Comparative Assessment of Fluoride Levels in Individuals with Chronic Fluoride Exposure and Type 2 Diabetes

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ABSTRACT

Kolar district a semi urban area located in Karnataka with an exponential increase in the incidence of diabetes mellitus and associated microvascular complications. The district is declared to be fluoride endemic and fluoride has been shown to increase blood glucose levels and impair glucose tolerance in animal models. Impaired glucose tolerance often is a precursor of type 2 diabetes. The aim of the study is to find the link between fluoride and diabetes, also if there is any association of fluoride in Diabetic nephropathy and retinopathy. Materials and Methods: All the subjects recruited were from the rural part of Kolar district whose major occupation is agriculture. As evident from the results of present study, in group III (Diabetic nephropathy) serum fluoride (0.313±0.154) is increased and urine fluoride (0.3±0.6) excretion is decreased. In group IV (diabetic retinopathy) cases both serum fluoride concentration (0.88±0.742) and urine fluoride excretion (0.92±0.61) are proportionately increased. In group III; diabetic nephropathy, fasting insulin is directly proportional and microalbumin is inversely proportional to serum fluoride levels. These observations conclude that in diabetes fluorosis primarily affects eyes if not kept in check it causes detrimental effect on renal tissue at later stages leading to non- skeletal fluorosis.

Background

Diabetes mellitus (DM) is metabolic aging disorder prevalent due to decreased insulin/ no insulin production by pancreas or due to inceased resistance or decreased sensitivity to insulin by the peripheral cells [1]. Incidence of Type 2 Diabtes Mellitus (T2DM) is increasing in our nation due mixed ethnicity and genetic disposition. India ranking number 1 in world population and by 2045 India will top the list in the type 2 DM incidence (T2DM).
Karnataka is a South Indian State with 14.5% of T2DM cases on an average in the year 2019 [2]. Kolar district is Southeastern district of Karnataka which is declared as fluorosis endemic district by National Programme for Prevention and Control of Fluorosis, National Health Mission, India. Groundwater is the main source of fluoride reahing the vegetation, animals, animal livestocks and human beings [3].

Fluorine is the 13th most abundant mineral which is most telctronegative halogen with the tendency to bind to highly electropositive elements in nature as well as in the living systems [4, 5]. Pioneers in the field of fluorosis since ages had potrayed the element to be essential micromineral whichprevents dentals caries due to its anti- cariogenic effect and hnce started fortifying the dental products with minor quantitites of fluoride [6]. Eventually, chronic exposure to minor quantities of fluoride exposure headed to detrimental effects on not only skeletal and dental tissues also to soft tissues called as non- skeletal fluorosis [7].
Several invitro studies has evidence that the fluorosis leads to increased insulin resistance impaired insulin sensistivity thereby causing disturbances in glucose metabolism leading to T2DM [8]. A study in Kolar district had proved that the chronic floride exposure is one of te causes for T2DM and its microvascular complications [9]. The present study documents the effect of fluoride on retinal health (diabetic retinopathy cases). Though majority of the populations are agriculturists, T2DM is prevalent without any history of heredity which has created the interest to evaluate their quality of living in the district. In toto, due to lacunae in data of non- skeletal fluorosis, aim of the study is to 'Evaluate the impact of fluoride in health and disease of chronic flouride exposed population of fluoride endemic area'.

METHODOLOGY

Materials & Methods

a. **Study Design:** Comparitive Cross Sectional Study
b. Total number of study subjects; n=363
   Group I: Healthy control subjects (n=96)
   Group II: Type 2 Diabetes Mellitus (T2DM) without microvascular complications (n=89)
   Group III: T2DM with nephropathy (n=87)
   Group IV: T2DM with ophthalmic complications (Cataract with/without Retinopathy) (n=91)
c. Study subjects: Patients attending diabetology, nephropathy and cataract clinic of the tertiary care hospital, were screened and recruited for the study. Patient attenders who were willing to participate in the study were enrolled for healthy control group. Subjects were explained about the study and after their written consent, 5 mL of blood and 10 mL spot urine sample was collected. After the analysis they were classified based on the regulatory body guidelined into T2DM, Diabetic Retinopathy (DR) and Diabetic Nephropathy (DN) cases and grouped.

