A Cross-Sectional Study of Serum and Urine Fluoride in Diabetes in Fluoride Exposed Population

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ABSTRACT

BACKGROUND

In addition to diet and nutrition, environmental changes may increase the incidence of diabetes mellitus and microvascular complication leading to 'diabetic kidney disease (DKD)'. One such factor considered in this study is fluoride. Increased incidence of DKD lead to estimation of fluoride in fluoride endemic areas. Fluoride and advanced glycation end products lead to the development of microvascular complication in patients of diabetes leading to diabetic kidney disease or diabetic nephropathy which in turn leads to increase in expression of sirtuin 1; a regulatory protein mediating deacetylation of histone proteins which was analysed.

METHODS

Subjects recruited for this cross-sectional study were divided as - group 1 (healthy controls), group 2 (patients with type 2 diabetes) and group 3 (diabetic nephropathy patients) with 50 subjects in each group. Routine parameters were analysed in biochemistry section of central laboratory.

RESULTS

In patients with diabetic kidney disease, serum and urine fluoride levels were similar (0.2 and 0.28 ppm); whereas, in other two groups, urine fluoride values were more than the serum fluoride values indicating better renal function and fluoride clearance. Least sirtuin 1 median range was observed in group 1 [22.65 (11.78 - 91.10)] and 36.9 (27.38 - 56.23) in group 3 lesser than group 2 with a value of [42.7 (30.17 - 52.93)].

CONCLUSIONS

Serum and urine fluoride estimation aids in assessing progression of disorder and hence helps in preventing complications. Fluoride may not be a cause of diabetes but may accelerate its microvascular complications as observed in this study. Sirtuin 1 levels in serum or plasma can be a marker for various damages caused by aging disorders. Sirtuin 1 correlation with diabetic parameters will help in health research to include it as a therapeutic target for various toxic conditions.

KEY WORDS

Advanced Glycation End product, Fluoride, Diabetic Kidney Disease, Sirtuin 1, Type 2 Diabetes Mellitus

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BACKGROUND

Quality of day to day living and livelihood of human being is influenced by readily available packed food also called fast food, due to rapid urbanization across the globe.[1] Lifestyle matters for body organs and cells to follow their routine for synthesis and secretion of biochemicals for bodily metabolism.[2] Lack of proper nutrients in food results in various aging disorders, majorly booming in health sector in recent days are cancer, metabolic syndrome and diabetes mellitus. Based on reports by World Health Organization (WHO), diabetes mellitus (DM) and its microvascular complication will be one of the causes for increase in morbidity and mortality by 2030.[3] India heading the list of highest diabetes cases will continue to be so till 2030 with about 2.5 folds increase in incidence compared to the year 2000.[3]

Type 2 diabetes mellitus (T2DM) is most common which is either due to improper secretion of insulin by pancreas or by improper utilisation of secreted insulin by peripheral cells.[4] Due to increase in prevalence of T2DM, there is an increased incidence of its microvascular complications such as diabetic retinopathy, diabetic kidney disease, diabetic neuropathy, stroke and cardio vascular diseases (CVD).[5,6] All of these diabetic microvascular complications are aging disorders, since, they are resultant of cell stress generated during hyperglycaemia leading to formation of free radicals which in turn forms a group of chemicals called reactive oxygen species (ROS).[7] Increase in ROS increases rate of apoptosis (programmed cell death) causing imbalance in proportion of cell death and cell division. During the imbalance, expression of an enzyme called 'sirtuin 1 (SIRT1)' increases which initiates autophagy of damaged cells wherever necessary.[8] Fyre in 1999 discovered 5 human homologues SIRT1-5 similar to yeast (*Saccharomyces. cerevisiae*) *sir2* (Silent Information Regulator 2) gene, one among them is SIRT1. SIRT1 is a NAD+ dependent deacetylase enzyme, deacetylating histones, transcriptional factors etc.[9-11]

