TITLE

ESTIMATION OF FLUORIDE AND SIRTUIN1 IN PATIENTS WITH DIABETIC NEPHROPATHY IN KOLAR DISTRICT OF KARNATAKA, INDIA

THESIS SUBMITTED

TO

Sri Devaraj Urs Academy of Higher Education and Research

For Awarding the Degree as

DOCTOR OF PHILOSOPHY

IN MEDICAL BIOCHEMISTRY

Under Faculty of Medicine

Submitted by
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Session
2017- 2020

DECLARATION BY CANDIDATE

I, **R. Sai Deepika,** hereby declare that thesis titled: **Estimation of Fluoride and Sirtuin1 in Patients with Diabetic Nephropathy in Kolar District of Karnataka, India,** is original research work carried out by me for the award of **Doctor of Philosophy** in Medical Biochemistry.

This study was carried out under the supervision of **Dr. Shashidhar KN,** Professor and Head, Department of Biochemistry and Co- supervision of **Dr. Raveesha A**, Professor and Head Department of General Medicine and **Dr. Muninarayana C**, Professor, Department of Community Medicine, Sri Devaraj Urs Medical College, A Constituent Institute of Sri Devaraj Urs Academy of Higher Education and Research.

No part of this has formed the basis for the award of any degree of fellowship previously elsewhere.

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Subject: Ethical clearance for Ph. D. Synopsis

The Central Ethics Committee of Sri Devaraj Urs Academy of Higher Education and Research, Kolar has examined Ph.D synopsis titled "**Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India**" and the detailed work plan of the project.

The central ethics committee has unanimously decided to approve the project and grant permission to investigator to carry out the research work. The interim and final report has to be submitted to the ethics committee after completion of the project for the issue of Central Ethics Committee certificate. Principal investigator should maintain the records of the Project and written consent form for not less than 5 year from the date of completion or termination of the project.

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This is to certify that original research work contained in the thesis entitled: **Estimation of Fluoride and Sirtuin1 in Patients with Diabetic Nephropathy in Kolar District of Karnataka, India,** in the subject of Medical Biochemistry is carried out by **R. Sai Deepika** (Reg No: **17PhD0302**) for the requirement of the award of degree Doctor of philosophy under Faculty of Medicine.

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Any part of this thesis has not been submitted elsewhere for the award of any degree of fellowship previously.

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ACKNOWLEDGEMENT

My foremost thanks and acknowledgement is to the Almighty, for His constant blessings throughout my study to complete my research successfully.

Firstly I thank my parents; Mr. R Ram Mohan & R. Sasikala and In- laws; Mr. NK Badarinath & Mrs. N. Nagarathna, for their moral, physical and financial support and encouragement to finish this work.

I deeply express my sincere gratitude to my research supervisor, Dr. Shashidhar KN, Professor and Head of Dept. of Biochemistry, for the steadfast academic, administrative and technical support in his busy schedule as vice- principal of college and director R & I of university.

I am very thankful for expert guidance in field of diabetes by Dr. Raveesha A, Professor and Head, Department of Medicine.

My sincere thanks to Dr. Muninarayana C, Professor, Department of Community Medicine in conducting study on fluorosis and guiding in research methodology and bio statistics.

It gives me pleasure to thank Honorable Chancellor Mr. Rajesh Jagadale, Honorable Vice Chancellor Dr. Pradeep Kumar Goud, and respected Registrar Dr. KNV Prasad SDUAHER for their support.

My sincere gratitude to Dr. PN Sreeramulu, Professor of General Surgery, Principal SDUMC and Dean- Faculty of Medicine, SDUAHER for his support and encouragement.

It is my duty to thank our Medical Superintendent Dr. Sheela S for permitting me to utilize hospital logistics and patient recruitment.

I am thankful to all the faculties who guided us in research methodology classes.

I am thankful to Director of academics Dr. Sarala N for her support in processing our progress report.

I take this opportunity to thank Dr. Sharath B, the PhD Coordinator for his immense support given in submitting my research work to the Academy.

I wish to express my warm and sincere thanks to Dr. Dayananda CD, Professor of Biochemistry and Dean Allied Health sciences.

I thank Mr. Ravishankar, Assistant professor, Department of Community Medicine for his help in designing the study.

I drop my special wishes to Dr. Hemalatha A, Professor Pathology in correcting my study proposal for approval.

I hold the responsibility to thank Dr. Suresh TN former co- coordinator R & I who took efforts and getting approval for start of study.

I learnt the basic designing of study according to human ethics from SDUAHER CEC chairman, Dr. Kiran Katoch, to whom I extend my thanks for word to word revision of study proposal and approving the study.

I thank Dr. Prabhavathi K, Dr. Susanna TY, Dr. Deena Mendez, Dr. Mamatha Kunder, Dr. Munilakshmi, Dr. Harish R, Dr. Rajeev Gandham and Mr. Bhuneshwar Yadav for their support and input for study.

I take this opportunity to thank B.Sc MLT students, Ms. Sireesha, Mr. Ravi Kumar, Ms. Nagina, for sample collection and analysis.

I thank all the technical and non-technical staff of our department Mr. Manohar, Mr. Pradeep Mr. Srininvas, Mr. Shaik Sarmadulla, Mr. Manjunath, Mr. Nagaraj, Mrs. Shashikala, Mrs. Gayathri, Mrs. Kavitha, Mrs. Bhargavi, Mr. Prasanth, Mr. Sunil Kumar, Mrs Veena, Mrs. Lakshmi, Mrs. Malleshwari, Mrs. Siddamma, Mrs. Lakshmamma, Mr. Narayanaswamy, Mr. Ramachandra and Mr. Manjunath.

I take this privilege to thank my Husband Mr. B. Panindra, my daughter Sreehitha. P, my sister in law Dr. B. Roopa, my brother Dr. SP. Sreedhara and my uncle Mr. RS Sreekanth for their kind co- operation in completing the study successfully.

R. Sai Deepika

ABBREVATIONS

AGEs	Advanced Glycation End products
AI	Atherogenic Index
CKD	Chronic Kidney Disease
CKD- EPI	Chronic Kidney Disease- Epidemiology Collaboration
CML	Carboxy Methyl Lysine
CVD	Cardiovascular disease
Cys C	Cystatin C
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
ELISA	Enzyme Linked Immunosorbant Assay
ESRD	End Stage Renal Disease
F	Fluoride
GDM	Gestational Diabetes Mellitus
eGFR	Estimated Glomerular Filtration Rate
HbA1c	Glycosylated Hemoglobin
HOMA IR	Homeostasis Model Assessment- estimated Insulin Resistance
IR	Insulin Resistance
IS	Insulin Sensitivity
KDIGO	Kidney Disease: Improving Global Outcomes
LDL	Low Density Lipoprotein
MAP	Mean Arterial Pressure
MODY	Maturity Onset Diabetes of Young
NAD	Nicotinamide Adenine Dinucleotide
NKF- KDOQI	National Kidney Foundation- Kidney Disease Outcomes Quality Initiative
NPPCF	National Programme for Prevention and Control of Fluorosis
nHDL	non- High Density Lipoprotein
ppm	parts per million
QUICKI	Quantitative Insulin- Sensitivity Check Index
ROS	Reactive Oxygen Species
SIRT	Sirtuin
TG	Triglycerides
TISAB	Total Ionic Strength Adjusting Buffer
T2DM	Type 2 Diabetes Mellitus
T1DM	Type 1 Diabetes Mellitus
WHO	World Health Organization

CONTENTS

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by polyuria, polydypsia, polyphagia and sudden weight loss (1). The clinical condition was first identified during 5^{th} century BC. Aretaeus a Greek physician defined DM as 'a siphon' meaning melting of flesh and limbs into urine. Contemporarily, the Indian physicians called it as honey urine, indicating sugar excretion from the body. In seventeenth century BC the word 'Mellitus' meaning 'Honey' was added by a Latin researcher (2).

Diabetes a global challenge needs to be addressed earlier to prevent the complications resulting out of it. This has been targeted by researchers in their own way.

Glycation end products and Advanced glycation end (AGE) products, oxidative stress markers and many more molecules have been incorporated in the early diagnosis and management of DM (3). However, all these molecules that have been studied include flaws. This created an interest to facilitate with a molecule "Sirtuin".

Since the present study has been carried out in a geographical population who are chronically exposed to fluoride, we tried to link fluorosis with DM and its complication. Further we confirmed with the glycation and advanced glycation end products (3 & 4).

Our inclusion of Sirtuin1 (Sirt1), Carboxy Methyl Lysine (CML), Fructosamine and Fluoride (F) molecules gave an impetus on the new knowledge generated that our objectives of the study construed with our findings and observations.

REVIEW OF LITERATURE

Diabetes Mellitus (DM) is a clinical syndrome characterized by unexplained weight loss, increase in thirst, frequent urination, increase in appetite and non- healing or delayed healing of wounds, blurring of vision. The basic biochemical markers for diagnosis of DM are fasting plasma glucose ≥126 mg/dL and post prandial plasma glucose ≥140 mg/dL (5).

American Diabetic Association (ADA) classified diabetes as (5, 6):

a. **Type 1 Diabetes Mellitus (T1DM):** absolute insulin deficiency due to auto immune destruction of β- cells of pancreas
b. **Type 2 Diabetes Mellitus (T2DM):** progressive destruction of insulin secreting β- cells of pancreas due to peripheral insulin resistance
c. **Gestational Diabetes Mellitus (GDM):** DM caused during 2^{nd} or 3^{rd} trimester of pregnancy
d. **Other types**: diabetes caused due to various other causes such as monogenic diabetic type, endocrine disorders, chemical induced onset etc.

World Health Organization (WHO) classified DM into (5):

1. **Type1DM:** β- cells destruction an immune mediated resulting in absolute insulin deficiency expressed during childhood and early adulthood
2. **Type2DM:** varying degrees of β- cells destruction and insulin resistance during adulthood
3. **Hybrid forms**
    a. **Immune mediated diabetes:** observed in adults similar to T1DM
    b. **Ketosis prone DM**: due to insulin resistance where, insulin requirement slowly diminishes with time and ketosis will be a predominant feature

4. **Other specific type**
    a. **Monogenic type:** gene mutation leading to changes either in β- cell function or insulin action
    b. **Defect in exocrine function of pancreas**: leading to increased secretion of hormones which are antagonists to insulin resulting in DM
    c. **Abnormality in endocrine part of pancreas:** which leads in secreting excessive hormone which are antagonists to insulin resulting in DM
    d. **Drug or Chemical Induced DM**: because of therapies affecting insulin secretion or destruction of β- cells
    e. **Infection related DM**: viral destruction of β- cells affecting insulin secretion
    f. **Autoimmune related DM:** which include other auto immune diseases and cause hyperglycemia either due to affected insulin secretion or due to insulin action
    g. **Other genetic syndromes:** which are due to chromosomal aberrations and may lead to DM
5. Hyperglycemia detected first time during pregnancy
    a. **Diabetes Mellitus in Pregnancy:** either type 1 or type 2 DM expressed during anytime of pregnancy
    b. **Gestational Diabetes Mellitus (GDM):** a clinical condition with hyperglycemia observed in pregnancy during 2^{nd} or 3^{rd} trimester

Even though the classifications are varied, the diagnostic criteria remains same (1,6)

1. Fasting Plasma Glucose ≥ 126 mg/ dL with a minimum fasting of 8 hours
2. 2 hours post- load glucose ≥ 200 mg/ dL
3. HbA1c (Glycated Hemoglobin) ≥ 6.5%

The major reason for classification of DM is to select appropriate therapeutic modalities. We have considered ADA classification as it is simple, understandable and suites our study. T1DM was considered to be young onset type but that's now obsolete since T2DM sets in during any stage of life. Though there is no much demarcation for the diagnostic criteria between T1DM and T2DM, ketoacidosis remains persistent in T1DM as it is completely dependent on insulin. Type 2 Diabetes Mellitus may also need insulin therapy based on their average blood sugar control and time of onset and any complications associated with it (1, 6).

According to International Diabetes Federation (IDF, 9th edition) 463 million people are recorded to be with diabetes in the year 2019 globally (7). Statistical data predicts with a fore thought that by the year 2045 there may be an increase of 51% of the population accounting for an estimate of 70 crore within age group of 20- 79 years across the world to be affected by diabetes (7). More so, there may be a 74% increase (from 8.80 to 15.3 crores) in T2DM in South East Asian (SEA) population vis- a- vis India (7). Particularly, 50- 70 years of age are majorly affected by diabetes in SEA countries. IDF statistics depicts that India continues to be ranked at 2nd position with a count of 13.4 crores of T2DM by 2045, of which 2.75 crores will be above the age of 65 years (7). In India, males are affected more than females (7).

International Diabetes Federation (IDF) data shows that South Indians are predominantly affected mainly due to higher intake of carbohydrate rich diet. A cross- sectional study carried out in India in the year 2017 has shown that Karnataka is at 4th position with 4.6% of type 2 DM (8). Based on a diabetes study by Wells JCK et al in the year 2016, India is presumed to be the capital of diabetes and the fact may be due to evolutionary perspectives of South Asian genetic makeup (9).

Studies conducted by researchers has documented that people with DM irrespective of good glycemic control may lead to microvascular and macrovascular complications. The pathophysiology of miscrovascular complication in diabetes mellitus can be linked to (3):

a. Neovascularization from Vaso Vasorum
b. Low- grade inflammation
c. Circulating Advanced Glycation Endproducts (AGE)
d. Oxidative Stress

Constant exposure of microvessels such as arterioles, capillaries and venules to diabetic melieu will stimulate neovascularism which may lead to formation of a new layer from vaso vasorum resulting in thickening of tunica intima (10). Thickening of blood vessel is observed in basement membrane capillaries of retina and glomeruli resulting in increased blood pressure and hypoxia (11, 12). In diabetes macro angiopathy or macrovascular thickening is due to uncontrolled dyslipidemia causing brain damage (stroke) or cardiovascular disease (11). Therefore, it is of utmost importance to monitor the balance of glucose and lipids in the blood to prevent complications of DM (11, 12).

Diabetic nephropathy a microvascular complication of T2DM is exponentially increasing across the nations and higher among Asian Indians (13). Schernthaner G et al. in the year 2013 has documented that around 30- 40% of T2DM patients end up in diabetic nephropathy (DN) which may be contributed by environmental or genetic factors (13). Although the underlying cause for DN in T2DM is still unclear, based on the studies we can derive that the difference in prevalence may be the result of metabolomics, proteomics, genetics or epigenetic factors (4).

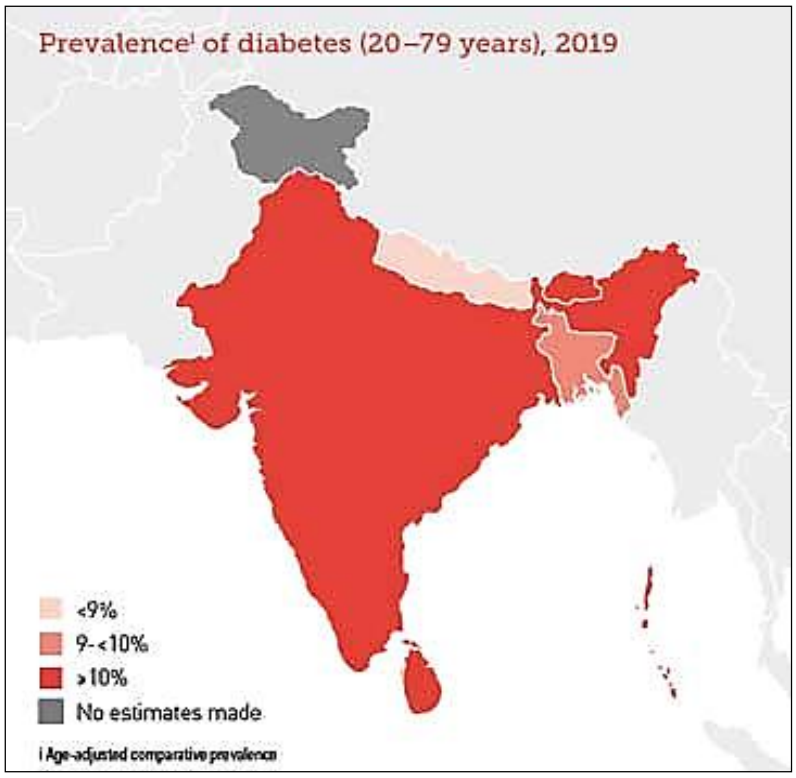


Fig 1: Prevalence of diabetes in India 2019
Source: International Diabetes Federation. IDF Diabetes Atlas, 9th ed. Brussels, Belgium: International Diabetes Federation; 2019

Advanced Glycation Endproducts (AGE)

Macromolecules such as proteins, nucleosides and lipids when bound to excess glucose units in blood derives 'Advanced Glycation Endproducts' (3). Advanced Glycation Endproducts

when accumulated in blood vessels or tissues may result in insulin resistance causing increase in body mass index (BMI); an initiator of diabetes (14). Uncontrolled AGE formation may be the root cause for diabetic complication at the earliest. Majority of the AGEs are acquired through food, very few are endogenously formed in body (14).

History of AGE

French scientist **Louis Camelle Maillard** in the year 1912 observed browning of food which led to explain the theory of Maillard reaction (14). In vitro, Maillard reaction involves various steps leading to formation of AGE (15). In vivo, the intermediate, amadori product will undergo rearrangement resulting in formation of AGE (15). During 1990's in vivo maillard reaction in diabetes and aging was discovered which was found to hasten the disorder (16). The theory of Amadori reaction was accepted during 1997 and till date 20 Advanced Glycation Endproducts are discovered (16).

AGE Mechanism of Action

AGEs are classified as

i. Receptor- dependent (17)

ii. Receptor- independent based on their entry into cells (18, 19)

i. Receptor- dependent

A multi- ligand immunoglobulin superfamily called Receptor for AGE (RAGE) is the receptor of AGE (3). RAGE is responsible for development of complications due to AGE by preventing binding of anti- RAGE molecules on the cell surface (3). On a concluding note, studies states that RAGE may not be an initiator of complications but a key component in accelerating inflammation and sustained tissue injury (17).

ii. Receptor- independent

AGE directly targets the proteins in cellular and blood vasculature skeletal proteins such as collagen and fibroblasts disturbing the structural integrity of basement membranes and vessel walls (18). The distortion of skeletal support is by excessive cross linking causing rigidity, leading to decreased reception of metabolites; carbohydrates (18). AGE can also modulate genetic expressions of a tissue linked to synthesis of matrix proteins (19). An action on vessel walls quenches nitric oxide leading to endothelial dysfunction resulting in atherosclerosis and hypertension (19). Inhibition of AGE by pharmacological inhibitors likely would decrease future complications example: amino guanidine (AG). Amino guanidine is a hydrazine compound which breaks the chain reaction in formation of collagen cross links thus preventing arterial stiffness (20). Recently, AGE inhibitors such as: pyridoxamine, OPB-198 and ALT- 946 are gaining momentum (21- 23).

Chemistry and Formation

Intracellular hyperglycemic environment activates various alternative pathways and direct glucose to enter Polyol pathway or protein kinase C pathway or produce Advanced Glycation endproducts (AGE) (24). Majorly produced and very much influential molecule to accelerate diabetes to its complications vis- a- vis; 'Carboxy Methyl Lysine (CML)' is considered in this study. Studies conducted by Brownlee et al are associated with diabetic nephropathy and AGE which has demonstrated increased rigidity of renal filtering apparatus (25, 26).

The primary triggering factor for production of AGE is the oxidative stress caused by hyperglycemic milieu. Glodberg T et al documented that not only carbohydrates rich food, even foods containing higher fat content may have a higher AGE especially Carboxy Methyl Lysine (CML) as it is formed by lipoxidation (27). Increased AGE and Advance Lipoxidation

Endproducts (ALE) are associated with increased incidence of Cardiac and Renal failure (27). These data indicate that precaution to be taken to avoid processed, baked and deep fried food. Processed, baked and deep fried food contains higher concentrations of CML (27).