Selection of Type 2 Diabetes subjects were based on the Americal Diabetic Association (ADA) Guidelines with fasting blood suger > 126 mg/dL and Post pradial > 200 mg/dL [1]. The basis of selection of diabetic nephropathy cases was based on the ADA and Kidney Disease: Improving Global Outcomes (KDIGO) guidelines which states that albumin creatinine ratio (UACR ≥ 30 mg/g) or eGFR< 60ml/min/1.73m^2 or abnormal blood pressure or increased serum creatinine values (>1.7 mg/dL) shall be classified under chrnic kidney disease with diabetes [10]. Diabetic retiopathy cases were selected and included only if hey were satisfying the criteria layed by All India Ophthalmological Society (AIOS) National Committee for Diabetic Retinopathy Awareness and Screening which was maily based on fasting blood ugar, HbA1c and vision acuty after cataract [11].

d. Methodology: Following parameters were analysed in the biological samples collected
   Plasma glucose, Serum Urea, Serum Creatinine, Serum Uric Acid, Serum Sodium, Serum Potassium, Serum Magnesium, Urine Micro albumin, Serum & Urine Fluoride, HbA1c%, Plasma Insulin.

Statistical Methods

Samples analyzed were tabulated in the Microsoft Excel sheet and performed statistical analysis package of SPSS version 23 such as student t test, One Way ANOVA and Pearson's Correlation to find out the significance ($p<0.05$).

RESULT

Table1: Demographic data expressed as Mean ± SD of all the groups

Groups Variables	Group I Non- Diabetic no microvascular complications (n=96)	Group II T2DM without microvascular complications (n=89)	Group III T2DM with nephropathy (n=87)	Group IV T2DM with ophthalmic complications (n=91)	p Value
Systolic Blood Pressure (mm/Hg)	121.48± 5.89	120.33± 8.11	137.54± 19.86	126.32± 17.21	0.001
Diastolic Blood Pressure (mm/Hg)	78.54± 4.89	77. 93± 6.28	93.43± 63.77	80.20± 8.05	0.001
Body Mass Index (Kg/m^2)	23.76± 2.86	23.48± 2.08	22.80± 2.23	23.93± 3.51	0.028

$p<0.05$ considered as significant

Table 2: Diabetic Profile and renal profile expressed as Mean ± SD of all the groups

Groups Variables	Group I Non- Diabetic no microvascular complications (n=96)	Group II T2DM without microvascular complications (n=89)	Group III T2DM with nephropathy (n=87)	Group IV T2DM with ophthalmic complications (n=91)	p Value
Fasting Blood sugar (mg/dL)	92.21± 13.259	176.6± 62.60	167.82± 62.52	200.78± 69.00	0.001
Post prandial Blood Sugar (mg/dL)	110.76± 12.993	262.31± 96.34	262.83± 84.04	285.40± 96.80	0.001
HbA1c (%)	5.540± 0.5003	8.98± 2.18	8.39± 2.04	10.48± 2.64	0.001
Insulin	11.50± 6.84	14.92± 12.90	10.9± 11	26.7± 16.1	0.001
Urea (mg/dL)	19.55± 6.30	25.39± 12.24	84.12± 38.99	23.5± 9.45	0.001
Serum Creatinine (mg/dl)	0.69± 0.20	0.66± 0.21	3.86± 2.239	0.69± 0.307	0.001
Uric Acid (mg/dL)	5.00± 1.46	4.33± 1.71	5.94± 4.69	4.23± 1.72	0.001

Sodium (meq/L)	136.72± 2.05	136± 2.94	133.34± 10.06	135.45± 2.71	0.001
Potassium (meq/L)	7.03± 19.00	4.3± 0.463	4.35± 0.958	4.23± 0.56	0.556
Magnesium (mg/dL)	1.96± 0.18	1.75± 0.23	1.955± 0.38	1.72± 0.234	0.001
Microalbumin (mg/g)	13.46± 10.85	64.16± 97.27	532.22± 549.6	54.38± 58.38	0.001

p<0.05 considered as significant

Table 3: Serum and Urine fluoride expressed as Mean ± SD of all the groups

Groups **Variables**	**Group I Non- Diabetic no microvascular complications (n=96)**	**Group II T2DM without microvascular complications (n=89)**	**Group III T2DM with nephropathy (n=87)**	**Group IV T2DM with ophthalmic complications (n=91)**	**p Value**
Serum Fluoride (ppm)	0.633± 0.183	0.665± 0.29	0.313± 0.154	0.88± 0.742	0.001
Urine Fluoride (ppm)	0.89±0.55	0.7±0.53	0.3±0.6	0.92±0.61	0.001

p<0.05 considered as significant

Table 4: Comparing means between groups by post- hoc bonferroni test

Variables	**Mean±SD**	**Groups**	**P value**
Systolic Blood Pressure (mm/Hg)	Group I 121.48±5.89	II	1.000
		III	0.000
		IV	0.051
	Group II 120.33±8.11	III	0.000
		IV	0.018
	Group III 137.54±19.86	Group IV 126.32±17.21	0.000
Diastolic Blood Pressure (mm/Hg)	Group I 78.54±4.89	II	1.000
		III	0.009
		IV	1.000
	Group II 77. 93±6.28	III	0.002
		IV	1.000