There are several other mechanisms contributing to increasing incidents of vascular damage in hyperglycaemic called advanced glycation end products (AGE). AGE are produced non-enzymatically as a part of body metabolism and also acquired through diet.[12] Since AGE promotes T2DM to its major microvascular complication especially, diabetic kidney disease or diabetic nephropathy, it leads to increase in expression of sirtuin 1.[13] One such significant AGE in research in present era is carboxy methyl lysine (CML) which is formed either due to improper glucose metabolism and / or externally through diet.[14] The majority of CML in tissue is derived from lipid peroxidation reactions, even during hyperglycemias when concentrations of glucose and Amadori products on protein are increased.[15] The mechanism of formation of CML and other AGEs during carbohydrate oxidation reactions is still uncertain.[16] In addition to diet and nutrition, environmental changes also has scope in increasing incidence of disorders, one such factor considered in this study is fluoride (F).

Chronic F exposure leads to various types of fluorosis such as dental fluorosis, skeletal fluorosis and recent studies are carried out on non-skeletal fluorosis. There are various animal studies on non-skeletal fluorosis concluding that deposition of F in blood vessels and organs leads to various complication in F endemic areas.[17] Kolar is considered F endemic area due to increased water F level of about 0.6 – 4 ppm in villages around the district.[18] After metabolism, excretion of F is mainly through renal tissue, partly through faeces and sweat.[17] Therefore, decrease in F clearance indicates decreased filtering capacity of kidneys which is estimated in this study for comparison between controls, T2DM and diabetic kidney disease.

METHODS

Study subjects were recruited from diabetology outpatient clinic of RL Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College. Research was carried out from January 2019 to February 2020. A total of 150 study subjects were divided into 3 groups with 50 subjects in each group, this cross-sectional study was approved by central ethics committee (CEC) of the academy.[18] The sample size was taken based on the convenience of the study. Patients under dialysis, CKD due to hypertension, nephritis, sepsis were excluded. All subjects included in this study were residents of the district for minimum of 5 years.[18]

Subjects were instructed to fast for a minimum of 8 hours for investigating blood parameters in biochemistry section of diagnostic laboratory which include fasting blood glucose (FBS), blood urea, serum creatinine and after 2 hours of meal post prandial blood glucose (PPBS) was analysed by fully automated VITROS 5, 1 FS (USA). Glycosylated haemoglobin (HbA1c) was estimated by high-performance liquid chromatography (HPLC) using Bio-Rad D10, sirtuin 1 was measured by sandwich enzyme-linked immunosorbent assay (ELISA) (kits procured from Sincere Biotech China). Serum and urine fluoride were estimated by Orion Thermo Scientific Fluoride Ion Selective Electrode (ISE). Estimated glomerular filtration rate (eGFR) was calculated according to Kidney Disease Outcomes Quality Initiative (KDQIO) 2012 guidelines and anthropometric parameters and demographic data were also recorded.[13] Written informed consent was obtained from study subjects approved by CEC and under the norms of Declaration of Helsinki.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 22 by International Business Machines (IBM) was applied for data analysis. Routine parameters were represented as mean ± S.D sirtuin 1 is presented as median (25- 75 percentile). To find significance for normally distributed data, analysis of variance (ANOVA) was applied. Distributed variables. P-value < 0.05 was considered significant.

RESULTS

In the present study, all the subjects selected were age and gender matched. As shown in Table 1 and Table 2, T2DM complications arise during latent stages of disorder, hence, age is significant with P-value < 0.001 between group 1 & 2 and group 1 & 3. Mild increase in blood pressure was observed between group 1 and 2 in contrary, major significant

difference was observed between group 1 & 3 and group 2 & 3 which makes it evident that DKD cases were hypertensive.