In- vivo formation of AGE occurs in three phases (17):

a. Initial phase
b. Proliferative phase
c. Latent phase

a. Initial phase

Reaction between **carbonyl groups** of reducing monosaccharides such as glucose or fructose or ribose and **amino group** of amino acids preferably lysine or hydroxylysine cysteine of hemoglobin or serum albumin forms **unstable Schiff's base** (28- 30**).** Non- enzymatically rearranged of Schiff's base forms a **stable Amadori product** (27). Examples of Amadori products are: Glycated hemoglobin (HbA1c), Glycated serum albumin (Fructosamine, GSA), histone and various plasma proteins (28- 31).

b. Proliferative phase

Multiple self rearrangements of Amadori products in presence of metal ions form various **harmful Carbonyl intermediates** which are the precursors for AGE. Those intermediates are ***α*- dicarbonyls or oxaloaldehydes** which includes: glyoxals (GO), methylglyoxals (MG) and 3- deoxyglucosone (3DG) (32). Direct oxidation or fragmentations of Schiff's base forms GO. Autooxidation of carbohydrate, lipid peroxidation and decompostition of triose phosphates results in formation of MG. Rearrangement and hydrolysis of Amadori product results in DG formation (33). Accumulation of these carbonyl compounds causes **'carbonyl stress' (33)**

c. Latent phase

Various chemical reactions such as hydration, dehydration, oxidation, non- oxidation, glycation, glycosylation, fructolysation and acid hydrolysis may result in formation of irreversible product called Advanced Glycation Endproduct.

Glyoxal Lysine Dimer (GOLD), Methyl Glyoxal Lysine Dimer (MOLD), Deoxyglucosone Lysine Dimer (DOLD) is formed by reaction between respective amadori product and lysine condensation reaction (32).

Degradation of lysine rich proteins will end up in production of Carboxymethyl Lysine (CML), an AGE whose aggregation destructs the skeletal structure of renal cells making it non-functional for regular metabolism (33).

Characteristics of Carboxymethyl lysine (CML)

a. Carboxymethyl lysine (CML) is an AGE formed by both, glycoxidation and lipoxidation (32)
b. Sources of Carboxymethyl lysine includes invivo (endogenous) and invitro through diet (exogenous) (27)
c. It is a non- fluorescent, non- crosslinked glycated product affecting retinal protein and basement membrane of glomerular apparatus (24, 34)
d. Formation of CML is by direct rearrangement of Amadori product and after oxidation of Glyoxal (GO) (17)
e. Highly available in most of the processed, bakes, broiled and deep fried food (27)

Since CML interferes with renal filtration and basement membrane functions, of late it is studied in depth about dietary modifications in diabetes and its microvascular complications.

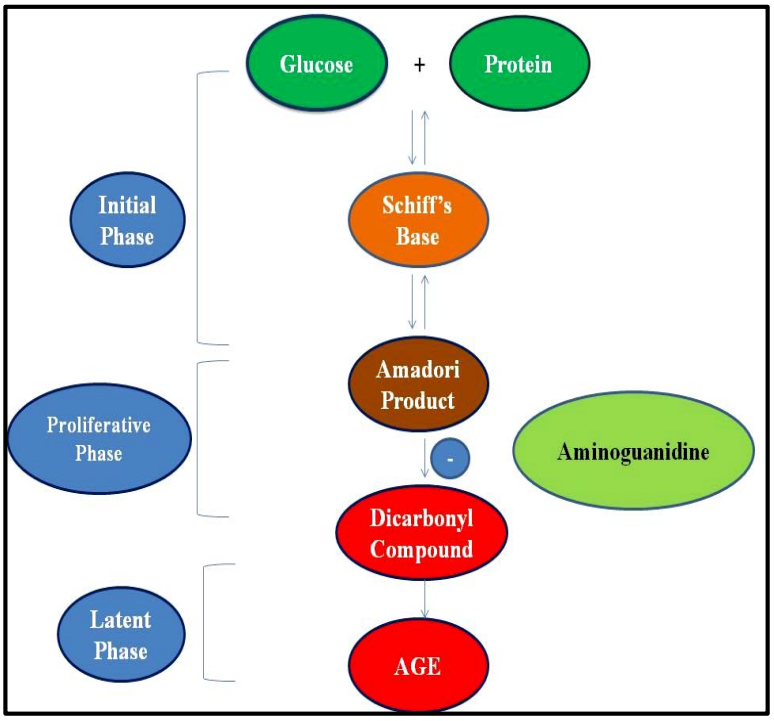


Fig 2: Formation of AGE

Source: Varun Parkash Singh, Anjana Bali, Nirmal Singh, and Amteshwar Singh Jaggi. Advanced Glycation End Products and Diabetic Complications. Korean J Physiol Pharmacol 2014; 18: 1- 14. http://dx.doi.org/10.4196/kjpp.2014.18.1.1) [17]

FLUORIDE

Lifestyle, diet, environmental factors etc, varies from, place to place. Kolar district is non-exceptional in this regard. Kolar district in Karnataka is fluorosis affected due to a deep borehole water source and scanty rainfall. Kolar is a semi- arid area located 595m to1474m above Mean Sea Level and situated 13°08'08.5"N 78°07'57.4"E (4). Despite the scanty rainfall and various other factors leading to decreased water source, the agriculturists of this belt grow a major crop known as finger millet (ragi) (4). Majority of the fruits and vegetables grown in this area are Mango, Grapes, onions, potatoes, cabbage, green leafy etc. Chronic exposure to toxic salts in the deep water is known to accumulate in body leading to toxicity and other consequences (35). Major minerals found in deep water leaching the rocks are Fluoride and Arsenic (35, 36). Since there are

many studies emerging related to ill- effects with respect to fluoride of non- skeletal tissue and its complications made us to evaluate fluoride effect on insulin resistance and diabetes.

Chronic fluoride exposure results in dental, skeletal and non- skeletal fluorosis. This may be the reason for dental problems in children and early osteoporosis in elders with increased incidences of diabetes and cancer without any family history (37, 38). As a preventive measure, the district health officers had installed community water purifier and created awareness among villagers. Installation of fluoride filters and water purifiers has contributed to eliminate and filter out other essentials minerals along with F (39). To assess, analyze and prevent the ill- effects of these filtered out minerals there is need for a molecule which has diagnostic, prognostic and therapeutic value.

Chemistry

Fluorine, a yellow colored pungent gas is one of the lightest halogens with atomic number 9 and mass number 18 ($^{18}F_9$) (40). Fluorine in aqueous or dissolved state forms highly electronegative fluoride (F) ion, with electronegativity of 4.0 (41). Fluoride (F) is considered as a reference atom for electronegativity in the periodic table (41). Electronegative property of F helps in formation of complexes with Aluminium, Magnesium, Calcium, Sodium and Mica (42).

Occurrence

Fluoride can be found in water, soil and also as a molecule released from the industrial effluents. Physiochemically, F is found as sodium fluoride with pH 7.4 and as gaseous hydrofluoric acid with vapour pressure of >1 atmospheric pressure (atm) (37). Naturally occurring forms of F are fluoroappetite, cryolite, topaz, fluorite etc (37, 43).

Major routes of fluoride exposure are from air, water, vegetables and/ or fruits grown in soil with high fluoride content.

Air

Industrial effluents involving manufacturing of coal, asbestos, aluminium etc emits fluoride as its effluents which are the major source of F in air (44, 45). Generally, 0.5 ng/$m^3$is sensed in air around the industries emitting fluoride (43).

Water

Industrial effluents dumped in nearby water bodies will lead to elevation in the fluoride content of groundwater (37). Domestic and industrial sewage also accounts for water pollution. These issues addressed recently by governing bodies in developed countries. Marier JR et al have documented that primary water bodies such as natural well and borehole water contain approximately 1ppm of fluoride (44). However, the developing countries are also concentrating of late in the issues pertaining to water and sewage pollution. These factors have led to the irrigation of vegetation with fluoride rich water.

Soil and Plants

Earth crust contains approximately 0.08% of F and accounts for the 13^{th} abundant element. Fluoride from the soil is absorbed by the roots in complex forms than as ionic form, till the shoot including the vegetables or cereals (43, 46). The degree of absorption and tolerance varies between plants (47). Accumulation of fluoride in plants is dependent on the concentration of fluoride in water which is used for irrigation, pesticides and also pH of soil (48). In neutral pH fluoride will not be readily available for absorption by plants (48). Studies had proven that anti- oxidant in plant balances the ROS generated by fluoride toxicity and hence prevents damage to its other parts (47).

In summary, plants are loaded with fluoride from air, water and soil which at optimum level can nullify its effect by its in built anti- oxidant mechanism.

Distribution

WHO statistics has documented major countries rich in fluoride contamination are South Africa, United Arab Emirates (UAE), India, China, Australia, Afghanistan, New Zealand, Parts of Russia and North America (37). Indian researchers conducted studies in Rajasthan, Andhra Pradesh, Uttar Pradesh, Tamil Nadu assessed the fluoride toxicity and its consequences (49- 51). Fluoride is considered as an environmental toxin and known to cause chronic and metabolic disorders (38). Verma et al in the year 2016 demonstrated in their study as Kolar; a district in eastern part of Karnataka as fluoride endemic area (4). According to WHO, two- third of the Indian states are fluoride endemic and one- third are affected by fluoride ailments. Further they have observed that the school going children are mainly affected by dental fluorosis (52).

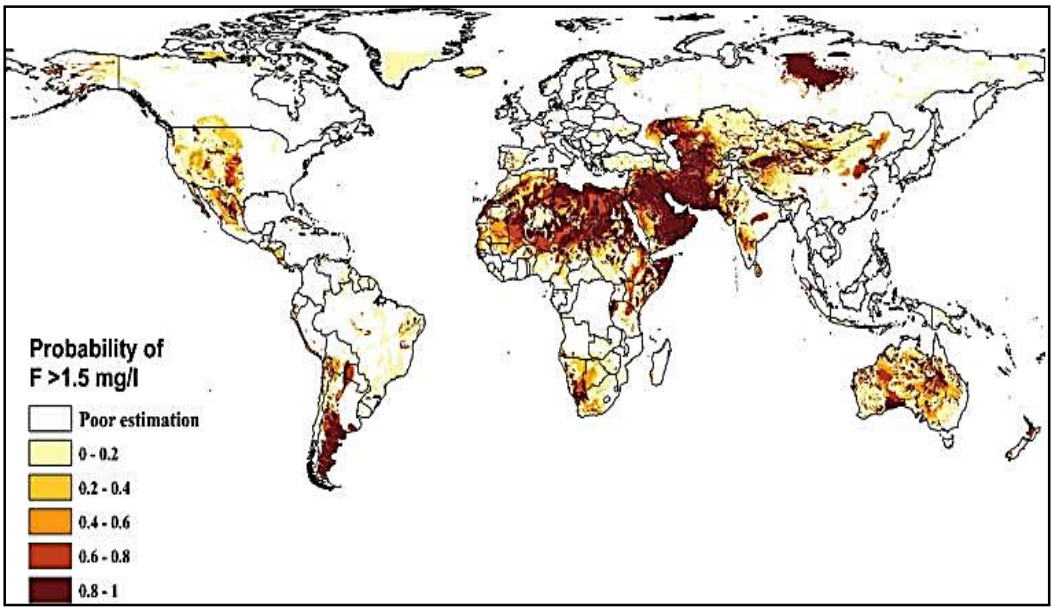


Fig 3: Fluorosis around the World

Source: Manouchehr Amini, Kim Mueller, Karim C. Abbaspour, Thomas Rosenberg, Majid Afyuni, Klaus N. Møller, Statistical Modeling of Global Geogenic Fluoride Contamination in Groundwaters Environ. Sci. Technol. 2008, 42, 3662–3668[53]

Fluoride and its Pathological Role

Dental Fluorosis

Physiological role of fluoride in optimum level at 0.5 to 1.5 ppm helps to prevent tooth decay and was added in the yesteryear in the tooth paste (37). However, of late it has been withdrawn and the statutory bodies has made it mandatory to publish the caption ‘use peanut size and not to swallow’.

Fluoride maintains acidic environment in oral cavity preventing tooth decay by bacteria *Streptococcus mutans* as experimented by Sananda et al (45). Constant use of fluoridated toothpaste in fluorosis endemic area led to fluoride toxicity in children leading to dentine discoloration, enamel erosion, tooth aches and resultant tooth loss. To assess the severity of dental fluorosis, there are various mathematical formulae. Till date, accepted equations are Dean’s and Thylstrup & Fejerskov (TF) indices (54, 55). Dental indices are based on dentine morphology of opacity and cavities in the enamel, based on which scoring is given from 0- 9 in Dean’s index and 0- 5 in TF index (54, 55)

Skeletal Fluorosis

Skeletal fluorosis in adults is exhibited as bone pain, fractures and marble n bones (37). Generally, fluoride replaces hydroxyl group of hydroxyappetite in bone matrix resulting in fluoroappetite, which is a more stable compound than hydroxyappetite (56).

Skeletal fluorosis was diagnosed by performing fluoride triple test, which includes (39):

a. Coin test
b. Chin to chest test
c. Hands on Occiput

Accumulation of fluoride in bone matrix is also pH dependent. Alkaline pH of serum tends fluoride to be absorbed more by bone forming complex with Ca and phosphate resulting in fluoroappetite (56). Gradually, chronic fluoride exposure due to increased groundwater content of fluoride not only damage dental and skeletal tissue but also soft tissue termed non- skeletal fluorosis leading to abnormality in metabolic pathways and chronic disorders.

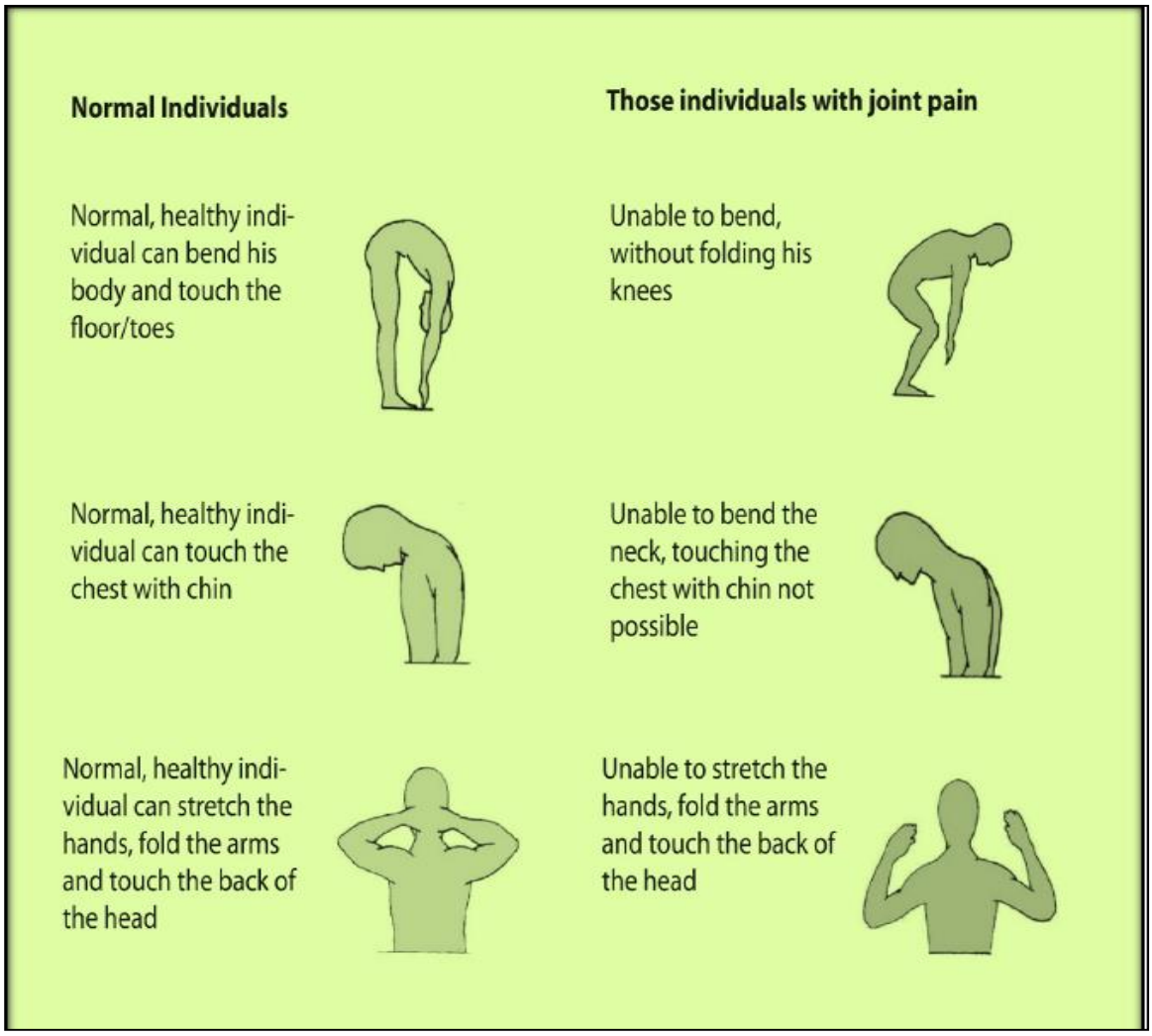


Fig 4: Fluoride Triple Test

Source: National Programme for Prevention and Control of Fluorosis (NPPCF) Revised Guidelines. Directorate General of Health Services Ministry of Health & Family Welfare Government of India. 2014; Annex 4(b) [39]

Fluoride Chemistry

Native form of fluoride is called fluorine which is the 13th most abundant and an electro negative halogen. Aqueous form of fluorine in nature is called the fluoride (F) and constitutes 0.1% of earth's crust minerals (43). Fluoride being highly electronegative tends to get easily associated with the electropositive ions such as hydrogen and sodium in biological system resulting in formation of Hydrogen fluoride (HF) and Sodium fluoride (NaF) (38). In biological membrane HF easily penetrates than ionic fluoride. Because of a very low pH of the stomach 40% is absorbed as HF (38). In airway epithelium fluoride enters through anion channels. Approximately 45% of the ingested fluoride is transported via facilitated diffusion (38, 57).

Fluoride Intake

Fluoride, an essential micro mineral and a trace element whose major function helps increase hardness of bones and teeth (58). Deficiency of F may lead to dental caries and osteoporosis (59). This in earlier days has led to fortification of tooth paste and drinking water. However, due to its increased concentration in the drinking water because of deep borehole water source led to toxicity diseases or symptoms example: dental fluorosis (59). Fluoride of late is termed as a *double edged sword* because when used in limited quantity it has an anticariogenic and antibacterial property in protecting dentine by inactivating the *Streptococcus mutans* (45, 60). In contrary, if the fluoride reaches the toxic levels it may pose adverse events on the biological system (61). Apart from dental and skeletal fluorosis, non- skeletal fluorosis is documented as thyroid disorders, male infertility and chronic kidney disease is most prevailing (62- 64). Finger millet and vegetables, a major agricultural product cultivated in this belt and the cattle consume the same fluoride contained water might have led to high fluoride content in produced milk (47, 48, 65). The other modes of F ingestion are the tea leaves which are known to contain >3.5ppm of

F (66). The same has been proved by the study conducted by Wu Jun-hua et al, where they documented that fluoride via tea consumption is directly proportional to urinary fluoride excretion (66). These findings make it clear that crops grown in higher water fluoride levels absorb fluoride proportionally leading to high fluoridated product. S. N. Ramaiah et al had derived conclusions in their study conducted at Malur Taluk of Kolar district that vegetables grown in this geographical belt which is F contaminated and the water which contains a minimum level of fluoride (35). SN Ramaiah et al study is in concordance with Chittaranjan Das et al who had documented that potatoes grown in fluoride endemic areas will also have high fluoride leading to accumulation of fluoride in tissues (48). This is also true with Kolar district as potato is one of the leading vegetable cultivated by farmers. Accepted serum fluoride level with respect to water exposure is approximately 0.15 mg/L. This has been experimentally proved by Singer and Amstrong (67).

Fluoride Excretion

Urinary fluoride excretion is primarily dependant on pH gradient across the tubular cells. It has been documented that higher the pH, higher will be the urine fluoride excretion (68). Circulating serum F levels are less in alkaline environment compared to acidic. This indicates that diabetic keto- acidosis may favor fluoride absorption and enhance fluorosis. Wu Jun-hua et al have proved that the ingestion and excretion of fluoride is directly proportional. The authors have concluded that the major route of fluoride excretion is through urine (66). Buzalaf, M.A et al have observed that approximately 60% of the ingested fluoride is filtered out by the kidneys (69). In regions where there is high prevalence of chronic kidney disease and cancer, fluoride acts as an accelerator to worsen the disease (70, 71). Continuous exposure of renal cells to fluoride makes the glomeruli and its supporting cells susceptible to irreversible renal damage leading to improper filtering of the metabolites and causing disturbance in bodily homeostasis (70). According to US

National research council ≥ 0.1ppm of fluoride in serum or urine is an indication of impaired glucose metabolism causing diabetes in later stages of life (71). Though there are many repair pathways activated to prevent mitochondrial oxidative stress of cells, chronic exposure is a hindrance for treatment.