	Group III 93.43±63.77	Group IV 80.20±8.05	0.027
Body Mass Index (Kg/m^2)	Group I 23.76±2.86	II	1.000
		III	0.157
		IV	1.000
	Group II 23.48±2.08	III	0.479
		IV	1.000
	Group III 22.80±2.23	Group IV 23.93±3.51	0.024
Fasting Blood sugar (mg/dL)	Group I 92.21±13.259	II	0.000
		III	0.000
		IV	0.000
	Group II 176.6±62.60	III	1.000
		IV	0.018
	Group III 167.82±62.52	Group IV 200.78±69.00	0.000
Post prandial Blood Sugar (mg/dL)	Group I 110.76±12.993	II	0.000
		III	0.000
		IV	0.000
	Group II 262.31±96.34	III	1.000
		IV	0.271
	Group III 262.83±84.04	Group IV 285.40±96.80	0.301
HbA1c (%)	Group I 5.540±.5003	II	.000
		III	.000
		IV	.000
	Group II 8.98±2.18	III	.238
		IV	.000
	Group III 8.39±2.04	Group IV 10.48±2.64	.000
Insulin (µIU/mL)	Group I 11.50±6.84	II	.297
		III	1.000
		IV	.000
	Group II 14.92±12.90	III	.136
		IV	.000

	Group III 10.9±11	Group IV 26.7±16.1	.000
Urea (mg/dL)	Group I 19.55±6.30	II	.358
		III	.000
		IV	1.000
	Group II 25.39±12.24	III	.000
		IV	1.000
	Group III 84.12±38.99	Group IV 23.5±9.45	.000
Serum Creatinine (mg/dL)	Group I 0.69±0.20	II	1.000
		III	.000
		IV	1.000
	Group II 0.66±0.21	III	.000
		IV	1.000
	Group III 3.86±2.239	Group IV 0.69±0.307	.000
Uric Acid (mg/dL)	Group I 5.00±1.46	II	.529
		III	.103
		IV	.294
	Group II 4.33±1.71	III	.000
		IV	1.000
	Group III 5.94±4.69	Group IV 4.23±1.72	.000
Sodium (meq/L)	Group I 136.72±2.05	II	1.000
		III	.000
		IV	.655
	Group II 136±2.94	III	.005
		IV	1.000
	Group III 133.34±10.06	Group IV 135.45±2.71	.046
Potassium (meq/L)	Group I 7.03±19.00	II	1.000
		III	1.000
		IV	1.000
	Group II 4.3±0.463	III	1.000
		IV	1.000

	Group III 4.35±0.958	Group IV 4.23±0.56	1.000
Magnesium (mg/dL)	Group I 1.96±0.18	II	.000
		III	1.000
		IV	.000
	Group II 1.75± 0.23	III	.000
		IV	1.000
	Group III 1.955±0.38	Group IV 1.72±0.234	.000
Serum Fluoride (ppm)	Group I 0.633±0.183	II	1.000
		III	.000
		IV	.000
	Group II 0.665±0.29	III	.001
		IV	.000
	Group III 0.313±0.154	Group IV 0.88±0.742	.000
Urine Fluoride (ppm)	Group I (0.89±0.55)	II	1.000
		III	.000
		IV	.000
	Group II (0.7±0.53)	III	.001
		IV	.000
	Group III (0.3±0.6)	IV (0.92±0.61)	.000
Microalbumin (mg/g)	Group I 13.46±10.85	II	1.000
		III	.000
		IV	1.000
	Group II 64.16±97.27	III	.000
		IV	1.000
	Group III 532.22±549.6	Group IV 54.38±58.38	.000

p<0.05 considered as significant

Table 5: Correlation of Blood Pressure, Diabetic and Renal parameters of 3 groups

Variables	Group II		Group III		Group IV	
	R Value	P value	R Value	P value	R Value	P value

