developing skeletal fluorosis^{8,9}.

Diabetic nephropathy patients are exposed to an acceleration of their disease due to water fluoridation. Particularly uncontrolled diabetic patients, because of polydipsia consume large volumes of water and were accumulate more Fluoride content in bones^{10,5}. The mechanisms by which Fluoride induces diabetes most likely include antagonism to calcium and magnesium centered biochemistry¹⁰. Insulin secretion (both basal and glucose-stimulated) by isolated islets of Langerhans *in vitro* is inhibited as a function of fluoride concentrations¹¹.

“We hypothesize that elevated serum Fluoride levels might contribute to the disturbances in mineral ion homeostasis that are observed in patients with DN. As this study was conducted in rural population which is a fluoride endemic area, majority of the people's occupation in this study was agriculture; due to increase in fluoride in ground water and being the source for agriculture might be reason for fluorosis¹². Kidneys are the major route of excretion of fluoride and is thus the primary organ affected¹³. DN is documented as a more prevalent disorder in fluoride endemic areas

Sample was analyzed for the following parameters

Sl. No	Parameter (Unit)	Detection limit	Analytical range	Assay Imprecision (%)	Instrumentation/ Method
1	Blood Glucose (mg/dL)	20	20-625	1.4-1.7	Vitros 5.1 FS; OCD United States (Newyork)
2	Serum Creatinine (mg/dL)	0.05	0.05-17	1.6-2.6	Vitros 5.1 FS; OCD United States (Newyork)
3	Urea (mg/dL)	2.00	2.00-120	1.5-1.8	Vitros 5.1 FS; OCD United States (Newyork)
4	Albumin (g/dL)	1-12	4.0- 5.0	1- 12	Vitros 5, 1 FS Turbidimetric Immunoassay
5	Sodium (mEq/L)	75- 250	135- 145	NA	Vitros 5, 1 FS, Ion Selective Electrode
6	Potassium (mEq/L)	1-14	3-5- 5-1	NA	Vitros 5, 1 FS Ion Selective Electrode
7	Fluoride (ppm)	NA	NA	NA	Ion Selective Electrode Thermo Scientific Orion-5

across the world and this is true even with Kolar district, Karnataka, India. This is of particular concern since the incidence of dental fluorosis has increased due to increased Fluoride– uptake from multiple fluoridated sources. The ubiquitous presence of Fluoride in food and beverage products regardless of the degree of water fluoridation suggests that the overall Fluoride exposure in individuals with Chronic Renal Insufficiency and diabetes may need to be more closely monitored. Studies conducted till date concentrated mainly on dental and skeletal fluorosis¹⁴. However, Studies related to fluoride, diabetes and diabetic nephropathy is very low and not clear. These issues created an interest in us to study serum fluoride and renal parameters in diabetic nephropathy subjects with minimal investment and maximal outcome!

Aims and Objectives

1. To estimate and compare Blood Glucose and Fluoride levels within study groups
2. To correlate serum renal parameters with fluoride among study subjects

MATERIALS AND METHODS

Study design is Prospective case control study, conducted at RL Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Constituent of Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka. Study was approved by institutional ethical committee to start the study & informed consent was obtained from all the study subjects. Total of 90 subjects in the age group 45-75 years of either gender, were included and the study subjects were recruited after confirming inclusion and exclusion criteria.

Inclusion Criteria

- Subjects of clinically proven Type 2 DM with or without diabetic nephropathy.
- Population exposed to high fluoride levels.

Exclusion Criteria

- Patients with Diabetes Mellitus not living in Kolar and not exposed to Fluoride
- Patients taking drugs or other factors known to cause diabetes and/ or diabetic nephropathy
- Patients undergoing any type of dialysis
- Acute kidney injury due to any cause
- Patients with hepatobiliary disorders leading to proteinuria / albuminuria.