Non- Skeletal Fluorosis

In the year 1937 Roholm observed that acute exposure to large doses of fluoride or continuous exposure to small doses of fluoride over years may damage not only teeth and bones but also vital organs and systems (72). Susheela in the year 2001 demonstrated an electron microscopy screening of cells of few organs exhibiting fluoride depositions in soft tissues and erythrocytes convincing that non- skeletal fluorosis are also an emerging illness to consider (73). Mondal et al observed that there was increasing incidents of non- skeletal fluorosis compared between 20^{th} and 21^{st} century (74). This increase may be due to lowering of water table leading to rocks leaching and hence fluoride dissolution from rocks into the groundwater. Ultimately, increasing rain water harvesting, small water body creation and rain water aggregation as a measure to increase the ground water led to decrease of water fluoride exposure. Major symptoms of non- skeletal fluorosis include GI issues such as loss of appetite, nausea, constipation. Other symptoms may be bone aches, headaches, accumulation of F in skeletal muscles leading to calcification (71, 75). The major impact of chronic fluoride exposure is observed in reproductive organs, thyroid, kidney and brain (62, 63, 71, 76, 77).

Fluoride in the form of sulfonyl fluoride, perfluoro octane and sodium fluoride are labeled as endocrine disruptors (78). These molecules interrupt the action of endocrine hormones such as insulin, via receptor inactivation or by damaging the hormonal structure (79, 80). Biochemically, fluoride inhibits enolase enzyme. Enolase enzyme is a metalloenzyme dependant on magnesium

ion as a cofactor which helps in reversible reaction of conversion of 2 phosphoglycerate to phospho enol pyruvate (58, 80). This enzyme is inhibited by F ion competitively in the cellular glycolytic pathway resulting in substrate accumulation that is glucose (80). Interestingly, increased blood glucose levels tends to increase insulin secretion leading to Autophagy or apoptosis of beta- cells of langerhans and hence causing insulin deficiency during increased serum fluoride (81, 82). These critical findings prove that the theory chronic or acute fluoride exposure leads to diabetes.

Research on fluoride has proved that it enhances oxidative stress and altered anti oxidants leading to the molecular damage (38). For the future progeny, antioxidants provided through a nutritious diet, for the elderly generation, fortified food and immune boosting drugs shall support as an adjunct for vitamins and minerals in enhancing the anti oxidants in the body. There are few non- essential antioxidants which are generated in the body naturally through metabolism. Nevertheless, our metabolism may need some trigger factors to synthesize the anti oxidants. The trigger factor may be an oxidant suppressor or an antioxidant activator such as minerals and vitamins. Antioxidants can be proteins, enzymes, vitamins, minerals and/ or lipids.

Extensive cellular damage by oxidants or free radicals will end up in programmed cell death or apoptosis or cell eating cell (autophagy) (82). Apoptosis or autophagy is an essential cellular repair mechanism which helps preventing extension of damage by activating cascade of pathological processes (83). Increase in repair mechanism can also arise due to deficiency of molecular oxygen (hypoxia) (84). Neighboring healthy cells will be activated to secrete chemicals to digest damaged cell and prevent chain reactions of damage (83). One such secreted enzyme centered in this study belongs to a group of proteins called **‘Sirtuin’** (82)**.**

SIRTUIN1

Sirtuins were first discovered in *Saccharomyces cerevisia*e (baker's yeast) as a regulatory protein coded by Silent Information Regulator 2 (Sir2) gene (85). Sir2 were known for life span modulation in lower animals such as yeast, *Drosophila melanogaster*, *Caenorhabditis elegans* etc (85, 86). Sirtuins in mammals are homologues of Sir2 gene of prokaryotes and said to regulate health and longevity (85).

History of Sirtuin1 (Sirt1) Discovery

Sirtuin1 is one of the molecules recently identified and still in research to combat the mitochondrial and oxidative damage. Sirtuin1 is a NAD^+ dependent histone deacetylase enzyme (85).

Mammalian sirtuins are seven membered family; Sirt 1- 7 (87). Structurally, all sirtuins are proteins which contain a catalytic core of 275 amino acids (88). Each class of sirtuins consists of unique C and N terminal amino acids. Sirtuins and Sir2 coded proteins are considered to be evolutionarily conserved. However, there may be few changes in the core domain based on the Phyla (89). Phylogenetic classification of sirtuins revealed that it has 60 different types of core domain. Based on these 7 classes of sirtuins they are distributed to four sub- classes; I- IV (89).

Sirt 1- 3 belongs to sub- class I which is seen in fungi and protozoan. Sirt 4 belongs to sub-class II which is discovered in insects, nematodes, bacteria, fungi. Sirt 5 belongs to sub- class III which is widely distributed in bacteria and archea. Sub- class IV consists of Sirt 6 and 7 which is seen more in metazoans, plants and vertebrates (41).

Sub- cellular localization	Sirt1	Sirt2	Sirt3	Sirt4	Sirt5	Sirt6	Sirt7
Nucleus	Class I NHD					Class I NHD & ART	Class I Function not determined
Cytoplasm		Class I NHD & ART					
Mitochondria			Class I NHD & ART	Class II ART	Class II NHD		

Sirtuins classifications, sub- cellular localization and functions

NHD: NAD^+ dependent histone deacetylation, ART: ADP Ribosyl Transferase

Apart from structural and Phylogenetic modifications, there is also a different sub cellular localization of sirtuin. Sirtuin1, 6 and 7 are related to nucleus. Sirt1 is associated with euchromatin rarely found in cytoplasm. Sirt6 belongs to heterochromatin. Sirt7 is restricted to nucleolus (89). Sirt2 prominently resides in cytoplasm (89). The mitochondrial sirtuins are sirtuins 3, 4 and 5 (89). Sirtuins are found attached to either DNA or RNA in the subcellular localization and decides its functions.

Sirtuins are defined as 'NAD^+ dependent histone deacetylases or ADP- mono ribosyl transferases'. Sirt1 and 5 are NAD^+ dependent histone deacetylases (89). Sirt4 and 6 are ADP-mono ribosyl transferases (89). Sirt 2 and 3 can perform to both the functions to certain extent which may be due to the core domain makeover of amino acids (90). Sirt7 is still under research for its robust function.

Existing knowledge on sirtuins till date is majorly carried out on laboratory animal models. There is a need to bring sirtuin1, an evolutionarily conserved molecule to lime light and reveal its importance in health and disease. In an animal study conducted by M Suzuki and JD Barlett, have observed that fluoride induced cell (Ameloblast cells) toxicity had led to Autophagy of cells via sirtuin1 activated pathway. Sirt1 expressed beneficial effect in prevention of dental fluorosis of other neighboring healthy cells (82).

Molecular studies on sirtuin1 have laid the foundation for human studies particularly in diabetes mellitus. As mentioned, since sirtuin1 is involved in glucose homeostasis, lipid metabolism, inflammatory responses, oxidative stress, cell senescence and apoptosis, higher reactive partners, dual role in nucleus as well as cytoplasm and most old and evolutionarily conserved class of protein among sirtuins has triggered interest to further estimate sirtuin1 in serum.

The method we considered is sandwich Enzyme Linked Immuno Sorbent Assay (ELISA). This method enables us to assess the trend of sirtuins in healthy controls, type II diabetes mellitus and diabetic nephropathy study subjects who were chronically exposed to fluoride.

Since the longevity of an organism majorly depends on cellular and tissue health; research and therapeutics evaluated sirtuin1 as an anti- aging, longevity and a regulatory protein. Sirtuins play a vital role in regulating factors related to aging at genetic level. However, its isoforms which is distributed in the subcellular organelles are involved in maintaining the cellular integrity. Initially, Sinclair DA and Guarente in the year 1997 authored a paper on the causes of aging in yeast and concluded that extra chromosomal rDNA circle is associated with Sir2 gene (85). Since

1999 Matt Kaeberlein et al are working on Sir2 gene to further explore its benefits as a longevity gene (86).

Sirtuins are called the homologue of Sir2 gene of yeast. It has been considered as the most ancient conserved protein of longevity in all organisms from unicellular to multicellular (90). Research on sirtuin1 is gaining momentum to find its evolution and other isoforms. Among the sirtuin family, sirt1 and 6 have nodalized in aging disorders.

Biochemistry of Sirtuin1

Sirtuins, a group of enzymes mediates NAD^+ dependent histone deacetylase and ADP-ribosyl transferase reactions. Sirtuins are involved at genetic level; DNA and RNA, leading to various crucial modifications in cell. The modifications results in causing stress, aging, survival mechanisms and metabolic functions (91).

Proteins acted upon by sirtuin1 in human beings include:

i. Histone proteins as an anti-transcriptional factor involving H1, H2 and H3 (92)
ii. FOXO3a, p300 and PGC- 1α as anti- transcriptional factor (93, 94)
iii. Anti- apoptotic factor on p53, NFκB, TNFα (95, 96)
iv. Pro-transcriptional factor on FOXO1&4 and HIV tat (93, 97)

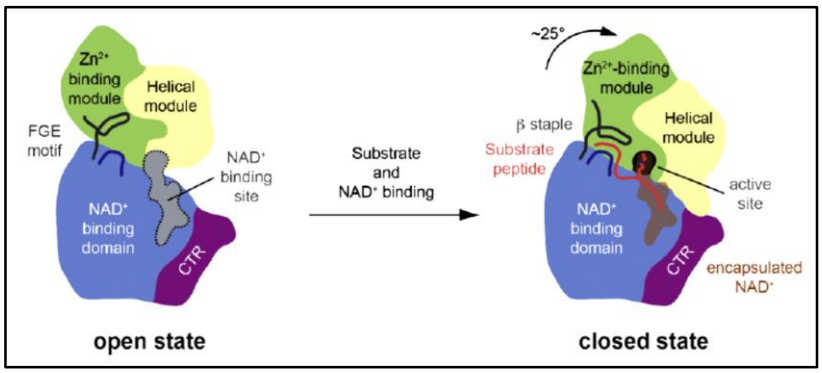


Fig 5: Structure of Sirtuin1

Source: Davenport AM, Ferdinand M. Huber and André Hoelz. Structural and Functional Analysis of Human SIRT1. J. Mol. Biol.2014; 426: DOI: 10.1016/j.jmb.2013.10.009 (98)

NAD^+- dependent histone deacetylation of proteins by sirtuin1 result in formation of novel product called; O- acetyl ADP ribose (AADPR) (91). AADPR acts as a signaling molecule. AADPR is known to form a complex with the sirtuin. AADPR can be a potent biological inhibitor of sirtuin1. Evidence suggests that macro H2A, a variant incorporated into regions which are silenced by X- chromosome inactivation may also bind to AADPR. However, the molecular basis and effects of AADPR needs further evaluation (91).

The major motive behind involvement of NAD^+ is, its hydrolysis yields energy for lysis of acetyl lysine containing amino acid residue and coupling with consecutive product formed and produce diffusible AADPR and a free protein with lysine residue and a molecule of nicotinaminde (91). Isotope labeled experiment on sirtuin1 reaction in mammals proves that NAD^+ levels also regulate sirt1 concentration and its activity (99). It is documented that mammalian NAD^+ levels of 200 to 500 μM is sufficient to maintain k_m value of sirt1 for deacetylation and hydrolysis of NAD^+(99). The site of NAD^+ in sirt1 binding is well within the Rossman fold (C pocket). The highly conserved histidine residues in the Rossman fold helps recognize the –OH group of NAD^+ in sirt1 (100). These histidine residues are also important in initiating nucleophilic attack to activate imidate product formation and proceed with the deacetylation reaction (91). ADP ribosyl transferase reaction can be affected by involvement of electro negative elements such as fluoride analogues (mono, di or tri fluoro compounds) which could be substituted at α position of acetyllysine decelerating the reaction (100).

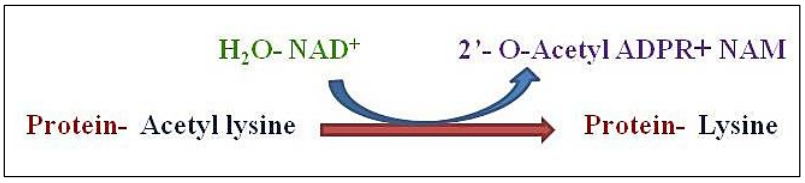


Fig 6: General reaction of sirtuin1

Source: Sai Deepika Ram Mohan and Shashidhar Kurpad Nagaraj. Sirtuin1: Serendipitous and Enigmatic Molecule. Int J of Current Medical and Pharmaceutical Research. 2018; 4 [11(A)]: 3828-36[(101)]

The uniqueness in sirtuin catalysis is formation of imidate and nucleophilic exchange reactions that can be reversed during the process of deacetylation by NAD^+ based on the availability of nicotimamide in the cell (91). Therefore, it can be concluded that NAD^+ acts as an inhibitor during deficiency of Nicotinamide within the cell (99). Till date, with human this reaction is observed only for sirtuin1 (99).

Sirtuin1 Activators

Activators of sirtuin1 include: resveratrol, isonicotinamide etc. These activators, activates and/or enhance the activity of sirtuin1.

'Resveratrol', is a polyphenol compound found in skin of grapes and tea. Resveratrol is a plant origin molecule which aids in activation of sirtuins when administrated in large doses (102, 103). Resveratrol increases sirt1 and PCG-1α levels decreasing cell stress (103). On the other hand, resveratrol is not specific to sirt1. In this regard a very large dose of resveratrol is observed to inhibit other essential enzymes such as cyclooxygenases (104).

Nicotinamide depressors ex: isonicotinamide (INA) will make NAD^+ to lose control on sirt1 in vivo without reacting with imidate and inhibits deacetylation by reacting with acetyllysine group. Isonicotinamide, which is non- toxic and least expensive, binds weakly and readily replaces NAD^+ there by activates sirt1 (102, 105).

Sirtuin1 inhibitors

Down regulation of sirtuin1 expression is by its inhibitors such as NAD^+, EX-527 and acridinedione derivatives**.** These inhibitors may be either a substrate inhibitor or an enzyme inhibitor. One such sirtuin1 inhibitor explained above is **NAD^+** which might reverse the ongoing reaction to the initial step depending on availability of nicotimamide invariable of the reaction

progress (102). There are few controversial studies which states that Sirt1 induces carcinogenesis by suppressing p53 pathway (105). Inhibition of sirt1 by p53 activating compounds such as: **EX-527, NSAIDs**, **acridinedione derivatives** acts as sirt1 inhibitor. These molecules are highly specific to sirt1 (105- 108). EX-527 and acridinedione derivatives, considered as anti cancerous and avoiding deacetylation of p53 by sirt1 (105- 108).

Mechanism of aging in sirtuin

Physiologically, sirtuin1 deacetylates histones 3 and 4 at lysine residues of 9 and 16 respectively. In vitro studies have documented that sirt1 leads to activation of various transcriptional factors which are responsible for anti- apoptotic and pro- transcriptional activities (92, 109).

Metabolically, sirtuin1 regulates gluconeogenesis through liver by deacetylating forkhead box protein O1 (FOXO1), cAMP response element-binding (CREB)-regulated transcription coactivator 2 (CRTC2) and activation of fatty acid oxidation pathway by acting on PCG-1α (97, 103, 110). Sirt1 regulates insulin secretion via synthesis of uncoupling protein 2 (UCP2) which leads to glucose stimulated insulin secretion (111). According to previous findings it is evident that sirt1 contributed majorly to obesity and type 2 diabetes.

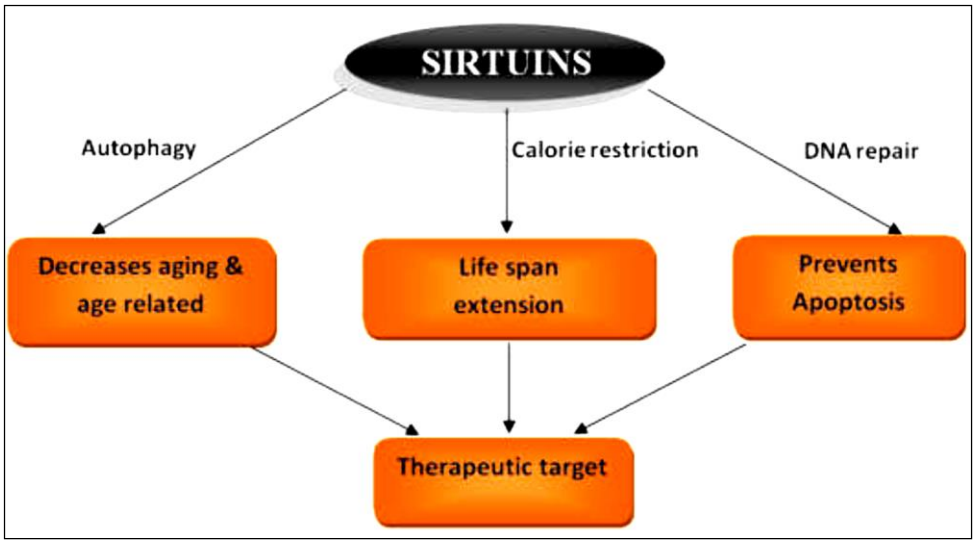


Fig 7: Physiological Role of Sirtuin1

Source: Sai Deepika Ram Mohan and Shashidhar Kurpad Nagaraj. Sirtuin1: Serendipitous and Enigmatic Molecule. Int J of Current Medical and Pharmaceutical Research. 2018; 4 [11(A)]: 3828- 3836[101]

Genetically, Sirtuin1 is coded by chromosome 10 q21.3 gene (112). Quantification of such coded protein/ enzyme secreted within the cell can be estimated in the urine, serum/plasma and other body fluids while transported or leaked (113). In a study conducted by Ozlem Gok et al in Turkey population, stated that Sirtuin1 can be a potential biomarker in T2DM diagnosis along with microRNA 181a and 132 which affects sirt1 physiological activity (114). Hence, there is also a need to find correlation between microRNA and alterations in sirt1 activity in diabetes which may give clue for its epigenetic modifications.

Pathologically, theory of autophagy activation in other tissue cells shall be considered to prevent the carryover of fluoride accumulated in cells to its cell lines. However, to draw any conclusions it needs to be substantiated by molecular studies combining fluoride and sirtuin1. This may give an impetus of sirt1 in therapy of Fluoride toxicity.

Autophagy is a cellular repair process which involves digestion of unwanted protein aggregates, poorly functioning organelles and damaged cells by lysosomal proteins mediated

digestion (83). It was reported by Kai- Peng et al that increase in AGE levels may decrease the half- life of sirtuin1 from 6 hours to 3 hours (115). Therefore, to prevent molecules such as AGE and ROS which generate free radicals, initiation of autophagy may help in rescuing the damaged cells by preventing apoptosis.

Genomic and proteomic study of sirtuins has proved that, sirtuin1 has the highest propensity and disorderness pertaining to its terminal regions which increases its interaction with proteins and hence exhibits wide range of functions in tissues (89). Sirtuin1 being a nuclear protein acts during inflammatory responses. Recent findings suggests that sirtuin1 (sirt1) shuttles between nucleus and cytoplasm during oxidative stress (116). Sirtuin1 also has major role in diabetes and lipid metabolism (114). This has created interest to further do research on serum sirtuin1 concentration in human subjects in type 2 diabetes mellitus.

Probable Outcomes of the study

Since fluoride concentration is increasing day on day in drinking water of Kolar district, along with incidence of Type 2 DM and other aging disorders this study was planned as a preliminary trial.

In this population, correlation studies on fluoride with insulin and diabetes have not been undertaken till date.

Our study may help in early detection of diabetes and prevention of the same if association of fluoride and sirtuin1 in biological sample is established when the patient reports to the hospital. If the framed hypothesis is right then Fluoride can be considered as enhancer molecule of diabetes and sirtuin1 can be considered as a counter action molecule for Fluorosis. This may help in therapeutic usage of sirtuin1 in aging disorder, as it is considered as an anti- aging protein.

OBJECTIVES

Research question

1. Will there be any alteration in serum sirtuin1 in diabetes and diabetic nephropathy? To find if there is any association with fluoride levels.
2. Can plasma Sirtuin1 values alter Carboxy methyl lysine and Serum Fluoride values?
3. Does Sirtuin1 affect diabetic profile parameters in cases and how?