Parameters	G1 (Controls) N = 50 Mean ± S.D.	G2 (T2DM) N = 50	G3 (DKD) N = 50 Mean ± S.D.	P Value
Age (in years)	41.5± 8.8	54.5 ± 7.6	56.84 ± 7.8	0.000
SBP (mmHg)	122± 5.2	125.04 ± 12.6	137.32 ± 19.3	0.000
DBP (mmHg)	78.36 ± 4.7	82.6 ± 7.8	89.2 ± 11.4	0.000
BMI (kg / m²)	23.8 ± 2.5	22.9 ± 2.02	22.6 ± 1.4	0.054
FBS (mg / dL)	94.94 ± 10.92	188.94 ± 68.91	179.46 ± 72.43	0.000
PPBS (mg / dL)	117.12 ± 15.94	249.04 ± 96.69	269.4 ± 95.39	0.000
HbA1c (%)	5.45 ± 0.545	9.35 ± 2.5	8.14 ± 2.14	0.019
CML (µg / mL)	1.23 ± 0.62	1.95 ± 0.92	1.92 ± 0.89	0.000
Blood urea (mg / dL)	18.8 ± 6.4	29.38 ± 14.62	71.14 ± 25.71	0.000
Serum creatinine (mg / dL)	0.68 ± 0.18	0.69 ± 0.22	3.43 ± 1.61	0.000
eGFR (mL / min / 1.73m²)	107.26 ± 19.07	101.3 ± 21.6	28.54 ± 17.29	0.033
Sirtuin1 (ng / mL) ǂ	22.65 (11.78 - 91.10)	42.7 (30.17 - 52.93)	36.9 (27.38 - 56.23)	0.098
Serum fluoride (ppm) ǂ	0.67 (0.64 - 0.74)	0.6 (0.5 - 0.67)	0.2 (0.15 - 0.26)	0.000
Urine fluoride (ppm) ǂ	1.02 (0.57 - 1.6)	0.84 (0.54 - 1.1)	0.28 (0.2 - 0.54)	0.004

Table 1. Comparison of Demographic Data, Diabetic and Renal Profile

ǂ: Median (25 - 75 percentile) Kruskal-Wallis; to derive the P-value of non-parametric data between all three groups

Parameters	G1 (Controls) N = 50 Mean ± S.D.	G3 (DKD) N = 50	P-Value
AGE (in years)	41.5 ± 8.8	56.84 ± 7.8	0.000
SBP (mmHg)	122 ± 5.2	137.32 ± 19.3	0.000
DBP (mmHg)	78.36 ± 4.7	89.2 ± 11.4	0.000
BMI (kg / m²)	23.8 ± 2.5	22.6 ± 1.4	0.004
FBS (mg / dL)	94.94 ± 10.92	179.46 ± 72.43	0.000
PPBS (mg / dL)	117.12 ±15.94	269.4 ± 95.39	0.000
HbA1c (%)	5.45 ± 0.545	8.14 ± 2.14	0.000
CML (µg / mL)	1.23 ± 0.62	1.92 ± 0.89	0.000
Blood urea (mg / dL)	18.8 ± 6.4	71.14 ± 25.71	0.000
Serum creatinine (mg / dL)	0.68 ± 0.18	3.43 ± 1.61	0.000
eGFR (mL / min / 1.73 m²)	107.26 ± 19.07	28.54 ± 17.29	0.000
Sirtuin1 (ng / mL) ǂ	22.65 (11.78 - 91.10)	36.9 (27.38 - 56.23)	0.220
Serum fluoride (ppm) ǂ	0.67 (0.64 - 0.74)	0.2 (0.15 - 0.26)	0.000
Urine fluoride (ppm) ǂ	1.02 (0.57 - 1.6)	0.28 (0.2 - 0.54)	0.000

Table 2. Comparison of Demographic Data, Diabetic and Renal Profile between Group 1 and Group 3 Subjects

ǂ: Median (25 - 75 percentile), comparison of median between group 1 & 3 by Mann-Whitney U test
Comparison of means between group 1 and group 3

Significant differences were observed in mean of FBS, PPBS, HbA1c and CML of group 2 & 3 with group 1. In group 3, major significant difference was found in renal profile parameters; blood urea, serum creatinine and eGFR. Blood urea and serum creatinine was increased in group 3 compared to control and diabetic group in concordance with American Diabetes Association (ADA) criteria of T2DM and DKD. In contrast, estimated glomerular filtration rate (eGFR) was drastically decreased in DKD which was < 30 (28.54 ± 17.29) in accordance with the KDIGO guidelines[13]. Sirtuin 1 and fluoride were represented as median (25 - 75 percentile) since it has a broad range and hence not normally distributed. Least sirtuin 1 median range was observed in group 1 [22.65 (11.78 - 91.10)] and 36.9 (27.38 - 56.23) in group 3 lesser than group 2 with a value of [42.7 (30.17 - 52.93)]. Also, there was a significant difference of serum and urine F between groups except between the groups 1 & 2.