- Gestational diabetes mellitus
- Patients with Type 1 DM or Monogenic Diabetic Syndrome

Sample Size Calculation

Mean difference of Fluoride is calculated with 80% power and 95% confidence interval, thus minimum sample size is 30 per group [4].

- Group I (n=30): Age and gender matched clinically proven healthy controls living in Kolar
- Group II (n=30): T2DM without CKD
- Group III (n=30): T2DM with CKD

Duration of Study

The study was carried out for a duration of 3 months from July 2019 to September 2019

Method of Collection

An overnight of minimum 8hrs fasting, 5ml venous blood was collected from all the recruited subjects. All possible standard precautions were taken while collecting the blood samples. Sterile disposable needle and vacutainer was used for sample collection. Correct procedure was followed at each step from selection of site of venipuncture to pressure used to transfer into vacutainer. On the whole the occurrence of hemolysis was prevented.

Fasting Blood Glucose, Postprandial Blood Glucose, urea, Creatinine, Sodium, Potassium and Albumin were done with Vitros 5.1 FS dry chemistry auto analyzer from Ortho Clinical Diagnostics (OCD) United States, based on the principle of “reflectance photometry”.

Serum Fluoride was analyzed in ISE Thermo Scientific Orion-5 Instrument¹⁵.

The samples were analyzed in batches, routine Bio-Rad internal quality controls samples were run and confirmed.

Statistical Analysis

The collected data were tabulated in MS excel and the analysis was performed using the statistical package SPSS version 20.0. All the quantitative variables were analyzed using one way Analysis of Variance test, p value < 0.05 was considered as statistically significant.

RESULTS

Mean age of subjects enrolled in this study were of 55.86± 11.87, 55.96± 9.90, 55.63± 8.02. Fasting blood glucose, post prandial blood glucose values were higher in T2DM without CKD group as compared to the controls and T2DM with CKD.

The mean values and p value is mentioned in (Table 2). Renal profile when compared between the three groups (Table 2). Urea, Creatinine and Potassium were significantly higher in T2DM with CKD as compared to controls and T2DM without CKD. In our study the mean value of serum Fluoride was

Table 1. Comparison of Demographic data between three Groups

Parameters	Groups	Mean	Standard Deviation	'p' value with Significance
Age (years)	Controls	54.86	11.87	0.156
	T2DM without CKD	55.96	9.90	
	T2DM with CKD	55.63	8.02	
Body Mass Index (Kg/M ²)	Controls	22.78	2.90	0.006*
	T2DM without CKD	24.31	3.41	
	T2DM with CKD	22.43	1.49	
Systolic Blood Pressure (mmHg)	Controls	122.00	3.31	0.001*
	T2DM without CKD	124.72	6.03	
	T2DM with CKD	148.32	17.27	
Diastolic Blood Pressure (mmHg)	Controls	80.33	2.99	0.001*
	T2DM without CKD	80.83	3.46	
	T2DM with CKD	86.06	10.92	

*Significance

Table 2. Comparison of Biochemical Parameters between three Groups

Parameters	Groups	Mean	Standard Deviation	'p' value with Significance
Fasting Blood Sugar (mg/dL)	Controls	94.4333	5.92	0.001*
	T2DM without CKD	179.27	53.86	
	T2DM with CKD	148.00	41.05	
Post Prandial Blood Sugar (mg/dL)	Controls	108.27	17.72	0.001*
	T2DM without CKD	236.50	64.83	
	T2DM with CKD	250.43	72.85	
Urea (mg/dL)	Controls	19.33	6.21	0.001*
	T2DM without CKD	20.03	8.73	
	T2DM with CKD	74.20	37.94	
Serum Creatinine (mg/dL)	Controls	0.7367	0.14	0.00*
	T2DM without CKD	0.6320	0.15	
	T2DM with CKD	3.5967	2.34	
Albumin (mg/dL)	Controls	4.1100	0.31	0.004*
	T2DM without CKD	3.9367	0.43	
	T2DM with CKD	3.0567	0.76	
Sodium (mEq/L)	Controls	137.57	3.32	0.003*
	T2DM without CKD	136.77	2.26	
	T2DM with CKD	129.30	16.72	
Potassium (mEq/L)	Controls	4.03	0.31	0.008*
	T2DM without CKD	4.24	0.38	
	T2DM with CKD	4.47	0.95	
Fluoride (ppm)	Controls	0.0949	0.12	0.001*
	T2DM without CKD	0.6318	0.59	
	T2DM with CKD	0.5128	0.30	