Objectives of the study

1. To find the correlation between fluoride and sirtuin1 in blood and urine of patients with diabetic nephropathy and correlate it to extent of kidney damage
2. To compare Carboxy methyl lysine (CML) and sirtuin1 levels in blood between groups
3. Finding Correlation between insulin resistance and sensitivity with special parameters among groups

METHODOLOGY

i. **Study Design**: The present study is a comparative cross- sectional study with three groups; Group 1, Group 2 and Group 3

All the parameters in methodology section will be compared between the three groups and also within the group.

ii. Study area

Type 2 diabetic patients attending OPD, department of general medicine and diabetology were recruited for the study after confirming inclusion and exclusion criteria.

All the study subjects are residents of Kolar districtfor a minimum period of three years.

Informed written consent was obtained from all study subjects.

Age and gender matched non diabetics and healthy subjects of same area were included as controls.

iii. Duration of the study: 3years (2017- 2020)

iv. Material and methods

Sample Size Calculation

Mean difference of SIRT1 is calculated with 80% power and 95% confidence interval, thus minimum sample size derived was 70 per group [117].

Formula for comparing means:

$$\mathbf{N= [2S_p^2(Z_{1-\alpha/2}+ Z_{1-\beta})^2] /\mu_d^2}$$

$S_p^2=(S_1^2 + S_2^2)/2$

S_1^2: Standard Deviation (SD) in first group

S_2^2: SD in second group

μ_d^2: Mean differences between samples

α: Level of Significance $1-\beta$: Power

Study Groups

Group1 (n=70): Age and gender matched healthy controls

Group2 (n=70): Diabetic Nephropathy

Group3 (n=70): T2DM without nephropathy

Inclusion criteria:

1. Subjects clinically proven with Type 2 DM with or without diabetic nephropathy
2. Subjects living and surviving in the same environment with high fluoride condition

Exclusion criteria

1. Patients with diabetes mellitus not living in Kolar and not exposed to fluoride
2. Patients taking drugs or other factors known to cause diabetes and/ or diabetic nephropathy
3. Patients undergoing renal dialysis
4. Acute kidney injury due to any cause and other renal pathologies
5. Patients with other type of diabetes

v. Statistical analysis: SPSS version 20 (IBM) was used to perform statistics.

All the variables which are normally distributed (Parametric) were represented as Mean ± SD and those which are non- parametric were represented as Median (25^{th} to 75^{th} percentile)

a. **Parametric variables include:** Age (in years), BMI (kg/m^2), BP (mmHg), FBS(mg/dL), PPBS (mg/dL), HbA1c (%), Blood urea (mg/dL), Serum creatinine (mg/dL), Albumin (g/dL), Sodium (mEq/L), Potassium (mEq/L), Total Cholesterol (mg/dL), HDL (mg/dL), nHDL (mg/dL), Insulin Sensitivity (QUICKI).

Parametric tests

- Descriptive statistics: Calculating mean ± standard deviation (mean ± SD) for normally distributed data

- **An**alysis **O**f **Va**riance (ANOVA): To find asymptotic significance by calculating probability-value (p- value) between all 3 groups
- Post- Hoc test: To find where exactly the difference in mean lies between two groups and the significance is calculated by Tukey's test
- Pearson's correlation (r): To find the trend between two variables (either positively correlated or negatively correlated**)**

b. **Non- Parametric variables include:** Uric acid (mg/dL), Triglycerides (mg/dL), LDL (mg/dL), VLDL (mg/dL), Sirtuin1 (ng/mL), Carboxymethyl Lysine (ng/mL), Fructosamine (ng/mL), Cystatin C (mg/L), Serum fluoride (ppm), Urine F (ppm), eGFR of creatinine (ml/min/1.73m^2), eGFR of Cystatin C (ml/min/1.73m^2), Fasting Insulin (IU/L) and Insulin Resistance (HOMA- IR).

Non- Parametric tests

- Descriptive statistics: Based on the frequency of data distribution, the values were divided into quartiles with 25th percentile and 75th percentile
- Kruskal- Walli's: To derive the p- value of non parametric data across all three groups. Mann-Whitney U and Tukey HSD test to find significance between groups
- Spearmann's Rho (ρ): To find whether the parameters are negatively or positively correlated

c. Normality of distribution of variables was assessed by Kolmogorov- Smirnov test.
p- value is < 0.05 indicates the variable in not normally distributed. If the p-value is >0.05 it is considered that the variables are normally distributed in the Gaussian curve.

Sample collection

After explaining the whole procedure in the patient's understandable language and providing him/her with the patient information sheet, informed written consent complying with the

Declaration of Helsinki2012 was obtained from the study subjects. The present study was approved by **Central Ethics Committee vide No: SDUAHER/KLR/CEC/35/ 2018- 19 dated: 14- 05- 2018.**

Patients who agreed to participate in the study and complied with the inclusion criteria were included in the study. Under Strict aseptic precautions, study subjects were allowed to be seated in comfortable position. After confirming 8 hours of fasting the blood sample was collected. Fasting sample was split into parts for biochemical analysis. For plasma glucose sample was segregated in NaF tube. For HbA1c, whole blood in EDTA tube. For routine biochemical parameters and research molecules serum sample was collected in Plain tube. Two hours post-prandial blood sample was collected. Corresponding urine sample was also collected from the study subjects for urine fluoride analysis.

All samples were properly labeled, analyzed and stored at appropriate conditions till further analysis. Quality assurance was carried out as per the criteria laid down in **Clinical Diagnostic Laboratory Services (CDLS)** of RL Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medicla College, a constituent of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India.

Methodology

All the routine investigations were carried out by fully automated Vitro 5, 1 FS, a fusion analyzer. Fasting insulin was analyzed by Vitro eCI based on the principle of electro chemiluminescence. Glycated Hemoglobin was estimated by BioRad D10 based on the principle of HPLC at Biochemistry section of CDLS.

Manual parameters were analyzed at the Research Laboratory of the Department of Biochemistry. Blood pressure was measured before fasting blood collection by using mercury

sphygmomanometer. Height was measured by manual Stadiometer and weight was recorded from digital weighing scale to calculate BMI (kg/m^2).

Fluoride triple test was performed, which includes (39):

a. Coin test
b. Hands on Occiput and
c. Chin to chest test was performed by study subjects to diagnose for any pain or skeletal disability due to fluoride exposure and accumulation

Following are the parameters and methodology

Sl. No.	Parameter	Methodology
1	Serum Sirtuin1	ELISA
2	Serum and Urine Fluoride	Fluoride ISE
3	Serum Carboxy Methyl Lysine (CML)	ELISA
4	Plasma Glucose (mg/dl)	Vitros 5, 1 Fs
5	Glycated Hemoglobin (HbA1c %)	BioRad D10
6	Serum Insulin (μIU/mL)	Vitros eCI
7	Serum Fructosamine (μmol/L)	ELISA
8	Serum Urea (mg/dL)	Vitros 5, 1 Fs
9	Serum Uric acid (mg/dL)	Vitros 5, 1 Fs
10	Serum Creatinine (mg/dL)	Vitros 5, 1 Fs
11	Serum Cystatin C (mg/L)	Mispa i2 (semi- auto)
12	Serum Sodium (meq/L)	Vitros 5, 1 Fs
13	Serum Potassium (meq/L)	Vitros 5, 1 Fs
14	Serum Triglyceride (mg/dL)	Vitros 5, 1 Fs
15	Serum Total Cholesterol (mg/dL)	Vitros 5, 1 Fs
16	Serum High Density Lipoprotein(mg/dL)	Vitros 5, 1 Fs
17	Serum Albumin (mg/dL)	Vitros 5, 1 Fs

ELISA: Enzyme Linked Immuno Sorbent Assay, ISE: Ion- Selective Electrode

Sandwich Enzyme Linked Immuno Sorbent Assay (ELISA)

Sandwich ELISA is preferred over other types since it do not need sample purification before processing. There is usage of two types of antibodies (capture and detecting antibodies) for one type of antigen. These antibodies make sandwich ELISA a precise method to measure antigen in complex mixture such as serum, urine and other body fluids. The only drawback is, it demands high detecting antibodies for the epitope for newly discovered antigens.

Principle: Human serum containing the analyte was compared with the purified recombinant standards provided with the kit. Samples were added to the microtitre wells coated with immobilized antibody. Addition of HRP conjugate with Antibody- Antigen- Enzyme- antibody complex, addition of colouring substrate (TMB) and later the stop solution will develop yellow colour. The colour is read spectrophotometrically to measure the concentration of analyte in human sample. Intensity of the colour is directly proportional to the concentration of analyte in the sample.

Procedure

1. **Preparation of Standard Wells**

   A set of 10 wells, from well number 1 to 10 are labeled as standards.

   The replicates of standards and the mean of the replicates were plotted in a standard linear graph.

   Steps in preparation of standard wells:

   Step1: Wells lebelled 1 and 2, standard solution of 100 µL and standard diluent 50 µL were added

   Step2: Wells labelled 3 and 4, solution mixture of 100 µL from wells 1 and 2 were added respectively. Standard diluent of 50 µL was added to each well. Solution mixture of 50 µL was discarded to maintain a uniform volume of 50µL in each well

Step3: Wells labelled 5 and 6 were added with 100 µL of solution mixture pipetted from wells 3 and 4 respectively. Standard diluent of 50 µL was added to each well

Step4: Wells labelled 7 and 8 were added with 100 µL of solution mixture taken from wells 5 and 6 respectively. Standard diluent of 50 µL was added to each well

Step5: Wells labelled 9 and 10 were added with 100 µL of solution mixture taken from wells 7 and 8 respectively. Standard diluent of 50 µL was added to each well. Solution mixture of 50 µL was discarded to maintain a uniform volume of 50 µL in all the wells.

2. **Well labeled 11 was set as blank** for colour comparison with standards and tests. Take precaution NOT to add Horse Radish Peroxidase (HRP) as it is a colour developing enzyme and may lead to analytical bias
3. **Sample Addition**

   Sample diluent of 40 µL was added to all the test wells numbered 12 to 96.

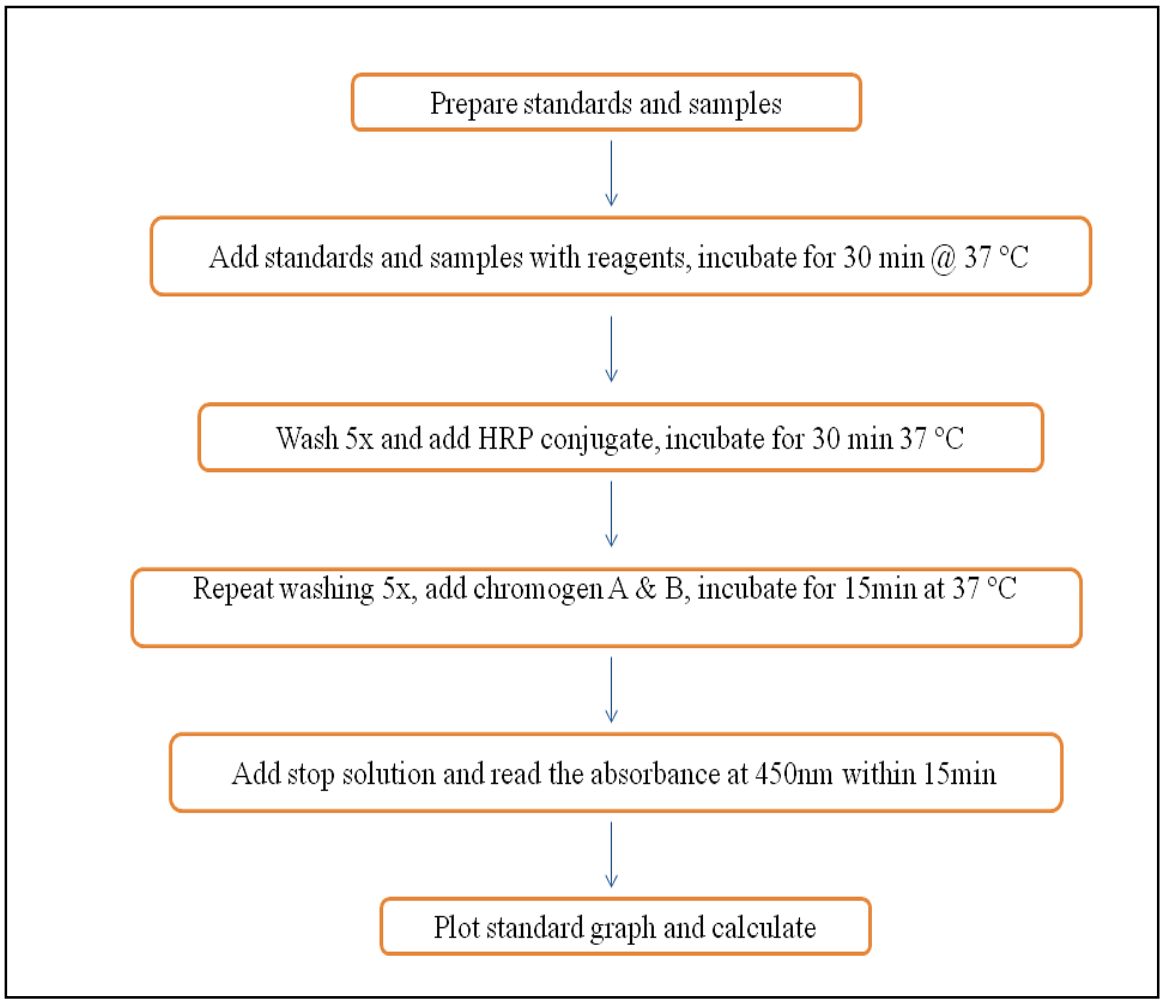
   Test samples of 10 µL of the study subjects were added from 12^{th} well onwards till 96^{th} well.

   Add the samples without touching sides of the wells using fresh TIPS for each sample with gentle mixing.

   This solution mixture was incubated for 30 minutes at 37 ºC.
4. **Washing:** 20 mL of 30x wash buffer (Tris- HCl) was prepared by making its volume to 600 mL with double distilled water. Automated ELISA plate washer was used to wash the plates for **5 consecutive times** with 30 seconds incubation. Each time after wash the plate was drained thoroughly by dry patting.
5. HRP conjugate of 50 µL was added to each well **except the blank well** and incubated for 30 minutes at 37 ºC.

6. Washing was repeated as done before with same wash buffer.
7. **Colour development and stop:** TMB chromogen substrate A and B were added to all the wells one after the other with gentle mixing which gives yellow colour and incubated in dark for 15min at 37 ºC. Stop solution was added after 15min incubation which turns the titre into blue colour.
8. **Assay:** Blank well was set to zero and the absorbance was read at 450 nm within 15min of addition of stop solution. Colour intensity is directly proportional to the concentration of the solution.
9. **Calculation:** Standard concentration was plotted in X- axis (horizontal line) and OD value was plotted along the Y- axis (Vertical line). A standard graph was generated as a straight line. The OD values were substituted in the equation to find the concentration of substance.

Flow chart of assay procedure



ESTIMATION OF ANTI- AGING AND REGULATORY PROTEIN; SIRTUIN1 (115)

Sirtuin1 a regulatory protein of Histone Deacetylase is localized in nucleus and cytoplasm. It is an Ubiquitous molecule found in all type of cells. Sirt1 is estimated by polyclonal antibodies coated wells of sandwich ELISA. Circulating sirtuins play a major role in preventing vascular damage caused by oxidative stress. Sirtuin1 is measured with kit procured from Sincere Biotech. A standard graph was plotted. The concentrations of the standards were labeled on the x- axis and corresponding OD values on the y- axis.

The concentration of standards was extrapolated on the standard graph line which was derived by using the equation y= mx+ c

Method: Double antibody sandwich ELISA with wavelength λ_{max} = 450 nm

Catalogue Number: E13651608 (Type II)

Interpretation: Concentration of human Sirtuin1 is expressed in ng/ml

Detection range (ng/mL): 0.65- 50

Minimum detection limit (ng/mL): 0.65

Intra- assay precision: ≤9%

Inter- assay precision: ≤15%

Storage specifications:

- Reagent: -20˚C upto ≤ 12 months
- Serum sample: -20˚C for 1- 3 months

  -80˚C for 3- 6 months

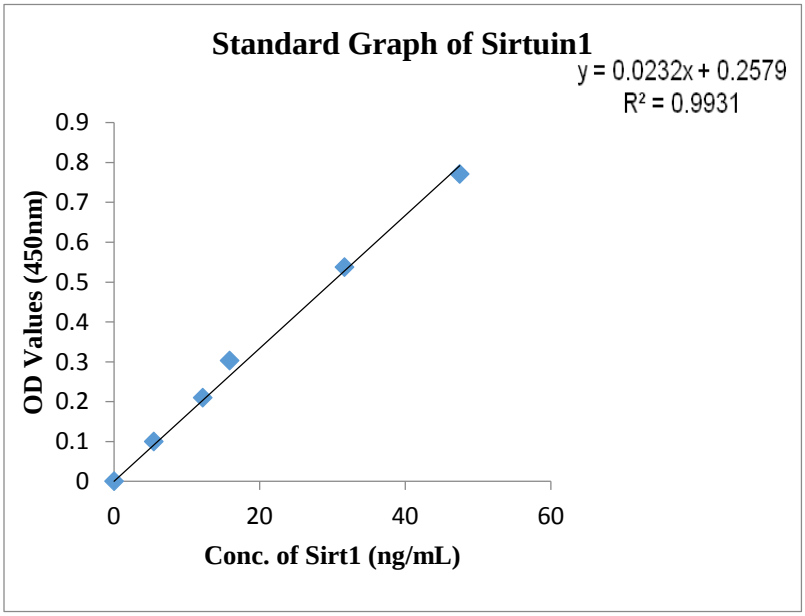
Reference range: Serum values are impended for humans

Standard curve was plotted against OD values in y- axis versus serial standards from S0 to S5. Concentration of Sirtuin1 present in test samples was calculated by using formula: y = 0.0232x + 0.2579 (R^2 = 0.993)

Table 1: Concentration and optical density of Sirtuin1

Concentration of standards increase exponentially in with a standard base limit of 4 ng/mL and there on in multiples upto 32ng/mL restricted to 48ng/mL in view of Job's effect after this concentration

Sl. No	Standard Concentration (ng/mL)	OD value at 450nm	Obtained Concentration (ng/mL)
S1	48	0.771	49.22
S2	32	0.537	29.47
S3	16	0.303	17.11
S4	8	0.21	8.15
S5	4	0.1	5.45
S0	Blank	0	0



Graph 1: Standard graph for Sirtuin1

Carboxy Methyl Lysine (CML) (118)

Standard curve for CML was plotted against OD values in y- axis versus serial standards. Concentration of CML present in test was calculated by using formula:

$y = 0.0001x + 0.024$ ($R^2 = 0.976$)

Method: Sandwich ELISA. Color change with wavelength λ_{max}= 450nm

Catalogue Number: E13651946 (Type II)

Storage

- Reagents: 4 ˚C if unused for ≤ 6 months
- Samples: -20 ˚C till analysis

Detection range (ng/ mL): 64.5- 5000

Minimum detection limit (ng/ mL): 64.5

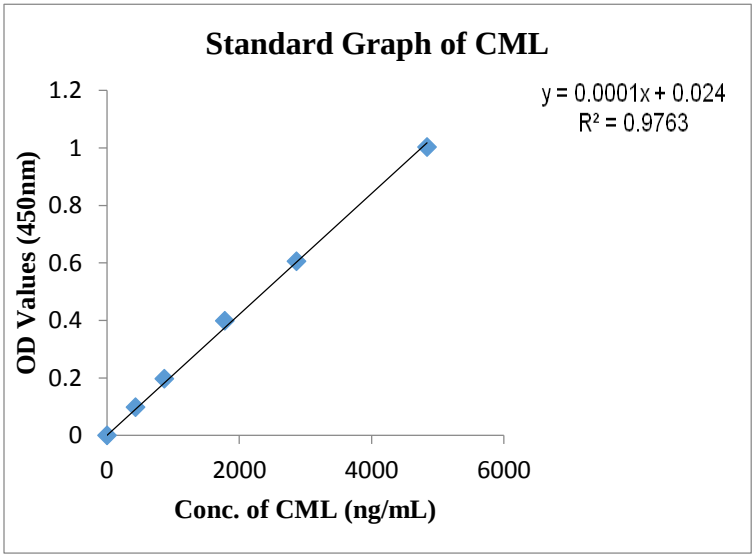
Intra- assay precision: ≤9%

Inter- assay precision: ≤15%

Reference Range: Serum values are impended for humans

Table 2: Concentration and optical density of Carboxy Methyl Lysine (CML) Concentration of standards increase exponentially with a standard base limit of 400 ng/mL and there on in multiples upto 3200 ng/mL restricted to 4800 ng/mL in view of Job's effect after this concentration.