Systolic – Diastolic Blood Pressure (mm/Hg)	0.526	0.001	0.678	0.001	0.26	0.009
Systolic Blood Pressure (mm/Hg) – Microalbumin (mg/g)	-0.124	0.224	0.198	0.04	0.241	0.017
Fasting Blood sugar- Post prandial Blood Sugar (mg/dl)	0.802	0.001	0.839	0.001	0.759	0.001
Post prandial Blood Sugar (mg/dl)- HbA1c (%)	0.433	0.001	0.617	0.001	0.264	0.009
Fasting Blood sugar (mg/dl)- HbA1c (%)	0.529	0.001	0.62	0.001	0.264	0.009
Urea - Serum Creatinine (mg/dl)	0.298	0.003	0.71	0.001	0.418	0.001

$p<0.05$ considered as significant

Table 6: Calculated Parameter expressed as Mean ± SD

Variables	Group I	Group II	Group III	Group IV	p-value
HOMA- IR	2.98± 1.78	5.72± 4.3	5.68± 5.037	5.26± 4.85	0.001
QUICKI	0.34± 0.028	0.3± 0.34	0.31± 0.039	0.31± 0.035	0.001

$p<0.05$ considered as significant

Table 7: Correlation between insulin and microalbumin with fluoride

Variables	Group II		Group III	
	R Value	P value	R Value	P value
Insulin (IU/L)	0.078	0.006	-0.06	0.003
Microalbumin (mg/g)	-0.014	0.001	0.083	0.006

$p<0.05$ considered as significant

DISCUSSION

Fluorosis ; hypermineralization of skeletal, dental and non- skeletal tissues are now a disorder of concern. To evaluate the fluorosis, basic demographic details of the subjects were recorded. Systolic and Diastolic Blood Pressure (BP) showed marginal increased in type 2 Diabetic groups with microvascular complications (Groups 2 and 3). Increase in blood pressure of patients with diabetic microvascular complications is a proven fact as per the studies conducted [12, 13]. From table 2, there was no significant difference Systolic blood

pressure (SBP) and Diastolic blood pressure (DBP) between groups 1 and 2. Whereas, in groups with microvascular complications, only the SBP was significantly high giving a clue that the SBP is the parameter which will be affected during renal injury and/ or retinal injury which is accordance with the study conducted by Noshad et al and Sanbao et al. [12, 13]. All the subjects recruited were from the rural part of Kolar district whose major occupation is farming. The BMI of the subjects were almost the same across all the groups however little raised in retinopathy group (23.93± 3.51). The BMI is significantly increased in group 4 when compared with group 3 indicating that there are chances of retinopathy cases to as to be affected with any other diabetic complication if not in control as documented by Polemiti et al [14]. After these essential demographic record, Diabetic and renal profiling were assessed to confirm the grouping of type 2 diabetic cases.

As a basic diabetic profile fasting blood sugar (FBS), post prandial blood sugar (PPBS) and HbA1c were analyzed and the values are tabulated in table 2. All the three parameters were increasing across the groups significantly and higher in group 4 (200.78± 69.00, 285.40± 96.80 and 10.48± 2.64). Therefore, the basic diabetic profile can be correlated with the increased BMI in Diabetic Retinopathy group (DR). From table 2, FBS was significantly increased in all the diabetic groups when compared with controls (92.21±13.259). On the other hand, the FBS was insignificantly increased in group 4 (200.78±69.00) when compared to groups 2 (176.6±62.60). Similarly, PPBS of groups 3 (262.83±84.04) and 4 (285.40±96.80) were increased when compared with group 2 (262.31±96.34) were increased but not significant indicating that the metabolism of glucose after a meal in all the cases are almost the same. Close monitoring of PPBS may prevent microvascular complications which were also documented by Fu S et al for cardio vascular stiffness [15]. Glycated Hemoglobin (HbA1c) was significantly increased in all the cases when compared to the control group (5.540±.5003). On contrary, the value was not significant between groups 2 (8.98±2.18) and 4 (10.48±2.64). In a study conducted by Su Jb et al, increased HbA1c led to increased diabetic peripheral nephropathy which is one among the microvascular complication which implies the same in our study with respect to nephropathy and retinopathy [16]. Glucose metabolism mainly depends on hormones; insulin and glucagon. In our study we considered insulin to assess insulin resistance and sensitivity, fasting insulin was analyzed which was very high in DR cases compared to other groups. Insulin was significantly varied in groups 3 (10.9±11) and 4 (26.7±16.1) when compared with groups 1 (11.50±6.84) and 2 (14.92±12.90). This increase which in turn may be compared with BMI giving a clue for insulin action on cell, hyperinsulinemia may lead to increased metabolic disturbances thereby aggravating the disorder [17]. Thus, comparison of diabetic profile with the demographic details there are few important novel outcomes affecting progression of Type 2 Diabetes Mellitus to microvascular complications and finally to the end stage of the complications.