*Significance

Table 3. Correlation of Serum Fluoride with Demographic and Biochemical Parameters

Fluoride	Pearson Correlation	Age	BMI	SBP	DBP	FBS	PPBS	Urea	Scr	Alb	Sodium	Potassium
		-0.09	-0.03	0.003	-0.33	0.28	0.44*	0.107	0.08	0.102	0.005	0.101
	Sig. (2-tailed)	0.62	0.83	0.989	0.07	0.13	0.013	0.572	0.67	0.591	0.979	0.597
	N	30	30	30	30	30	30	30	30	30	30	30

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

BMI: Body mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBS: Fasting Blood Sugar; PPBS: Post prandial Blood Sugar; Scr: Serum Creatinine; Alb; Albumin

significantly higher in T2DM without CKD group as compared to control and T2DM with CKD groups (Table 2).

DISCUSSION

Diabetic and renal profiles of biochemical parameters were in concordance with WHO and KDIGO (Kidney Disease: Improving Global Outcome) guidelines^{16, 17}. Due to failure in renal apparatus, filtering capacity of creatinine and urea by nephrons from blood is decreased leading to an increased serum urea and creatinine. Similarly, albumin which is an essential element to assess nutritional status of DN cases. Decrease in serum albumin indicates its excretion through damaged nephrons of DN cases leading to poor energy generation and metabolism¹⁸. Major cations such as Na and K are also considered in renal profiling to assess their electrolyte balance. Since, sodium plays a key role in maintaining blood pressure increase in sodium in DN may increase BP however, there is no such drastic increase in Na hence can be concluded that increases in BP in DN may be due to other hormonal disturbances in renal physiology. One of the causes for congestive heart failure in diabetics is DN due to increased BP either as a consequence of electrolytes imbalance or due to defective renal mechanism of blood pressure regulation¹⁹. KDQIO (Kidney Disease Quality Initiative Outcome) has a set of guidelines in managing DN which is to be considered as important tools to avoid further complications of DN¹⁹.

Few studies on serum Fluoride has concluded that it is one of the factors responsible for developing diabetic complications. Chronic

water Fluoride exposure causes skeletal, dental and non- skeletal fluorosis one among them is diabetes mellitus and its complications²⁰. This study also supports the hypothesis of increase serum Fluoride increases DM and DN which is evident from the results. Estimation of urinary Fluoride gives a clear idea on its excretion pattern by kidneys and extent of renal parenchymal injury which is a drawback of this study.

CONCLUSION

The strength of our study include significant elevation of fluoride in diabetes without CKD group due to poor regulation of glucose metabolism and compared to other two groups.

Based on previous literatures; measures to control dental fluorosis is initiated among local population. However, we also have to concentrate on suppressing the effect of fluoride on organs and vascular system (non-skeletal) in community with chronic fluoride exposure. Our study may benefit policy makers and clinicians to decrease the magnitude of present condition by implementing strategies at community level such as

1. Analysis of serum/ urine fluoride for the subjects attending medicine OPD department and who are at risk
2. Installing water defluoridation plants at every village as they and livestock are mainly exposed to fluoride
3. Creating awareness among residents by conducting regular camps and routine medical checkups
4. Estimate fluoride levels of potable water at regular intervals, which would help decrease its

effect among living system.

Evaluation of these parameters may help in early diagnosis and management and may help better patient care and nation as well.

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