Sl. No	Standard Concentration (ng/mL)	OD value at 450nm	Obtained Concentration (ng/mL)
S1	4800	1.003	4838.25
S2	3200	0.606	2865.75
S3	1600	0.399	1778.25
S4	800	0.198	865.75
S5	400	0.098	430.75
S0	Blank	0	0



Graph 2: Standard graph for Carboxy Methyl Lysine (CML)

Fructosamine (119)

Standard curve for fructosamine was plotted against OD values in y- axis versus serial standards. Concentration of fructosamine present in test samples was calculated by using formula: $y = 0.0081x + 0.2356$ ($R^2 = 0.9881$)

Method: Sandwich ELISA, change with λ_{max}= 450nm.

Catalogue number: QY-E01291

Detection Range (ng/mL): 7.8- 500

Detection Limit (ng/mL): 7.8

Reference Range (ng/mL): 100- 285

Intra- assay precision: ≤9%

Inter- assay precision: ≤15%

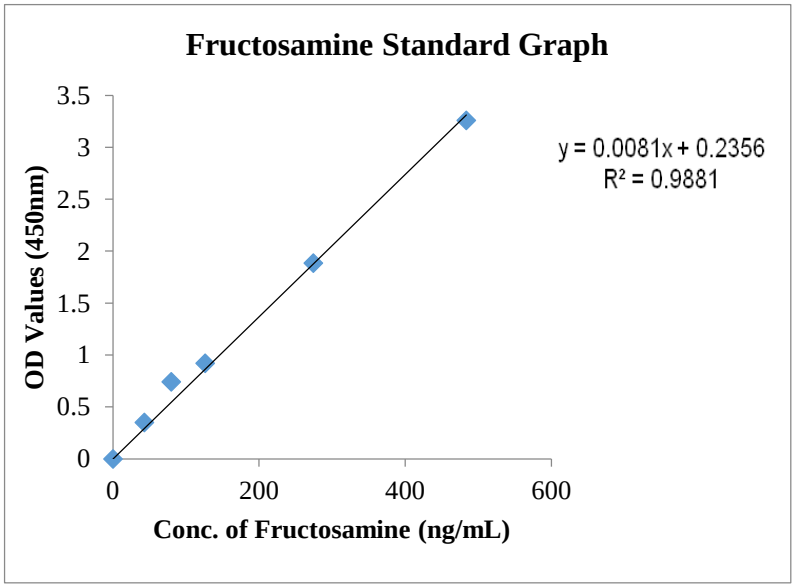
Storage

- Reagents: 2- 8˚C until expiry
- Samples: - 20 ˚C till analysis

Table 3: Concentration and optical density of Fructosamine

Concentration of standards increase exponentially in with a standard base limit of 31.2 ng/mL and restricted to 500 ng/mL.

Sl. No	Standard Concentration (ng/mL)	OD value at 450nm	Obtained Concentration (ng/mL)
S1	500	3.2575	483.75
S2	250	1.8835	274.36
S3	125	0.92	126.25
S4	62.5	0.741	79.85
S5	31.2	0.633	49.06
S0	Blank	0	0



Graph 3: Standard graph for Fructosamine

Fluoride Ion Selective Electrode (ISE) (120)

Principle: Sensing element or electrode membrane (internal electrode) is made of Lanthanum Fluoride bored with Europium Fluoride plays a role in forming perforations in crystal lattice. In liquid medium, forms passage of Fluoride for reaction. Filling solution (external electrode) constituting of AgCl or KCl compensates the F passage by maintaining equilibrium of anions during reaction. Filling solution conductivity of F ions is read on voltmeter through silver wire connected. This forms the basis for **Potentiometric measurement of ions**.

Standards: Fluoride standard of 100ppm and 1000ppm is commercially available from which desirable fluoride standards can be prepared. Also, fluoride (F) standards of 1ppm, 2ppm, 10ppm and 100ppm combined with Total Ionic Strength Adjusting Buffer II and III which are supplied by the manufacturers. .

TISAB II is used for analysis of serum and urine sample in 1:1 dilution (sample: buffer)

TISAB III is used for water analysis in 9:1 dilution (sample: buffer)

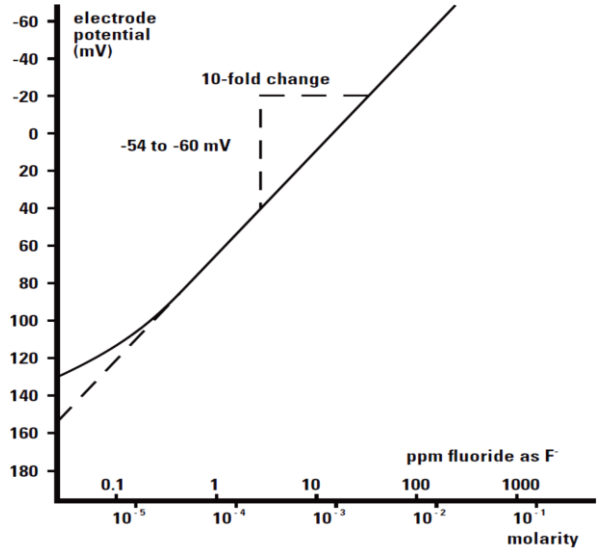
Calibration

Calibration of fluoride analyzer by;

1. Direct calibration
2. Serial Dilution
3. Calibration with only fluoride standard (Sodium Fluoride)
4. Direct calibration using ISE instrument

For our study, the instrument was calibrated by direct calibration using ISE instrument. Since the measured sample in the study is serum and urine, calibration with TISAB II was carried out. A 10mL fluoride standard with TISAB II, was added to a polyethylene beaker and measured its concentration at -54 to -64 mv slope. If the displayed value is ±1 ppm to the original value of

standard, the instrument is calibrated. If the values are away from the actual standard; set up must be changed to the desired standard value keeping the slope as the same. Calibration curve is plotted in the system generated automatically with voltage reading in millivolts versus Fluoride in ppm. Only 2 coinciding standard concentrations are sufficient to prove the linearity for calibration.



Typical direct calibration curve

Procedure:

1. Before setting up instrument, unscrew the electrode to expose the internal electrode. The electrode must be rinsed with deionised water and dry with soft tissue paper (do not touch with bear hand)
2. After washing and drying, screw it tightly; carefully add filling solution (external electrode) from the hole given till 2cm below the filling hole
3. Immerse the sensing element such that a minimum of a centimeter is into the test solution contained in polyethylene beaker
4. Wait for the stable reading, once **'ready'** you can note the value of fluoride concentration

5. Again, rinse the electrode with distilled water and wipe sensing element with filter paper carefully do as to remove any particle or test solution adhered
6. Repeat the same procedure for all the samples.

Method: Fluoride Ion Selective electrode (ISE)

Instrumentation: Thermo- Scientific Orion

Units of Measurement: Expressed as ppm or mole per liter or mg/L

Standards of different concentrations are provided by manufacturers were used

Detection limit: 0.02 ppm

Permissible limit: 1- 1.5ppm

Quality compared based on the provided standards

Reference range (67)

- Serum: 0.02- 1.65 mg/L
- Urine: 10 mg/L [203]

Cystatin C (Cys C) (121)

Method: Nephelometry and Photometry

Instrumentation: Agappe Mispa i2 Bench top semi automatedanalyzer

Principle of the instrument

Nephelometry is the measure of turbidity of a solution due to immune particles agglutination.

Photometry is the measure of absorbance of light passing through varying colored solutions

Principle of the Test

Cystatin C in the test sample binds to the specific polyclonal rabbit anti- Cystatin C antibody which had been adsorbed on to latex particle and agglutinates. The extent of agglutination is directly proportional to concentration of Cystatin C in sample.

The color development and the intensity of the color is measured at λ_{max} = 650nm

Procedure:

1. Mispa i2 is manufactured by Agappe as bench top, bed side semi- auto analyzer
2. Switch on the instrument before 10minutes of usage
3. The analyzer is programmed by inserting a smart card for Cys C (Cystatin C) specifications. Smart card was inserted in the slot provided in the instrument
4. Add 200 µL of R1 into civette containing tris buffer. Maintain pH of the mixture at 8.5 ± 0.3
5. Add 5 µL of test sample along the sides of cuvette to prevent bubbles
6. Place the cuvette in slot provided in the instrument.
7. After 250s of incubation, instrument will prompt you to add R2 which contains polystyrene latex particle coated with polyclonal anti Cys C antibody
8. Using the sensor pipette attached to the instrument, 40 of µL R2 was added to the cuvette
9. Result will be displayed in mg/L and printed by internal printer

Calibration: Smart card is incorporated with all calibration details required for the test

Quality check could be done by

1. Agappe Cys C control (Product No. 11634001)
2. Known concentration of standard solution
3. Split sample analysis
4. Serial dilution
5. Pooled sample
6. Recheck, repeat of already analyzed samples

However, in our study we used the Agappe control standards.

Product code: 12009021

Detection range (mg/L): 0-10

Detection limit (mg/ L): 0.1

Storage:

- Serum: ≤ -20 ˚C till analysis
- Reagents: -4 ˚C till expiry of kit

Quality control (QC) and quality assurance (QA):

Sirtuin 1, fluoride, carboxy methyl lysine, Fructosamine and Cystatin C which are newer parameters we followed the protocol:

I. The performance verification certificate was considered from the kit manufacturer and confirmed the minimum and maximum detectable range following the dilution protocol as per the kit insert
II. Specific standards by manufacturers were used to maintain quality for fluoride analysis

Glucose (121)

Method: Glucose Oxidase Peroxidase (GOD-POD) enzymatic method.

Measured at wavelength λ_{max}=540nm

Instrumentation: Vitros 5, 1 FS

Minimum detection limit (mg/dL)

- Serum: 20- 625
- Urine: 20- 650

Reference range (mg/dL)

- Serum: Fasting blood sugar: 80- 110

  Post- prandial blood sugar: 100- 140

  Random blood sugar: 90- 180

- Urine: ≤ 30

Storage:

- Reagents: ≤ -18 ˚C until expiry

  Onboard: ≤ 1 week

- Sample: ≤ -18 ˚C upto 1 year

Glycosylated Hemoglobin (HbA1c %) (122)

Method: High Pressure Liquid Chromatography (HPLC)

Instrumentation: Bio Rad D10

Units of measurements:

- **National Glycohemoglobin Standardization Program (NGSP) units**

  %A1c = (IFCC × 0.09148) + 2.152

- **International Federation of Clinical Chemistry (IFCC) Units**

  HbA1c (mmol/mol) = {HbA1c [g/dL]/ Hb [g/dL]} × 1000

Detection range (%): 2- 25

Minimum Detection limit (%): 2

Reference Range (%)

- 6.1- 6.4 Pre- diabetic
- ≥6.5% are considered diabetic

Storage

- Reagent: Onboard ≤ 28 days

  Unopened 2-8 ˚C until expiry

- Sample: 2-28 ˚ C ≤ 3 days

Insulin (123)

Method: Immunometric Immunoassay

Instrumentation: Vitros enhanced Chemiluminescence (eCI)

Storage

- Reagent: Onboard stability: ≤ 12 weeks,

  2- 8 ˚C till expiry

- Samples: -20 ˚C upto 4weeks

Detection Range (µIU/ mL): 1- 300

Reference Range (µIU/ mL): < 25

Urea (124)

Method: Urease enzymatic method. Measured at wavelength λ_{max} = 670nm

Instrumentation: Vitro 5, 1 FS

Detection range (mg/ dL)

Serum: 4.29- 257.4

Reference range (mg/dL)

- Serum- Male: 19- 43

  Female: 15- 36

Storage

- Reagents: Onboard: ≤ 2weeks,

  ≤ -18 ˚ C until expiry

- Serum: -18 ˚ C upto 6 months

Creatinine (125)

Method: Enzymatic Sarcosine Oxidase.

Measured at wavelength λ_{max} = 670 nm

Instrumentation: Vitro 5, 1 FS

Detection range (mg/dL)

- Serum: 0.05- 140
- Urine: 1.2- 46.5 *

* after multiplying with dilution factor X 21

Reference range (mg/dL)

- Serum

Female: 0.66- 1.25

Male: 0.52- 1.04

- Urine* (mg/day)

Male: 1000- 2000

Female: 800- 1800

* creatinine concentration x 24hrs

Storage

- Reagent: Onboard: ≤ 2 weeks

  ≤ -18 ˚C until expiry

- Sample: ≤ -18 ˚C till analysis

Uric Acid (126)

Method: Uricase enzymatic method

Measured at wavelength λ_{max} = 670nm

Instrumentation: Vitro 5, 1 FS

Detection range (mg/dL)

Serum: 0.50- 17

Reference range (mg/dL)

- Male: 3.5- 8.5
- Female: 2.5- 6.2

Storage:

- Reagents: Onboard stability ≤ 2 weeks

  ≤ -18˚C for ≤ 6 months
- Sample: 18- 28 ˚C ≤ 3 days. Freezing not recommended

Serum Albumin (127)

Method: Turbidmetric immunoassay

Measured at wavelength λ_{max}= 540nm

Instrumentation: Vitro 5, 1 FS

Detection Range (g/dL): 1- 6

Reference range (g/dL): 4.0- 5.0

Storage:

- Reagent: ≤ -18 ˚C until expiry

  On board ≤ 1 week
- Sample: ≤ -18 ˚C upto 3 weeks

Sodium (128)

Method: Potentiometric measure by Ion Selective Electrode

Instrumentation: Vitro 5, 1 FS

Storage

- Reagent: ≤ -18 ˚C until expiry

  Onboard ≤ 10 days

- Sample: ≤ -18 ˚C upto 6 months

Detection range (mEq/ L): Serum: 75- 250

Reference range (mEq/L): Serum: 135- 145

Potassium (128)

Method: Potentiometric measure by Ion Selective Electrode

Instrumentation: Vitro 5, 1 FS

Detection range (mEq/ L): Serum: 1-14

Reference range (mEq/L): Serum: 3.5- 5.1

Storage

- Reagent: ≤ -18 ˚C until expiry

  Onboard: ≤ 2 weeks

- Sample: ≤ -18 ˚C upto 1 year

Total Cholesterol (129)

Method: Cholesterol oxidase enzymatic method

Measured at wavelength λ_{max}=540nm

Instrumentation: Vitro 5, 1 FS

Detection range (mg/dL): Serum: 50- 325

Reference range (mg/dL):

- Desirable: <200
- Borderline: 200- 239
- High: >240

Storage

- Reagent: ≤ -18 ˚C until expiry

  Onboard: 2 weeks

- Sample: ≤ -18 ˚C for ≤ 3 weeks

Triglyceride (130)

Method: Lipase hydrolysis enzymatic method.

Measured at λ_{max} = 540nm

Instrumentation: Vitro 5, 1 FS

Detection range (mg/dL): Serum: 10- 525

Reference range (mg/dL)

- Normal: ≤150
- Borderline: 150-199
- High: 200-499

Storage:

- Reagent: ≤ -18 ˚C until expiry

  Onboard: ≤ 1 week
- Sample: ≤ -18 ˚C for 6 months

Direct HDL (131)

Method: Cholesterol ester hydrolase enzymatic method.

Detected at wavelength λ_{max}=670nm

Instrumentaion: Vitro 5, 1 FS

Detection range (mg/dL): Serum: 5- 110

Reference range mg/dL

- Low: ≤ 40
- High: ≥ 60

Storage:

- Reagent: ≤ -18 ˚C until expiry

  Onboard ≤ 1 week

- Sample: ≤ -10 ˚C

Quality Assurance Scheme

- For routine diagnostic parameters both level 1 and level 2 internal quality assurance scheme (IQAS) supplied by Bio Rad USA is used on daily basis
- Level 1 is physiological range and level 2 is pathological range
- External quality assurance scheme (EQAS) samples were run procured from Bio Rad USA and the values were sent for proficiency testing (PT) and quality confirmed from PT provider (Bio Rad USA) was considered in our study
- The HbA1c PT provider is Bio Rad USA for both internal and external quality
- Three levels of quality testing were run for low range, physiological range and high range values.

CALCULATED PARAMETERS

Estimated Glomerular Filteration Rate (eGFR), Low Density lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), non- High Density Lipoprotein (nHDL), Homeostasis model assessment- estimated insulin resistance (HOMA-IR) and Quantitative Insulin- Sensitivity Check Index (QUICKI) were calculated considering their limitations.

i. **eGFR 2009, considering serum creatinine** (132)

To classify and consider diabetics under diabetic nephropathy group, we calculated estimated Glomerular Filtration Rate (eGFR). Serum creatinine and serum Cystatin C values were substituted in equation CKD- EPI 2009 and 2012.

eGFR (2009)= 141 min(SCr/k, 1)α max(SCr/k, 1)-1.209 x 0.993Age [1.018 if female] [1.159 if black], where SCr is serum creatinine (in mg/dl), k is 0.7 for females and 0.9 for males, a is 0.329 for females and 0.411 for males

eGFR 2012, considering serum creatinine and Cystatin C (132)

eGFR (2012)= 135 × min(SCr/K, 1)−α × max(SCr/K, 1)−0601 × min(SCysC/0.8, 1)−0375 × max(SCysC/0.8, 1)− 0.711 × 0.995age [× 0.969 if female] [× 1.08 if black]If female: K = 0.7, α =−0.248 If male: K = 0.9, α =−0.207

ii. LDL: **Friedewald Equation** (133)

Low Density Lipoprotein was calculated using Total Cholesterol (TC), High Density Lipoprotein (HDL) and Triglycerides (TG) derived. Limitation; total cholesterol value >400 mg/dL direct LDL was analyzed by fully automated Vitros 5, 1 FS

LDL= {[(Total cholesterol) – (HDL cholesterol)] – Triglyceride}/5

iii. Very Low density Lipoprotein (VLDL) (133)

Very Low Density Lipoprotein was calculated by dividing triglyceride by 5

VLDL= TG/ 5

iv. Non- HDL (nHDL) (134)

Non HDL is now gaining importance against VLDL and HDL since it has a major role in assessing lipid accumulation in vascular diseases

nHDLc = Total Cholesterol- HDL

v. Insulin resistance (IR) and Insulin sensitivity (IS) was calculated to assess functioning of target cells against secreted insulin.

Based on HOMA and QUICKI values treatment and administration of Insulin dosage can be decided. They are calculated using fasting glucose and insulin. In addition to diabetes such as, metabolic syndrome, obesity, hyperlipidemia, polycystic Ovarian Disease etc can be staged with their values. Therefore we considered to calculate HOMA IR and QUICKI

HOMA IR (Homeostasis model assessment-estimated insulin resistance) (135)

(Fasting plasma insulin x fasting blood sugar) /405

QUICKI (Quantitative Insulin-Sensitivity Check Index) (136)

1/[log (Insulin μU/mL) + log (Glucose mg/dL)

vi. **Mean Arterial Pressure (MAP)**: Average measure of blood pressure throughout a cardiac cycle considering diastolic and one- third of pulse pressure (SBP- DBP) (137)

MAP (mmHg)= DBP+ 1/3 (PP)

vii. Atherogenic Index (AI) of Plasma in recent days is gaining importance in predicting future complications due to abnormal lipid profile (138)

AI= log10 (TG/ HDL)

Vitros 5,1 FS

Vitros eCI

Flouride ISE

ELISA

BioRad D10

Mispa i2

RESULTS

The results of our study Titled: Estimation of Fluoride and Sirtuin1 in Patients with Diabetic Nephropathy in Kolar District of Karnataka, India are presented here.

Table 4: Demographic data

Parameter	Group1 (n= 70)	Group2 (n= 70)	Group3 (n= 70)	p-value
	Mean ± SD			
Age (in years)	50.71 ± 9.2	56. 04 ± 8.2	53.1 ± 8.2	0.08
SBP (mmHg)	122.1 ± 5.4	137.6 ± 17.6 [a,b*]	125.3 ± 11.02 [a]	**<0.001**
DBP (mmHg)	78.3 ± 4.6	87.53 ± 11.2 [a,b*]	82.23 ± 7.64 [a*]	**<0.001**
BMI (Kg/m^2)	24.1 ± 3.14	22.6 ± 1.6 [a*]	23.2 ± 1.83 [a*]	**<0.001**
MAP (mmHg)	92.8 ± 3.8	104.2 ± 12	95.2 ± 13.8	**<0.001**

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: T2DM
SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure,
BMI: Body Mass Index, MAP: Mean Arterial Pressure
a: comparison with healthy control, b: comparison with type 2 DM, p- values; * : <0.05

Subjects recruited for this study are age and gender matched and hence they are not significant. Group 1 consisted of 31 (44.3%) male, group 2 contained 47 (67%) male and group 3 with 37 (52.8%) male subjects of p- value 0.08 indicating that gender matched subjects were recruited for all the groups. Overall total number of males was 115 among 210 study subjects which constitute around 55%.