Table 3 depicts Spearman's correlation (ρ) analysis explaining the trend in of a parameter in comparison with the special molecule sirtuin 1. SIRT1 is correlated with AGE; CML and serum fluoride, showing a negative trend, indicating protective effect of increase in SIRT1 may decrease formation of CML. These parameters are first time compared with sirtuin 1 in human population hence, further in-vitro studies are required to study in detail the effects of sirtuin 1 on different tissues and correlate in different conditions.

Parameter	G2 (T2DM) N = 50 Spearman's Rho Correlation (ρ)	G3 (DKD) N = 50
CML (ng / mL)	- 0.137	- 0.19
SF (ppm)	- 0.062	- 0.312*

Table 3. Correlation of Sirtuin1 with CML and Serum Fluoride

CML: Carboxy Methyl Lysine, SF: Serum Fluoride, *P-value < 0.05

DISCUSSION

Basta et al. 2008 stated that circulating advanced glycation end products increases oxidative stress damaging major blood vessels especially in diabetes.[19] Carboxy methyl lysine (CML) is one of the AGEs responsible for renal damage, especially damaging the glomeruli and podocytes as stated by Semba RD et al. 2010a.[20] Although there is no much deference in the mean values of CML in this study, statistically there is a significant difference of means between control-diabetes and control-DKD values, on the other hand CML is not significant between groups 2 & 3 since both are diabetic groups. In a study by Semba RD, 2012 they considered urine CML values where serum CML was proportionate to urine CML.[21] Similarly, DKD cases in our study may excrete more CML through urine due to chronic hyperglycemia which may be a reason for decreased serum CML in group 3 than group 2 which may be an early indication of renal failure.

Apart from urine CML excretion, effect of fluoride on filtering apparatus also plays an important role. T. Kido et al. in the year 2017 stated that the constant exposure of glomeruli to fluoride results in impaired kidney function and decreased renal clearance of fluoride, which is also observed in this study.[22] In patients with diabetic kidney disease serum and urine fluoride levels were however similar (0.2 and 0.28 ppm), whereas in other two groups urine fluoride values were more than the serum fluoride indicating better renal function and fluoride clearance. Though there are no many molecular studies regarding the action of fluoride on renal cells, fluoride levels are to be considered in evaluating renal functioning in fluoride endemic area.

Further, to classify renal efficiency estimated glomerular filtration rate (eGFR) was calculated. In this study, DKD is categorised based on KGIDO 2012 guidelines considering only eGFR calculated with serum creatinine and eliminated those cases falling under 4th stage of DKD.[13] Patients under dialysis who were included in 5th stage (renal failure) were excluded from the study. eGFR equations are widely used in recent days to renal filtration evaluation since it is precise, non-invasive and affordable.[23] Barely based on serum creatinine values renal function cannot be predicted and in current study there are various factors dependent on renal clearance therefore eGFR calculation is recommended as documented by PC Fontela et al.[23] eGFR of DKD (28.54 ± 17.29) cases is drastically reduced when compared to group 1 and group 2 (107.26 ± 19.07 & 101.3 ± 21.6). Concurrently, as said above, urine fluoride is also decreased in this group and disproportionate with serum fluoride as compared to fluoride values in other two groups. Therefore, there might be a correlation between

fluoride and eGFR and hence, consideration of fluoride as one of the important factors along with age, gender and race in the equation of eGFR shall help in deciding the progression of disorder. Fluoride can concentrate in urine 50 folds more than plasma making kidney susceptible to renal damage.[24] Hence, fluoride acts as a nephrotoxin and further epidemiological and molecular studies will help in assessing fluoride correction to improve health.