The cases recruited for DN group were not on dialysis however, they were in initial stages of the disorder. Type 2 Diabetic cases admitted in general medicine ward with increased serum creatinine and urea levels were reassessed for the renal profile to confirm the microvascular complication. Diabetic retinopathy (DR) cases were recruited based on the opinion of the ophthalmologist on retinal condition (with/without cataract). The renal profile of DN group was increased compared to other groups confirming diabetic nephropathy an depicted in table 3 [18]. Urea (84.12± 38.99), Creatinine (3.86± 2.239) and uric acid (5.94± 4.69) were increased in group 3 however, Sodium, Potassium and Magnesium were within range in all the groups. Serum Fluoride was not significantly varied when compared between controls (0.633±0.183) and type 2 diabetes cases (0.665±0.29). The results of the current study projects the serum fluoride concentration of 0.88± 0.742 ppm in group IV (Diabetic retinopathy) which was increased when compared to other groups. The least was found in

Group III (Diabetic nephropathy) of 0.313± 0.154 ppm. The urinary fluoride concentration of all the groups were more than the serum fluoride indicating normal clearance of fluoride through renal system except in group 3 (0.3±0.6). The disproportionate serum and urine fluoride excretion is in nephropathy group may be due to renal damage leading to decreased urine fluoride values. Microalbumin was analyzed in the all the subjects. Microalbumin was sharply increased group 3 (532.22±549.6) indicating severe renal damage in initial stages also. Therefore, a significant increase was observed only between group 3 and other groups. In brief, estimation of these biochemical parameters gives a gist that in diabetic nephropathy fluoride clearance is hindered which gives an insight for further studies to evaluate the molecular damage in renal cells.

From table 3, the correlation of SBP with microalbumin was not significant in type 2 DM cases (-0.124) though it was significantly correlating in groups 2 (0.198) and 3 (0.241) indicating that microalbumin can be considered as a marker of renal dysfunction a well as early marker of detection of renal dysfunction in retinopathy cases [19]. Since all the 3 groups are diabetic and also have increased glucose levels, the correlation of FBS and PPBS with HbA1c remains as significant positive correlation. In contrast with microalbumin as biomarker of renal tissue damage, serum urea and creatinine are considered for initial evaluation of the state of kidney [20]. There is no much evidence stating the importance of correlation between parameters. From the present study correlation of urea and creatinine was significantly positive in all the three groups unlike microalbumin which was not significant in group 2 which proves that the trend of this duo is maintained since years as biomarkers of renal insufficiency and found better when compared to microalbumin and SBP.

Insulin profile of the present study includes HOMA- IR and QUICKI. HOMA- IR was increased in group 4 compared to other groups which is in concordance with fasting insulin values of all the 4 groups. Increase in resistance decreases insulin reception by cell and thereby impaired glucose metabolism [21]. Increase in sensitivity increases reception of insulin by cells. From table 4 it is deemed fit to accept that control subjects have increased insulin sensitivity than other groups [22]. Therefore, insulin profile shall be considered for better understanding of glucose metabolism of an individual [20, 22].

The major objective of the study is to find the effect of fluoride on renal efficiency and insulin action. From table 5, it is clear that in Diabetic nephropathy, due to increased serum fluoride and decreased urine fluoride, fasting insulin is directly proportional and microalbumin is inversely proportional to serum fluoride levels. These results indicate the renal damage is prevalent in fluoride endemic area as suggested by Deepika et al [23].

CONCLUSION

In summation, after the diagnosis of type 2 diabetes mellitus, management and prognosis of the disorder is of prime importance. The environmental factors also play a major role wherein, fluorosis is given prominence in fluoride endemic area as in the present study. There was no notable effect of fluoride on the routine diabetic investigations such as fasting blood sugar, post prandial blood sugar and HbA1c. However, correlation of fluoride with Fasting insulin, HOMA-IR, QUICKI, and microalbumin must be envisaged for better management of diabetic microvascular complications. As a concluding remark, insulin sensitivity and resistance can act as surrogate marker in managing diabetes thereby preventing its complications. The same may also hold good to assess the impact of fluoride on the insulin which may act as basis for further molecular studies on fluorosis.

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