Reference range of Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) is between 90- 120 mmHg and 60- 90 mmHg respectively. SBP is significantly increased by 17mmHg of upper limit and 7 units increase in DBP in group 2 cases compared to Groups 1 and 3.

Reference range of BMI is between 18.5- 25 kg/m^2. There was a significant decrease in BMI of cases compared to controls.

Majority of the subjects with diabetic nephropathy are males as evident from the above table. Data indicates a significant difference in blood pressure, Body Mass Index (BMI) and Mean Arterial Pressure (MAP) between groups.

Fluorosis triple test analysis was performed in all 3 study groups and the results are tabulated

Table 5: Fluorosis Triple Test Analysis represented as number of study subjects who could not perform the tests

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: T2DM.

	Non- compliance to Chin- to- chest	**Non- compliance to Hands on occiput**	**Non- compliance to Coin test**	**p- value**
Group1 n= 70	3	2	11	0.832
Group2 n= 52	5	15	38	0.425
Group3 n=70	2	5	31	0.557

If the subjects were able to perform the action they were considered negative (non- compliance) and if not performed they are positive.

To find the compliance of study subjects to tests and to find significance of dependents with serum fluoride, statistical Chi- square test was applied.

The results were coded as zero and 1 for the negative and positive respectively and to test the probability of non- performance of triple test by subjects due to fluoride exposure. It was observed to be statistically not significant across the groups.

In all the groups the basic diabetic and renal parameters were estimated. Values obtained were entered in table 6. All the parameters are represented as Mean ± SD.

Table 6: Basic Diabetic and Renal profile

Parameter	Group1 (n= 70)	Group2 (n= 70)	Group3 (n= 70)	p- value
Basic Diabetic Profile (Mean ± SD)				
FBS (mg/ dL)	93.94 ± 9.4	173.6 ± 64.02 [a*]	182.3 ± 67.7 [a*]	**<0.001**
PPBS (mg/ dL)	115.1 ± 16.2	271.2 ± 91 [a*]	273.1 ± 103 [a*]	**<0.001**
HbA1c (%)	5.5 ± 0.5	8.2 ± 2 [ab*]	9.2 ± 2.4 [a*]	**<0.001**
Basic Renal Profile (Mean ± SD)				
Blood Urea (mg/ dL)	19.6 ± 6.6	69.03 ± 27.5 [ab*]	27.6 ± 13.4 [a*]	**<0.001**
Serum Creatinine (mg/ dL)	0.66 ± 0.1	3.4 ± 1.5 [ab*]	0.68 ± 0.21	**<0.001**
Serum Albumin (g/ dL)	4.05 ± 0.4	2.8 ± 0.8 [ab*]	4.3 ± 0.9	**<0.001**
Sodium (mEq/ L)	137 ± 2.1	133.5 ± 5.1 [ab*]	135.8 ± 3.2	**<0.001**
Potassium (mEq/ L)	4.3 ± 0.41	4.6 ± 0.9 [a*]	4.4 ± 0.5	**0.016**

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: T2DM
FBS: Fasting Blood Sugar, PPBS: Post- prandial Blood Sugar, HbA1c: Glycated Hemoglobin
a: comparison with healthy control, b: comparison with type 2 DM, p- values; * : <0.05

ANOVA (Post- Hoc) test was applied to analyze the statistical significance between the groups and observed an excellent significance indicating a difference in values of primary biomarkers of diabetes and diabetic nephropathy.

Fasting Blood Sugar (FBS), Post- Prandial Blood Sugar (PPBS) and Glycosylated Hemoglobin (HbA1c) were found to be increased in groups 2 & 3 which proved that the study subjects are diabetic. In diabetic nephropathy group, serum urea and serum creatinine are significantly increased; serum albumin is significantly decreased compared to healthy controls and T2DM.

Serum Lipid parameters were estimated in all the groups to find significance between groups and the values are tabulated in table 7.

Table 7: Lipid Profile

Parameter (Reference Range)	**Group1 (n= 70)**	**Group2 (n= 70)**	**Group3 (n= 70)**	**p- value**
	Mean ± SD			
Total Cholesterol (mg/ dL) (120- 200)	171.4 ± 39.1	157.6 ± 59.6 [b*]	183.3 ± 59.6	**0.007**
HDL (mg/ dL) (40- 60)	39.3 ± 10.1	28.3 ± 10.3 [ab*]	40.9 ± 13.7	**<0.001**
nHDL (mg/ dL) (<130)	132.1 ± 39.5	125.7 ± 58.9	145.4 ± 56.8	0.077

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: T2DM
HDL: High Density Lipoprotein, nHDL: non High Density Lipoprotein
a: comparison with healthy control, b: comparison with type 2 DM, p- values; * : <0.05

Except nHDL all other parameters were significant when compared between groups. Most of the lipid parameters were decreased in group 2 which may be due to administration of statins and/ or due to diet restriction.

Reference value of nHDL is <130 mg/dL which is found increased in T2DM than other groups which indicates a higher risk of future complications of diabetes.

Non- HDL (nHDL), a known atherogenic marker, was considered in our study to find any association, which is calculated by substracting HDL from Total Cholesterol. Non- HDL was not observed to be statistically significant. This indicates that nHDL is independent of TC, TG, HDL, LDL and VLDL.

Serum uric acid, triglyceride, LDL and VLDL were not normally distributed and we considered these variables as non- parametric which are represented as median (25^{th} to 75^{th} percentile)

Table 8: Non- Parametric data

Parameter (Reference Range)	**Group 1 (n= 70)**	**Group 2 (n= 70)**	**Group 3 (n= 70)**	**p- value**
	Median (25^{th} to 75^{th} percentile)			
Uric acid (mg/ dL) (2.5- 7.2)	4.4 (3.7- 5.8)	4.0 (2.6- 5.6) [a*]	4.0 (3.07- 5.1) [a*]	**0.023**
TG (mg/ dL) (44- 150)	141 (93- 193.5)	148 (114.5- 211)	184 (119.5- 215.5) [a*]	**0.033**
LDL (mg/ dL) (100- 130)	98 (76.2- 122)	87 (50.7- 110.5)[ab*]	104 (86.8- 127.3)	**0.01**
VLDL (mg/ dL) (2- 38)	28 (17.7- 36.4)	30 (23- 42.2)	36.9 (24- 43.1) [a*]	**0.020**

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: T2DM
TG: Triglycerides, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein
a: comparison with healthy control, b: comparison with type 2 DM, p- values; * : <0.05

Uric acid; a non- protein nitrogenous substance is included as a renal profile parameter and is also considered as an anti- oxidant. Its values are known to be decreased in patients compared to controls.

All the lipid parameters are found significantly decreased in DN than other groups except VLDL which is slightly increased in DN than controls.

Triglyceride (TG) is significantly increased in group 3 compared to group 1

LDL is significantly increased in group 3 when compared with group 2 indicating T2DM patients needs vigilance ove this molecule

For Advanced biomarkers of diabetic nephropathy (DN) and fluorosis we analyzed serum sirtuin1, carboxymethyl lysine (CML), serum fluoride and urine fluoride. The values are emphasized in table 9.

Table 9: Advanced biomarkers of diabetic nephropathy (DN) and fluorosis

Parameters	Group 1 (n=70)	Group 2 (n= 70)	Group 3 (n= 70)	p- value
	Median (25^{th} to 75^{th} percentile)			
Sirtuin1 (ng/mL)	46.76 (12.4- 97)	34.74 (25.08- 53.2) [b*]	49.6 (33.71- 101.63)	**0.002**
Serum Fluoride (mg/L)	0.66 (0.62- 0.72)	0.24 (0.2- 0.5) [a*]	0.6 (0.56- 0.68) [a*]	**<0.001**
Urine Fluoride (mg/L)	0.89 (0.55- 1.49)	0.24 (0.16- 0.41) [ab*]	0.72 (0.53- 1.02)	**<0.001**
CML (ng/mL)	899 (625.25-1306.5)	1815 (1100- 2591.13)[a*]	1870 (1155.1-2272.5)[a*]	**<0.001**

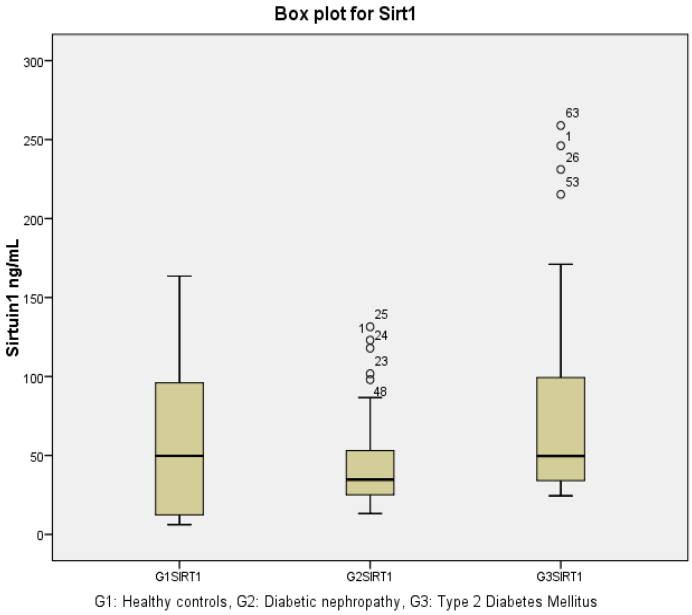
Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: T2DM
ppm: parts per million or mg/L
a: comparison with healthy control, b: comparison with type 2 DM, p- values; * : <0.05

Advanced biomarkers of DN and fluorosis have broad range of detection and were skewed in distribution. The estimated variables in view of skewness were considered as non- parametric and represented them as median (25^{th} to 75^{th} percentile). Reference ranges for the parameters are yet to be defined and need population or multicentric studies across the globe.

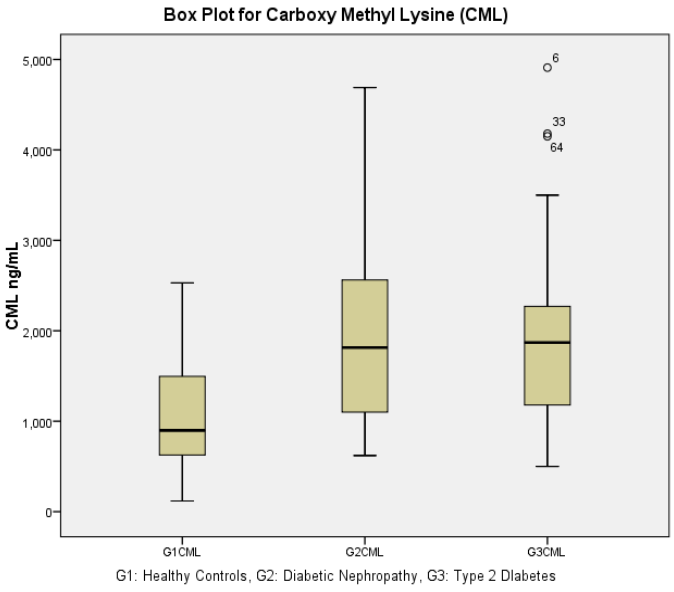
Sirtuin1 is significantly decreased in group 2 compared to group 3. Urine F excretion is decreased in groups 2 and 3 which is a predictor of decline in renal function. There was a drastic decline in levels of all the parameters in group 2 except the CML which is significantly increased in comparison with controls. Significant increase in CML of Group 3 was observed when compared to group 1. Level of significance between groups for all the parameters was fair and excellent

justifying the hypothesis and purpose of the study. The same has been depicted as box whiskers plot [graph 4 to 7].

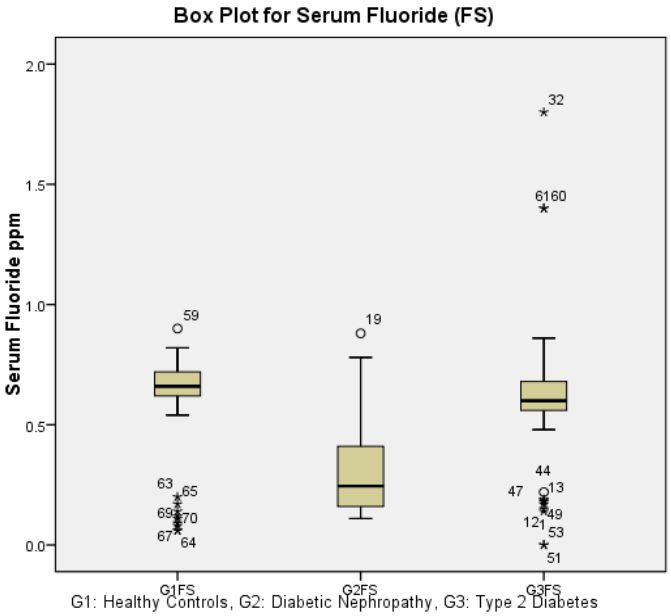
Graphs 4- 7: Box whisker plots of non- parametric variables



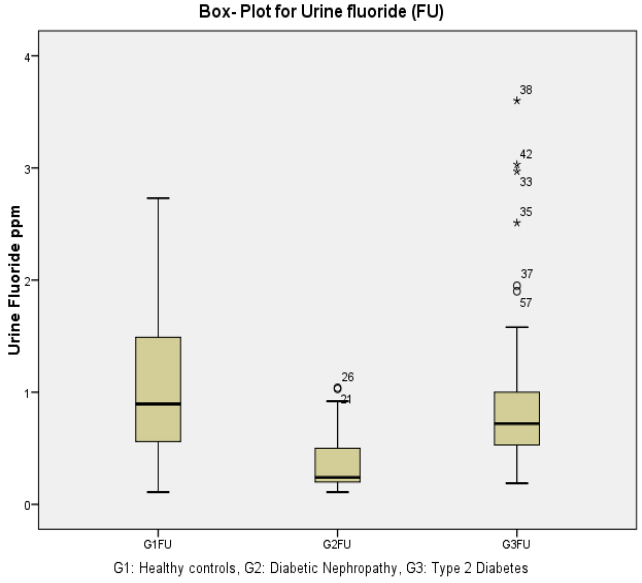
Graph 4: Box plot for sirtuin1



Graph 5: Box plot for CML



Graph 6: Box plot for serum fluoride



Graph 7: Box plot for urine fluoride

Whiskers extend from minimum to maximum values observed in the data. The box measures from 25^{th} to 75^{th} percentile while the line inside it represents the median. Outliers are clearly indicated out of the whiskers with asteriks (*). These graphical representations clearly give the gist of the important result regarding the biomarkers considered essential for our study.

Ratios can help predict future complication of a study subject, more so, with atherosclerosis and DN. To substantiate this, we tabulated the relevant parameters related to DM, DN and fluorosis which is represented in table 10.

Table 10: Ratios of renal, lipid parameters and advanced molecules

Parameter	Group1 (n= 70)	Group2 (n= 70)	Group3 (n= 70)	p- value
	Mean± SEM			
UA: Creatinine	7.4± 0.26	1.6± 0.14	6.6± 0.37	**<0.001**
Cys C: Creatinine	2.5± 0.2	1.6± 0.1	4.4± 0.4	**<0.001**
LDL: HDL	2.6± 0.1	3.3± 0.23	3± 0.16	**0.025**
TG:HDL	4.3± 0.3	6.7± 0.5	4.8± 0.3	**<0.001**
nHDL: HDL	3.6± 0.5	5.1± 0.4	4± 0.2	**<0.001**
Atherogenic Index	0.54± 0.03	0.74± 0.03	0.63± 0.03	**<0.001**
Sirt1: Serum F	192.9± 32.8	198.1± 20.5	193.3± 41.6	0.993
CML: Sirtuin1	57.9± 9.4	58.2± 4.7	40.1± 3.7	0.078

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: Type 2 Diabetes Mellitus
SEM: Standard error of mean UA: Uric Acid, Cys C: Cystatin C, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, nHDL: non High Density Lipoprotein

The data represented in table 10 depicts mean ± standard error of mean (SEM) for the entire population from which the study subjects were recruited.

Lipid ratios in recent days are gaining importance as it can directly correlate the bad with good cholesterol. Ratios of renal, lipid and advanced biomarkers are shown in table 10.

From the previous tables it is evident that lipid parameters of group 2 cases are decreased which is proportional to the increase in ratios. Proportionality check among sirtuin1, serum F and CML were made to find relevance and importance of deriving a ratio to assess aging.

- Since serum creatinine is high in DN patients, the ratios of Uric Acid: Creatinine and Cys C: Creatinine is inversely related to the severity of complication.
- Sirt1: Serum fluoride ratio was almost equal in all the three groups since all were exposed to fluoride, some amount of sirtuin1 action might be there
- CML: Sirtuin1 ratio was decreased in T2DM due to increased sirt1 and CML in T2DM. Since there are no defined or documented values on ratios for these advanced biomarkers viz, sirtuin1, CML and fluoride we have calculated them. These ratios have given insight and clue about these advanced biomarkers in diabetes, diabetic nephropathy and healthy controls. Since there is no document related to reference ranges of advanced biomarkers and requires a large multicentric, population and cohort studies.

Short term glycemic control, insulin estimation and calculation of diabetes indices were considered in our study to find its association with diabetic nephropathy and to correlate. The results are shown in table 11.

Table 11: Extended Diabetic profile

Parameters (Reference range)	**Group 1 (n= 70)**	**Group 2 (n= 70)**	**Group 3 (n= 70)**	**p- value**
	Median (25th to 75th percentile)			
Fructosamine (ng/mL) (yet to be derived)	100.2 (55.8- 172.4)	245.93 (0.16- 0.41)[a*]	329.9 (131.42-88.2)[a*]	**<0.001**
Insulin (µIU/mL) (2.6- 24.9)	9.9 (6.22- 14.4)	8.82 (5.14- 13.32)	9.11 (6.35- 15.62)	0.370
HOMA- IR <3.5 (Decreased IR)	2.34 (1.3- 3.2)	3 (1.99- 5.62) [a*]	4.35 (2.4- 7.1)[a*]	**<0.001**
QUICKI (mean ± SD) >3.2 (Increased IS)	0.34 ± 0.02	0.32 ± 0.03[a*]	0.3 (0.29- 0.34)	**<0.001**

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: Type 2 Diabetes Mellitus
HOMA-IR: Homeostasis Model Assessment- Insulin Resistance, QUICKI: Quantitative Insulin Check Index, IS: Insulin Sensitivity,
a: comparison with healthy control, b: comparison with type 2 DM, p- values; * : <0.05

Due to broad range of values of the variables, these are considered non- normally distributed and represented as median (25th to 75 th percentile).

Fructosamine (Glycated albumin) a marker of short term glycemic control was least in group 1 when compared to other groups 2 and 3. To our surprise we observed that fructosamine was less in diabetic nephropathy compared to T2DM. This gives a foresight that HbA1c is a marker for long term glycemic control and complications of T2DM.

Insulin, a hypoglycemic hormone has a broad reference range and is decreased in group2. Except fasting insulin all the parameters showed a significant difference in their values.

HOMA- IR was significantly increased in groups 2 and 3 when compared to control group indicating an increased insulin resistance. There is a significant decrease in QUICKI of group 2

subjects compared to group 1. Decrease in QUICKI of Group 3 subjects is of concern to prevent diabetic complication in future.

To support the findings derived from our study we analyzed Cystatin C and calculated eGFR 2009 & 2012 considering creatinine standalone and creatinine with cystatin C respectively. The values are documented in table 12.