As demonstrated by Barbier et al. cell and cell organelles are vulnerable to fluoride toxicity, rescuing cells from oxidative damage is the need of hour to prevent further health complications.[25] Sirtuin is nicotinamide adenine dinucleotide (NAD^+) dependent histone deacetylase a regulatory protein which initiates autophagy to prevent cellular damage.[26] Sirtuin 1 (SIRT1) is most studied type among sirtuins since, it is involved in majority of the metabolism and aging disorders. Hence, it is called as anti-aging molecule.[27,28] In an animal study by Suzuki et al. fluoride induction increased sirtuin 1 levels indicating that SIRT1 helps nullifying the fluoride toxicity and activate repair mechanisms.[29] In the current study, serum sirtuin 1 in group 2 (42.7 (30.17 - 52.93) was increased compared to other group 1 and 3 (22.65 (11.78 - 91.10) & 36.9 (27.38 - 56.23)) which is indication of salvage of cells exposed continuously to diabetic milieu and preventing them from further complications, provided calorie restriction must be followed in all subjects to maintain a proper homeostasis which is in support with Suzuki et al. Hence, from this study we can state that in deceased subject's SIRT1 in increased than in controls due to a chain link of hyperglycemia, oxidative stress, AGE and an additional environmental factor; fluorosis pertaining to this population. Therefore, a correlation of AGE and fluoride with SIRT1 of group 2 & 3 showed a negative trend indicating that SIRT1 amelioration is still active to prevent damage but is deteriorated in group3 since the cells in DKD are in irreversible stress due to various comorbid conditions.

CONCLUSIONS

Estimation of carboxy methyl lysine especially in DKD cases is of prognostic value and hence, aids clinicians in making decision for insulin therapy. Fluorosis is a major public health issue to be considered in disorders in fluoride endemic areas. Serum and urine fluoride estimation is useful in assessing the progression of disorder and hence can prevent further complications. Fluoride (F) may not be the cause for diabetes but may accelerate diabetes to progress to its microvascular complications as observed in this study. Sirtuin 1 levels in serum or plasma can be a marker for various damages caused by aging disorders. Sirtuin 1 correlation with diabetic parameters will help in health research to include it as a therapeutic target for various toxic conditions. Hence, sirtuin 1 is considered as a biomarker for aging disorders especially in diabetes and also as a preventive molecule which can be included for therapeutics in disorders involved in cellular damages.

Limitations

Water fluoride estimations of villages were not performed which might have given a clear picture regarding exposure and outcome. Also, comparison of parameters between F exposed and non-exposed population may give a clear picture of the scenario. Molecular study of sirtuin 1 could have explained correlation appropriately.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
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REFERENCES

[1] Popkin BM. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with non-communicable diseases. Am J Clin Nutr 2006;84(2):289-98.
[2] Oggioni C, Lara J, Wells JC, et al. Shifts in population dietary patterns and physical inactivity as determinants of global trends in the prevalence of diabetes: an ecological analysis. Nutr Metab Cardiovasc Dis 2014;24(10):1105-11.
[3] Wild S, Roglic G, Green A, et al. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27(5):1047-53.
[4] Nanditha A, Ronald CWM, Ramachandran A, et al. Diabetes in Asia and the Pacific: implications for the global epidemic. Diabetes Care 2016;39(3):472-85.
[5] De Boer IH, Rue TC, Hall YN, et al. Temporal trends in the prevalence of diabetic kidney disease in the United States. JAMA 2011;305(24):2532-9.
[6] Kim HJ, Byun DW, Suh K, et al. Association between serum cystatin C and vascular complications in type 2 diabetes mellitus without nephropathy. Diabetes Metab J 2018;42(6):513-8.
[7] Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107(9):1058-70.
[8] Salminen A, Kaarniranta K, Kauppinen A. Crosstalk between oxidative stress and SIRT1: impact on the aging process. Int J Mol Sci 2013;14(2):3834-59.
[9] Frye RA. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (Sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem Biophys Res Commun 1999;260(1):273-9.
[10] Nakae J, Cao Y, Daitoku H, et al. The LXXLL motif of murine forkhead transcription factor FoxO1 mediates Sirt1-dependent transcriptional activity. J Clin Invest 2006;116(9):2473-83.
[11] Imai S, Armstrong CM, Kaeberlein M, et al. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 2000;403(6771):795-800.
[12] Luft VC, Duncan BB, Schmidt MI, et al. Carboxymethyl lysine, an advanced glycation end product and incident diabetes: a case-cohort analysis of the ARIC Study. Diabet Med 2015;33(10):1392-8.
[13] Inker LA, Astor BC, Fox CH, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. Am J Kidney Dis 2014;63(5):713-35.

[14] Henle T. AGEs in foods: do they play a role in uremia? Kidney Int Suppl 2003;63(84):S145-7.
[15] Semba RD, Fink JC, Sun K, et al. Serum carboxymethyl-lysine, a dominant advanced glycation end product, is associated with chronic kidney disease: the Baltimore longitudinal study of aging. J Ren Nutr 2010;20(2):74-81.
[16] Fu MX, Requena JR, Jenkins AJ, et al. The advanced glycation end product, nepsilon- (carboxymethyl) lysine, is a product of both lipid peroxidation and glycoxidation reactions. J Biol Chemistry 1996;271(17):9982-6.
[17] Report of the Ad Hoc subcommittee on fluoride of the committee to coordinate environmental health and related programs Public Health Service. Health Risk Assessment of Fluoride. Department of Health and Human Services Public Health Service 1991: p. 43-8.
[18] Verma A, Shetty BK, Guddattu V, et al. High prevalence of dental fluorosis among adolescents is a growing concern: a school based cross-sectional study from Southern India. Environ Health Prev Med 2017;22(1):17.
[19] Basta G. Receptor for advanced glycation endproducts and atherosclerosis: from basic mechanisms to clinical implications. Atherosclerosis 2008;196(1):9-21.
[20] Semba RD, Nicklett EJ, Ferrucci L. Does accumulation of advanced glycation end products contribute to the aging phenotype? J Gerontol A Biol Sci Med Sci 2010;65(9):963-75.
[21] Semba RD, Ang A, Talegawkar S, et al. Dietary intake associated with serum versus urinary carboxymethyl-lysine, a major advanced glycation end product, in adults: the energetics study. Eur J Clin Nutr 2012;66(1):3-9.
[22] Kido T, Sugaya C, Yanagisawa H, et al. The effects on renal function, in an Institute of Cancer Research-derived glomerulonephritis (ICGN) mice, of the subacute administration of the fluoride ion in drinking water. Research Report Fluoride 2017;50(1 Pt 2):161-74.
[23] Fontela PC, Winkelmann ER, Ott JN, et al. Estimated glomerular filtration rate in patients with type 2 diabetes mellitus. Rev Assoc Med Bras (1992) 2014;60(6):531-7.
[24] National Research Council (NRC). Fluoride in drinking water: a scientific review of EPA's standards. Washington, DC: The National Academies Press 2006.
[25] Barbier O, Arreola-Mendoza L, Del Razo LM. Molecular mechanism of fluoride toxicity. Chem Biol Interact 2010;188(2):319-33.
[26] Sauve AA, Wolberger C, Schramm VL, et al. The biochemistry of sirtuins. Annu Rev Biochem 2006;75:435-65.
[27] Wątroba M, Szukiewicz D. The role of sirtuins in aging and age-related diseases. Adv Med Sci 2016;61(1):52-62.
[28] Xu C, Cai Y, Fan P, et al. Calorie restriction prevents metabolic aging caused by abnormal SIRT1 function in adipose tissues. Diabetes 2015;64(5):1576-90.
[29] Suzuki M, Bartlett JD. Sirtuin1 and autophagy protect cells from fluoride-induced cell stress. Biochim Biophys Acta 2014;1842(2):245-55.