Table 12: Extended Renal Profile

Parameters (Reference Range)	**Group 1 (n= 70)**	**Group 2 (n= 70)**	**Group 3 (n= 70)**	**p-value**
	Median (25th to 75th percentile)			
Cystatin C (mg/L) (0.5- 2)	0.9 (0.69- 1.2)	4.3 (3- 6.5) [a*]	2.3 (1.46- 3.69)[a*]	**<0.001**
eGFR (ml/min/1.73m^2) Median (25th to 75th percentile)				
CKD- EPI 2009 # (Normal Range: >60 ml/min/1.73 m^2)	109.5(99-116.25)	26(17.75-37.25)[ab*]	106 (93- 115.5)	**<0.001**
**CKD- EPI 2012 $** (Normal Range: >60 ml/min/1.73 m^2)	92.5 (74- 118.5)	60.5 (40- 91)[ab*]	118.5(95.8-37.35)	**<0.001**

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: Type 2 Diabetes Mellitus
CKD- EPI: Chronic Kidney Disease- Epidemiology
#: equation based on creatinine
$: equation based on creatinine and Cystatin C
a: comparison with healthy control, b: comparison with type 2 DM, p- values; * : <0.05

Due to skewness of data, the parameters were represented in median (25th to 75th percentile). Estimated GFR of group 2 is significantly decreased when compared between groups 1 and 3. The observed values predict that considering a unitary molecule creatinine in calculating eGFR and assessment of diabetic nephropathy is better rather than considering both creatinine and cystatin C. Moreover, Cystatin C is increased in DN which is resultant of renal damage. Cystatin C of group 3 is >2 mg/L (0.5- 2.0 mg/L) which is significantly higher than group 1 which is an indication of future renal damage.

Sirtuin1, a regulatory enzyme in protein deacetylation is known to fluctuate both physiologically and pathologically. This made us to find the extent of fluctuation in comparison with serum CML and serum fluoride; for which we correlated using Spearman's rho correlation. The values are tabulated in table 13.

Table 13: Correlation of Serum fluoride, Carboxy Methyl Lysine (CML) and Cystatin C with Sirtuin1

Parameters	**Group 1 (n= 70)**	**Group 2 (n= 70)**	**Group 3 (n= 70)**
	Spearman's Rho (ρ) correlation		
Serum Fluoride (mg/L)	-0.061	-0.292	-0.005
CML (ng/mL)	-0.188	-0.054	-0.153

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: Type 2 Diabetes Mellitus
CML: Carboxy Methyl Lysine

Sirtuin1 is considered as the biomarker of aging and T2DM. Correlation of the causative factors (CML & fluoride) for the fluctuation in sirt1 values are tabulated in table 13. Since the parameters were not normally distributed, spearman's rho correlation was applied and resulted in a negative correlation with a non- significant p- value. Negative correlation implies that an increase in Sirtuin1 may decrease the harmful effect of Fluoride and CML.

Spearman's rho correlation tool was applied to correlate the calculated values across groups 1, 2 and 3 between HOMA- IR, QUICKI and sirtuin1. The values are depicted in table 14.

Table 14: Correlation of HOMA- IR and QUICKI with Sirtuin1

Parameters	Group 1 (n= 70)	Group 2 (n= 70)	Group 3 (n= 70)
	Spearman's Rho (ρ) correlation		
HOMA- IR	-0.028	+0.047	+0.073
QUICKI	+0.098	-0.004	+0.022

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: Type 2 Diabetes Mellitus
HOMA- IR: Homeostasis Model Assessment- Insulin Resistance
QUICKI: Quantitative Insulin Sensitivity Check Index

Sirtuin1 plays a role in stimulation for secretion of insulin from pancreas and regulates insulin reception by target cells. Table 14 portrays the trend between the insulin related factors such as insulin resistance and sensitivity which are calculated parameters.

The values observed in table 14 indicate that HOMA- IR is inversely proportional to sirt1 indicating increase in sirt1 decreases the resistance index. QUICKI is directly proportional to sirtuin1 values indicating that increase in sirt1 increases insulin sensitivity.

Hence, sirtuin1 has a dual role in both diagnosis and prognosis of diabetes and its outcome. In group 2 the values are inversely related to sirt1. In group 3, the sensitivity is being maintained and the resistance is increased, which require further molecular evaluation.

Carboxymethyl Lysine (CML) an Advanced Glycation Endproduct (AGE) is known to increase in diabetes and diabetic nephropathy. This molecule is also documented to get affected by sirtuin1. To prove the documented observations in our population we considered simple linear regression analysis for prediction of CML levels. The findings are documented in table 15.

Table 15: Simple Linear Regression Analysis for predictors of CML

Parameters	Beta 95% CI (Lower, Upper)	p- values
Sirtuin1	-1.555 (-2.942, -0.168)	**0.028**
FBS	2.652 (0.387, 4.921)	**0.022**
HbA1c	42.601 (-21.824, 107.027)	0.194
Fructosamine	0.922 (0.506, 1.339)	**<0.001**

Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: Type 2 Diabetes Mellitus
FBS: Fasting Blood Sugar, HbA1c: Glycated Hemoglobin

Our findings proved that CML is inversely and significantly affected by sirtuin1 which is a positive sign for the proposed research question of our study. On the other hand FBS, HbA1c and fructosamine; diabetic markers are positively correlated which is obvious as CML is an AGE increased during diabetes. All the parameters are significant except HbA1c.

The values of certain biochemical parameters are known to affect the urine fluoride excretion. To prove this we have considered HbA1c as glycemic marker, serum creatinine for renal status, serum sodium and potassium as a part of electrolyte profile. Sirt1 as an advanced biomarker for T2DM and its complication, eGFR was calculated considering standalone serum creatinine and serum fluoride as the study population is fluoride exposed.

The values of simple linear regression analysis for aforesaid parameters are mentioned in table 16.

Table 16: Simple Linear Regression Analysis for predictors of urine F excretion

Parameters	Beta 95% CI (Upper, Lower)	p- value
HbA1c	-0.019 (-0.051, 0.013)	0.251
Serum Creatinine	-0.071 (-0.140, -0.001)	**0.046**
Sodium	0.02 (-0.019, 0.022)	0.877
Potassium	-0.144 (-0.267, 0.021)	**0.022**
Sirtuin1	-0.001 (-0.002, 0.000)	**0.01**
eGFR (2009)	0.004 (0.002, 0.007)	**0.002**
Serum fluoride	0.893 (0.589, 1.197)	**<0.001**

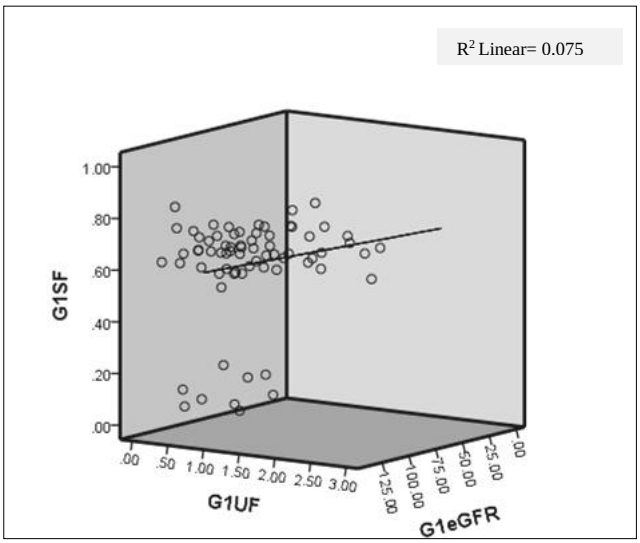
Group1: Healthy control, Group2: Diabetic Nephropathy, Group3: Type 2 Diabetes Mellitus
HbA1c: Glycated Hemoglobin, eGFR (2009): estimated glomerular filteration rate considering creatinine

Fluoride is absorbed by the calcified and mineralized tissues. Its major excretion is through urine. Fluoride is also under research for causing non- skeletal fluorosis such as diabetes, cancer etc. To assess all these probabilities of causation and effects, a linear regression was performed. Except HbA1c and sodium all the parameters were significant. Serum creatinine, potassium and sirt1 had a significant inverse effect on urine fluoride excretion whereas; eGFR and serum fluoride had a significant direct effect on its excretion.

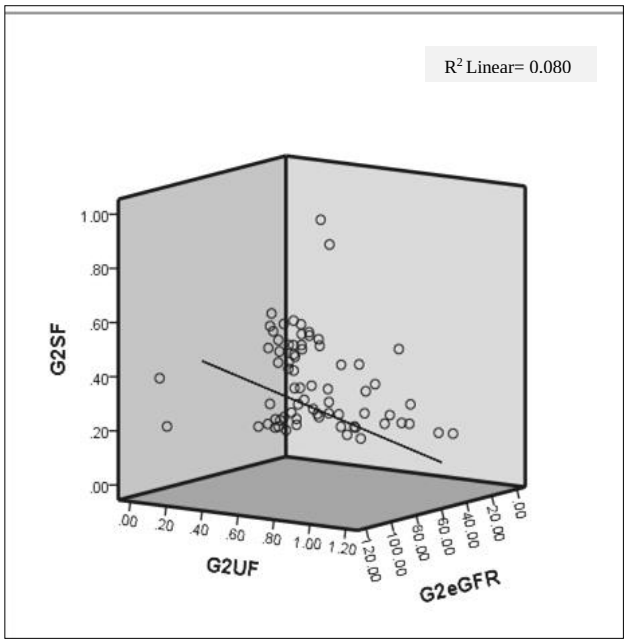
The present study is conducted in fluoride endemic area and recruited T2DM and DN cases. A correlation of fluoride exposure and excretion with eGFR were correlated in a 3 dimensional graph for group 1, 2 & 3 (triple axis).

Group 1 (controls) showed a better positive correlation indicating a normal functioning of renal apparatus represented in graph 8. Graph 9 represented a strong inverse correlation indicating decline in renal function in group 2 (diabetic nephropathy). A poor positive correlation was

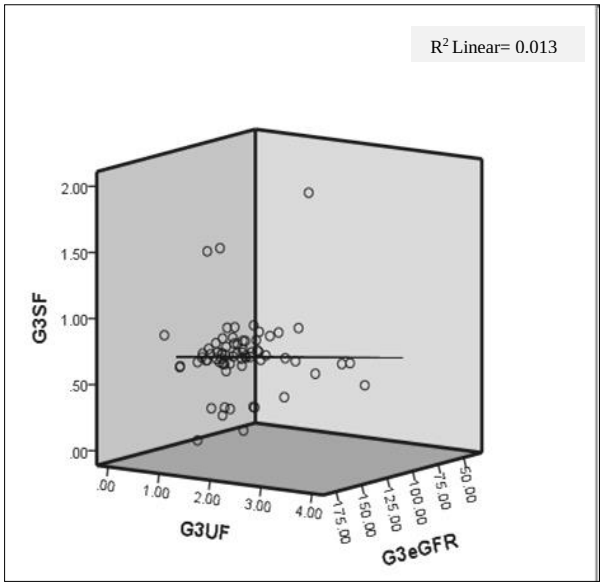
observed in group 3, conclusive that there may be a strong possibility of diabetic complication especially renal impairment at the earliest.



Graph 8: Correlation of serum and urine F with eGFR in group 1 (Healthy Controls)



Graph 9: Correlation of serum and urine F with eGFR in group 2 (Diabetic Nephropathy)



Graph 10: Correlation of serum and urine fluoride with eGFR in group 3 (Type 2 DM)

DISCUSSION

Biomarkers are the molecules which help predict the underlying physiological and pathological basis for health and disease. There are many biomarkers identified and established for various metabolic disorders and diseases. It is the time for research community to discover preventive molecules which shall be included in therapeutics, targeting a specific disease/ disorder and/or a permanent treatment to rule out the condition. As already mentioned, oxidative stress, insulin resistance and ecological imbalances are the common reasons for metabolic disorder which must be battled by either inducing or activating a cascade of processes which may mask disastrous activity in a system.

The present study involves a very important dimension in metabolic disturbance namely Type 2 Diabetes mellitus (T2DM) and Fluorosis. Though fluoride (F) is not the only important causative, it shall be considered as one of the triggering factor or accelerator to end up in complication. To diagnose diabetes mellitus and one of its microvascular complications; diabetic nephropathy, following parameters was analyzed:

- **Diabetic profile:** Fasting and post- prandial blood sugar, Glycated hemoglobin
- **Extended diabetic profile:** Fasting insulin, carboxymethyl lysine, fructosamine, HOMA-IR and QUICKI
- **Renal profile parameters in serum**: Creatinine, urea, uric acid, sodium, potassium and albumin
- **Extended renal profile:** Cystatin C and eGFR, calculated as per CKD- EPI 2009 and 2012 formulae (132)

In the present study, table 4 represents the demographic details of all the study subjects. The mean age of the study subjects were age and gender matched and there was no significance between groups. Prevalence of diabetes by age is well represented in IDF Diabetes Atlas 9th edition (7). It has been made clear that prevalence of diabetes is known to gradually increase from younger age of 20- 24 years to adult age of 44- 62 years and elderly age group of 64- 79 years (7). Blood pressure and Mean Arterial Pressure (MAP) of DN group was high compared to the other two groups indicating loss of renal control on blood clearance concurrent with findings by Guido Grassi et al (139). CKD cases in stages 2 and/ or 3 are at higher risk of landing up in dialysis due to hypertension, especially the increase in systolic blood pressure (139). As far as BMI, the study was carried out in rural area and the staple food being finger millets (ragi); which has low glycaemic index and most of the study subject's BMI were within desired range (140). The significant difference in the mean values of BMI between the controls (24.1 ± 3.14) and DN (22.6 ± 1.6) may be due to restriction in diet.

National Programme for Prevention and Control of Fluorosis (NPPCF) recommended triple test prior to radiological examination to assess skeletal fluorosis (39). Though the triple test was not of much significance physically and statistically, from table 5 most of the subjects in group 2 were unable to perform triple test especially the coin test. There are no studies supporting these criteria in DN and hence further radiological evaluation is necessary to prove the fact.

As T2DM is a predispoding condition for DN and DN patients are documented with damage of the renal tissue, 1α hydroxylation of Vit D metabolism might have got impaired. This impairment in Vit D synthesis would have resulted in altered calcium metabolism vis-à-vis Flourosis. These hypothethetical statements support the non-compliance of the fluorosis triple test.

The notable finding in our study is that the fluoride triple test was not significant across the groups.Which may be contributed to the high consumption of finger millet by the local population and is known to contain a high calcium. This dietary calcium might have compensated for the non-compliance of the “Fluorosis Triple Test”.

Diagnosis of Type 2 diabetes mellitus (T2DM) is made by estimation of fasting blood glucose, PPBS and HbA1c. For the diagnosis of T2DM and its complication DN we have considered the basic diabetic and renal profile. The observations are tabulated in table 6. From current study, basic diabetic profile with FBS (mg/dL) of groups 2 and 3 was 173.6 ± 64.02 and 182.3 ± 67.7 respectively compared with FBS of group1 (93.94 ± 9.4 mg/dL) which is in accordance with WHO and ADA (5, 6). The values of FBS, PPBS and HbA1c are significantly higher in groups 2 & 3 compared to group 1 with a significant p- value. However, comparison of FBS and PPBS between group 2 & 3 did not show much significance. This observation may be because of rigid control of diabetic status among the group 2 study subjects, awareness of future complications, vigil on the treatment modalities and constant monitoring. The HbA1 % values in group 3 (9.2 ± 2.4) versus group 1 (5.5 ± 0.5) and group 2 (8.2 ± 2) versus group 1 showed a significant difference and a higher values among group 2 & 3 study subjects compared to group 1. This indicates that the groups 2 & 3 study subjects did not had an appreciable long term glycemic control (6). As an interim remark, the basic tests performed proved to be of confirmation to test and classify study subjects as diabetics.

Routine investigations for renal function, basic renal profile was considered.We assessed creatinine, urea and albumin with electrolytes in blood such as sodium (Na) and potassium (K). Current study concentrated on essential parameters in serum such as: creatinine, urea, albumin, sodium and potassium (table 6). Among the basic renal parameters estimated in group 2; urea and

creatinine were 69.03 ± 27.5 and 3.4 ± 1.5 mg/dL respectively. Observed values of creatinine and urea showed a sharp increase. Serum albumin value was 2.8 ± 0.8 g/dL and was decreased in group 2. This is a clear sign of renal dysfunction coinciding with the literature of Gowda S et al (141). Since CKD stages 4 & 5 are excluded from the study, there are no much differences in Na & K as the study subjects are with the initial stages of glomerular damage.

As a measure to prevent cardiovascular complication of diabetes, there is a need to keep in check the lipid profile parameters. Thus we considered estimation of total cholesterol (TC), Triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), non HDL (nHDL) and very low density lipoprotein (VLDL). Studies have documented that there are chances for renal artery narrowing due to lipid accumulation leading to hypertension and other renal complications (142). As TC and HDL are normally distributed we considered mean ± SD. For TG and LDL as these are not – normally distributed we considered median (25^{th} to 75^{th} percentile) interquartile ranges. In our study lipid profile parameters such as TC, HDL, LDL, VLDL and nHDL were observed low in group 2 compared with other groups (table 7 & 8). There are many controversial studies on lipid levels in diabetic nephropathy. Study conducted by S. Palazhy et al. in Indian population and a study conducted by Hung- Chun Chen et al. in Taiwanese population documented that lipid molecules were elevated in severe DN (143, 144).

Our study findings are in agreement with Merlin C. Thomas et al, where the study was conducted on Caucasians (145). They documented that lipid values varied across different stages of DN and hence may not be constant for all subjects (145). On the other hand, group 3 (T2DM) subjects showed dyslipidemia indicating disturbances in lipid metabolism due to insulin resistance or poor diet control which if continued may lead to co- morbid conditions in accordance with Wu

L et al (142). Eventually, regular follow up of lipid profile in diabetes would be better if made mandatory by the clinicians to avoid further complications.

Advanced biomarkers such as, sirtuin1, carboxy methyl lysine (CML), urine and serum fluoride are considered in our study to find its correlation with diabetes and diabetic nephropathy. As advanced biomarkers were not normally distributed we considered median (25^{th} to 75^{th} percentile). The values are represented in Table 9 and describes a decrease in sirt1, serum and urine fluoride of group 2 [34.74 (25.08 to 53.2), 0.24 (0.2 to 0.5) and 0.24 (0.16 to 0.41)] compared to other groups. This uniquesness in our study indicates that sirt1, serum and urine F are better biomarkers for T2DM and DN.

Similarly, graphs 4, 6 & 7 represent the decrease of sirt1, serum and urine fluoride in diabetic nephropathy group. As a known fact, increased oxidative stress is also a cause for diabetes which may lead to serious consequences if not in control. CML cross linking obliterates the skeleton of cellular matrix of tissues activating oxidative stress and inflammation. This increased retention of CML in cells and tissues causes renal damage in DM (33). Our findings are consistent with the findings of Suzuki D et al (33). The CML of DN and T2DM are doubled when compared with control group indicating that CML is both a diagnostic and prognostic marker.

Sirt1 is considered as molecule with dual role with protective action and as a biomarker. Sirt1 action has been suppressed in DN subjects and decreased. Sirtuin1 modulates acetylation and deacetylation balances of multiple substrates and regulates various responses such as, apoptosis, cell senescence, endocrine metabolism, glucose homeostasis and aging (109, 57). Since DM is a known predisposing cause for diabetic nephropathy, sirt1 is known to play a key role in the assessment of DN our study finfings are in support of these documented statements.

From the total fluoride ingested in children, approximately 50% is absorbed compared to young and middle- aged people where it is 30- 40 % (81). From our study findings it can be hypothesized that early exposure to fluoride in younger age will have more impact during advanced age. In table 9 serum fluoride levels represented as median (25^{th} to 75^{th} percentile) are high in groups 1 and 3 [0.66 (0.62 to 0.72) & 0.6 (0.56 to 0.68)] and likewise its excretion is also proportionate [0.89 (0.55 to 1.49) & 0.72 (0.53 to 1.02)] unlike group 2 whose serum and urine fluoride levels [0.24 (0.2 to 0.5) & 0.24 (0.16 to 0.41) respectively] are equal which means renal clearance is decreased. Our findings suggest that the medical line of treatment with stringent monitoring of the DN shall not alter neither serum nor urine F. The same is represented graphically in graphs 6 & 7 (box and whisker plot). Among the major body fluids, fluoride toxicity is predominantly noted in serum. In healthy subjects, unabsorbed fluoride is mainly excreted through urine (147). Values in table 9 indicates, during renal damage fluoride excretion is hindered leading to increased serum fluoride and its complications.

Suzuki et al. has documented that increase in sirt1 acted as a protective molecule against fluoride damage by activating autophagy which gives a new hypothesis to be applied in human biology (82). CML values [median (25^{th} to 75^{th} percentile)] were almost equal in group 2 [1815 (1100 to 2591.13)] compared with group 3 [1870 (1155.1 to 2272.5)] but is decreased in group 1 [899 (625.25 to 1306.5)] which is represented in graph 5. As described by RD Semba et al, serum CML concentration influences renal function in maintaining AGE homeostasis and is usually found increased in diabetes and its complications especially in DN (148). Contrary to our study results, Semba RD et al stated that there is a need to evaluate about increase in serum and urine CML which could be a major determinant of AGE status (149). Urinary CML excretion is uncertain and not much associated with intake and the status of the individual's metabolism (149).

These observations has made clear that increased CML and F act as pro- oxidants restricting the effect of sirtuin1 on cellular damage causing further implications on increased insulin resistance and decreased insulin sensitivity which is in line with the findings of Jaime Uribarri et al (117).

To assess the advancement and/ or further complications of diabetes and DN we considered lipid ratios, renal ratios and advanced biomarker ratios. These ratios has given an insight of a highly significance across lipid and basic renal profile parameters. Uric acid metabolism is dependent on various factors and hence not considered as better marker for renal clearance.

Serum ratio of uric acid: creatinine may serve as the marker for renal performance in diabetic nephropathy. Uric acid: creatinine ratio is found significantly decreased in group 2 which is in accordance with Liubao Gu et al (150). Similarly, urea: creatinine ratio from the present study showed a 50% significant decrease in ratio due increase in serum urea and creatinine proportionately. As stated earlier, though cystatin C is a better surrogate marker on par with serum creatinine these ratios may help assess the upcoming burden on kidneys replacing cystatin C.

Lipid ratios in recent days are gaining weightage in metabolic disorders especially diabetes and metabolic syndrome (73). Ratio of lipid parameters with HDL gives a clue both in controls and T2DM to prevent accumulation of these molecules hence decreasing the chances of aging disorders. Atherogenic index (AI) described by Myat Su Bo et al. in T2DM with CVD cases was found higher when compared with T2DM which is in agreement with increased AI of diabetic nephropathy cases from the present study (138).

For every 1 part increase in serum fluoride 78 parts of sirtuin1 is increased in control group which further increased in cases as seen in table 10. This increase implies that serum fluoride as a causative molecule and sirtuin1 as a predictive (marker) molecule for prediction of disease

progression. The injuring product CML and the rescue protein sirt1 when calculated as ratios gave a similar outcome as with fluoride. CML: Sirt1 ratio of healthy group (19: 1) was very less than group 2 (52: 1) and group 3 (37: 1). These ratios indicate the worsening of kidneys in diabetic nephropathy (group2) and progressing towards damage in T2DM (Group3). These findings indicate that ratios of advanced parameters for assessment of DM and DN give a clue on progression of the disease status.

Apart from associating these special molecules to routine parameters, extended diabetic and renal profile were included to assess in depth the consequences of fluoride and CML in prognosis of the disorder. Analogous to HbA1c; Fructosamine is considered as a short term (2-3weeks) indicator of protein glycation and an upcoming parameter of interest (151). Though there are no population studies to define reference range for fructosamine its level in serum is directly proportional to plasma glucose levels indicating the level of glycemic control in diabetes (151). Robert M. Cohen et al. documented that HbA1c and Fructosamine have glycosylation gap but are good predictors of average glucose of long and short term controls respectively (151). This finding is proved from our study from table 11 that there is an increased Fructosamine level (ng/mL) [median (25^{th} to 75^{th} percentile)] in group 2 [245.93 (0.16 to 0.41)] & 3 [329.9 (131.42 to 88.2)] when compared to group1 [100.2 (55.8 to 172.4)].

Fructosamine is called the glycated albumin. Albumin is a multifaceted molecule. Serum albumin plays various important role as a carrier protein synthesized in liver and excreted through kidney. Fructosamine predicts the short term glycemic control as the half- life of albumin is 21days. The mean physiological reference range of serum albumin is maintained in control and T2DM group but decreased in DN, which may be due to increased albumin excretion in urine.

With increase in plasma glucose levels in cases, there is also a notable elevation of fructosamine in cases compared to controls.

Fasting insulin was analyzed to calculate homeostasis model assessment- insulin resistance (HOMA-IR) and quantitative insulin check index (QUICKI). Insulin values were decreased in DN and high in T2DM, there is no much significance in insulin values since it has a broad reference range. As we move down the table, HOMA- IR was calculated using online calculator with the formula derived by Matthews DR et al (135). HOMA- IR value ≥3.5 is considered as insulin resistance as per IDF (7). Though euglycemic glucose clamp is gold standard in measuring insulin sensitivity, there was an excellent correlation between euglycemic clamp and QUICKI documented by Katz A et al. (136). QUICKI value <0.32 indicates diabetes with decreasing insulin sensitivity concordant with values of Katz et al (136). Therefore, it is evident from previous studies and current study that, HOMA- IR and QUICKI can act as better surrogates of insulin action for assessment of diabetic status.

Working group of National Kidney Foundation (NKF) for assessment of renal efficiency in the prognosis of DN has recommended CKD- EPI (KDIGO), MDRD etc, to calculate the estimated Glomerular Filtration Rate (eGFR); by considering creatinine and Cystatin C (132). Serum creatinine is considered the marker for diagnosis of renal impairment (132). Though eGFR is a gold standard marker for renal function analysis, serum creatinine is considered as a marker to assess the state of renal impairment (132). As serum creatinine depends on rate of its synthesis, extra renal eliminations, muscular mass, gender etc, a better marker cystatin C specific to renal filtration is considered (152). The value of serum cys C (mg/L) were high in group 2 subjects [median (25^{th} to 75^{th} percentile)] [4.3 (3 to 6.5)] than in other groups which is in agreement with study by Yun Kyung Jeon et al. (152). Our study considered eGFR equations of creatinine and a

combination of creatinine and Cystatin C in which group 2 showed a decline in both the equations of eGFR represented as [median (25^{th} to 75^{th} percentile)] is [26 (17.75 to 37.25), 60.5 (40 to 91) respectively] confirm the deterioration of renal function.

Values depicted in table 12 shows that eGFR of groups 1 and 3 were within reference range and were not significant. Calculating eGFR by standalone creatinine values correlates better than the eGFR calculated considering creatinine- cystatin C values. Values obtained from CKD- EPI of 2012 in our study were less in group 1 [92.5 (74 to 118.5)], versus group 3 [118.5(95.75 to137.25)] which may be because of few outlier of cys C values. These findings construe that calculation of eGFR proposed in 2009 consider creatinine as a better indicator. However, serum Cystatin C maintains its reputation as a standalone surrogate marker in diagnosis of DN.

Correlation of vital parameters for DN in our study such as CML, Fluoride, Sirtuin1, Cys C, insulin resistance (IR) and insulin sensitivity (IS) were compared and documented in table 13. This indicates that the activation of sirtuin1 action on damaged cells**.** Further, CML decrease during increased sirt1 indicates the deacetylation of protein suppression mechanism. This is also supported by Jaime Uribarri et al. in their study conducted in New York population (118). Similarly, increased sirtuin1 protect cells from fluoride induced stress by activating autophagy as demonstrated by M. Suzuki et al. in an animal study (83). A complete negative correlation of the cause with sirt1 implies a consequence that there will be a decrease of rescue mechanism in increased AGE and fluorosis.

Other important component of this study is insulin action (IR and IS) which is derived by HOMA- IR and QUICKI calculation. Our findings predicted calculated values of insulin sensitivity and resistance which may indirectly indicate the cellular status in insulin reception.

From table 14 negative correlations indicate decrease in HOMA- IR values result in decreased resistance during increased sirtuin1 values and vice versa. Increased QUICKI value in general indicates increased insulin sensitivity. Therefore, correlation outcome of this study clearly indicates that though there is no significant correlation, the trend what we have observed is an evidence for the curative nature of sirtuin1 in insulin sensing of cells for glucose metabolism. Further, sirt1 is not only implicated in DM but also in other insulin related disorders such as, metabolic syndrome, obesity, hypothyroidism etc. Jaime Uribarri et al correlated CML as a contributor to increased IR. Suppression of CML shall help activate sirtuin1 protective mechanism and revert the changes back to normal which gives a lead to conclude this study (118).

Table 15 & 16 includes simple linear regression analyses to find the effect of parameters on CML and fluoride respectively. CML was significantly affected by Sirt1, fructosamine and FBS indicating variations in these parameters further increases CML values. Serum creatinine, potassium, sirt1, eGFR and serum fluoride affects urine fluoride excretion. Sirtuin1 being the common parameter to affect the values of CML and urine fluoride, more prominence needs to be given on sirtuin1 potency. Hence, sirtuin1 can be a marker for aggravating changes at molecular level in diabetes, diabetic nephropathy and fluorosis.

To assess the damage of glomeruli by serum F and resultant F excretion, correlation between the serum and urine fluoride with eGFR creatinine was plotted. Correlation of serum and urine F with eGFR showed a mild positive correlation in controls and T2DM (graph 8 and 10). This signifies that fluoride in blood is proportionally cleared through urine in healthy controls compared to DN (graph 9) and is negatively correlating with eGFR. This indicates that the fluoride clearance is dropped in DN intensifying the organ damage.

The findings of our study are comparable with the various studies conducted elsewhere considering sirtuin1, CML, Fluoride, HbA1c and Fructosamine. However, to derive any conclusions a large population study with a defined reference range considering all the criteria of the definition needs to be looked into.

CONCLUSION

From the present study we comprehend that fluoride and diabetes in combination may be a fast destructing disorder of normal metabolism. Fluorosis and diabetes is a globally prevailing epidemiological disorder and a challenge. There is a hitch for need of a biomarker and/ or a therapeutic molecule to trace or heal the consequences. The prime molecule of interest in this study; Sirtuin1 (sirt1) is now gaining momentum in the era of aging disorders and that is included in our study to find its trend in diabetes and fluorosis.

- The increase in serum sirtuin1 levels indicate the severity of cellular damage due to stress during hyperglycemia and fluoride toxicity
- Sirtuin1 can be considered as a biomarker of aging disorder such as type 2 diabetes mellitus which is evident from the present study
- As stated, Advanced Glycation Endproducts augments the pathological aging process. This is proved with Carboxy Methyl Lysine
- Correlation of CML, eGFR and Fluoride with sirt1 indicates that increasing sirt1 may defend the forthcoming damage of cells and hence could be considered in formulating therapeutic modalities
- In our study we could document that, fluoride and CML alters sirtuin1 values. Decrease in sirt1 value is a consequence of uncontrolled hyperglycemia and chronic fluorosis in Diabetic nephropathy
- The levels of sirtuin1 is directly proportional to its synthesis in Diabetes Mellitus (DM) and is inversely proportional in Diabetic Nephropathy (DN)
- Male prepondarance of 55% is observed in cases compared to females. This gives an impetus that females have better protection against Diabetes and diabetic nephropathy.

Increased ratios of renal, lipid and advanced biomarker ratios shall be considered as a risk factor for development of future complications of diabetes.

- Increased Uric Acid: Creatinine and Cys C: Creatinine in group 3 is a caution for T2DM to keep in check the three parameters to prevent any renal damage
- Uric acid: Creatinine ratio may act as a substitute, if there are any challenges in Cystatin C estimation

The mean, median and ratios of T2DM from the current study helps in prediction of future complication of diabetes mellitus.

Major source of CML and serum F is diet. Calorie restriction increases sirtuin1 levels. Hence a balanced and healthy diet may improve the overall quality of life preventing any ailments causing chronic complications.

To substantiate our findings and before giving a status to the molecules, sirt1 and fluoride; we propose invitro studies by using cell lines and to decide on therapeutic applications of sirt1.

Further invitro studies shall help find the actual point of action of siruint1 and fluoride to find remedies to fight against aging and its effects.

STRENGTHS AND LIMITATIONS

Strengths

- First study to elucidate relationship between urine and serum fluoride in diabetes and diabetic nephropathy in human
- Comparison between plasma CML, fluoride and eGFR with sirt1 in human
- Successful evaluation of fluoride ISE for serum and urine fluoride
- Identification of sirtuin1 as anti- aging molecule and to be used as marker for aging

Limitations

- Serum F estimation in population exposed to high F versus area with geographical location of permissible limits of F could have given a better correlation
- Potable water F with serum fluoride values comparison could have given better insights
- Genetic study on sirt1 or CML might have given a better conclusion regarding aging

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ANNEXURE- I

CASE HISTORY SHEET

TITLE OF THE STUDY: Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India

Case No:

Name: Mr/Mrs OP No:

Age: IP No:

Gender: Ward:

Date: Occupation:

Weight:

Address:

hone:

CHIEF COMPLAINTS:

HISTORY OF PRESENTING ILLNESS:

HISTORY OF STAY IN THE PRESENT ADDRESS:

TYPE OF WATER CONSUMPTION AND DURATION

Drinking:

Cooking:

PAST HISTORY:

Hypertension: yes/no if yes, duration:

Diabetes: yes/no if yes, duration:

Heart diseases: yes/no if yes, duration:

Liver diseases: yes/no if yes, duration:

Drug ingestion: yes/no if yes, duration & details:

Gestational diabetes: Yes/ No

Treatment history

FAMILY HISTORY:

Diabetic history: Mother/ Father/ Siblings/ Maternal Uncle/ Paternal Uncle

OCCUPATIONAL HISTORY:

Any change in occupational history?

Duration and reason

PERSONAL HISTORY:

Economic status: Below poverty level/above poverty level

Micturation (Urine appearance)

Bowel (Defaecation)

Smoking: yes/no if yes, duration:

Alcohol: yes/no if yes, duration:

Menstrual History:

GENERAL PHYSICAL EXAMINATION:

Ht: Wt: BMI: BP: Pulse:

Built : normal / below normal / well-built / obese/ athletic

Nourishment: well / poor nourished

Skeletal Examination:

Triple test: Chin to Chest test:

Hands on occiput test

Coin test

CLINICAL DIAGNOSIS:

INVESTIGATIONS:

BLOOD:

Plasma Sirtuin1: ng/mL

Serum Fluoride: mg/L

Plasma Carboxy Methyl Lysine:

Diabetic Profile

Plasma FBS: mg/dL

Plasma PPBS: mg/dL

Glycated albumin (Fructosamine): ng/mL

Glycated hemoglobin (HbA1c) (%):

Insulin assay μIU/mL

HOMA IR (Calculated)

QUICKI INDEX (Calculated)

Renal Function Tests

Serum Urea: mg/dl

Serum Creatinine: mg/dl

Serum Uric Acid mg/dl

Serum Sodium mEq/L

Serum Potassium mEq/l

Serum Cystatin C mg/dL

eGFR (calculated)

2009 mL/ min/ 1.73m^2

2012 mL/ min/ 1.73m^2

LIPID PROFILE

Serum Total Cholesterol: mg/dL

Serum Triglycerides: mg/dL

Serum HDLc: mg/dL

Serum nHDLc (calculated): mg/dL

Serum VLDL (calculated)	mg/dL
Serum LDL (calculated):	mg/dL

URINE

Fluoride	mg/L or ppm

Patient Information sheet

TITLE OF THE STUDY: Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India

Name of the Principal Investigator: Mrs. R. Sai Deepika

Investigator's statement:

I, Mrs. R. Sai Deepika pursuing Ph. D in the Department of biochemistry at Sri Devaraj Urs Medical College, constituent of Sri Devaraj Urs Academy of Higher Education and Research Kolar.

In Kolar district the fluoride concentration in drinking water is high. Majority of the population are agriculturists and are dependent on livestock and ground water for their survival. This study is to assess the association of fluoride and with T2DM and its related nephropathic microvascular complication. It is known that diabetic nephropathy is one of the major diabetic microvascular complications and is more prevalent in the local population. Findings in this study may help us to understand the complication better and may lead to better therapeutic interventions and the damages associated with it.

This study is also estimating sirtuin1; an enzyme biomarker for early detection, prognosis and better management of diabetic nephropathy. This is a preliminary study done in blood and urine, and in future an extension of this study may be carried on cell culture to establish the molecular mechanism of fluorosis on renal cells. Some of the stored samples from you may be used after proper labeling and anonimization, for this future study.

Sirtuin1 is a molecule expressed under physiological and pathological conditions such as aging, starvation and diabetic nephropathy respectively. Sirtuin1 also helps in prevention of glomerular cell damage. It has been proposed that it can be considered as a therapeutic adjunct to decrease the severity of diabetic nephropathy.

Diabetic nephropathy develops as a result of enormous oxidative stress and is mainly due to uncontrolled and/or chronicity of diabetes. Therefore estimating stress parameters and proteins that undergo glycosylation [glycosylated hemoglobin (%), albumin (fructosamine)] will also be estimated in blood and/or urine.

Procedure: In this regard, I will ask you few questions about your personal, past and family history. I also need to collect 6mL of blood and 10mL of spot urine samples for investigations. You are at liberty to ask any questions. An honest answer to my questions shall help us in better understanding the pathological process and may help in quality patient care. I assure you this will not take much of your precious time and also the investigations you undergo are not charged.

The information obtained from you shall be maintained strictly confidential unless otherwise compelled by law.The entire information, investigative report and other detail obtained from you is used only for research. However, during the course of study, if any issues need to be addressed and which are found accidentally, they will be intimated to you with a proper guidance for further management with standard patient care and would be referred to higher centers, for those treatment modalities which are not available in our Hospital.

If you agree and cooperate with me in carrying out the study using your information and sample I reassure that you will not be burdened financially and you are at liberty to withdraw from the study at any point of time.Your withdrawal and/or non acceptance to participate in this study will not affect the treatment or the rapport with the physician.

About 6mL of the blood using aseptic precautions will be drawn from you to estimate blood glucose, kidney function tests, sirtuin1, carboxy methyl lysine and fluoride levels. Besides the above, proteins that undergo glycosylation such as, glycosylated hemoglobin (%), fructosamine are also estimated in your blood. I also request you to give 10 ml of corresponding urine for estimation of fluoride, sugar and microalbumin.

For end stage renal disorder patients, RL Jalappa Hospital follows standard dialysis protocol. For study subjects, in addition to regular management I assure regular eye checkup for assessing the injury and function of the eye due to complications of T2DM. Further, if advanced treatment is required they will be referred to Higher Centre for management with proper guidance.

Further I also request you that the left out sample (Secondary sample) shall be stored with proper precautions (labeling, recordings and anonimization) and used for analysis later on if required. I also assure you that the publications from the present study in the present or future shall be done without disclosing your identity.
Feel free for any clarification pertaining to this study with the Principal investigator and Supervisor and Co- Supervisors
Mrs. R. Sai Deepika: 9036413299 (Principal Investigator)
Dr Shashidhar K.N: 09845248742 (Supervisor)
Dr. Raveesha. A: 9448448353 (Co- Supervisor)

PARAMETERS AND REFERENCE RANGES OF THE AREA

Sl. No.	PARAMETER	REFERENCE RANGES
1	Serum Sirtuin1	To be derived
2	Serum and Urine Fluoride	
3	Serum Carboxy Methyl Lysine (CML)	
4	Plasma Glucose (mg/dl)	FBS: 70- 110, PPBS: 70- 200
5	HbA1c %	≥6.5 are considered diabetic
6	Serum Insulin (mcIU/mL)	2.6- 24.9
7	Serum Fructosamine (mcmol/L)	200- 285
8	Serum Urea (mg/dL)	7- 20
9	Serum Uric acid (mg/dL)	2.5- 8.5
10	Serum Creatinine (mg/dL)	0.5- 1.4
11	Serum Cystatin C (mg/L)	0.5- 1.0
12	Serum Sodium (meq/L)	136–146
13	Serum Potassium (meq/L)	3.5–5.0
14	Serum TG (mg/dL)	30–200
15	Serum Total Cholesterol (mg/dL)	<200
16	Serum HDL (mg/dL)	30- 40
17	Serum LDL (mg/dL)	<90
18	Serum Albumin (mg/dL)	4.0- 5.0

Healthy Control Information Sheet

TITLE OF THE STUDY: Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India

Name of the Principal Investigator: Mrs. R. Sai Deepika

Duration of the study: 3 years (2017-2020)

Investigator's statement:

I, Mrs. R. Sai Deepika pursuing Ph. D in the Department of biochemistry at Sri Devaraj Urs Medical College, constituent of Sri Devaraj Urs Academy of Higher Education and Research Kolar.

In Kolar district the fluoride concentration in soil and drinking water is is high. Majority of the population are agriculturists and are dependent on livestock, ground water for their survival. This study is to assess the association of fluoride and its impact on T2DM and its related microvascular nephropathic complication, it is known that diabetic nephropathy is the major diabetic microvascular complication and is prevalent the local population. Findings in this study may help us to understand these complications better and may lead to better therapeutic interventions to contain the damge associated with it**:** sirtuin1 is a newer molecule expressed under physiological and pathological conditions such as aging, starvation and diabetic nephropathy. Since, sirtuin1 helps in prevention of glomerular cell damage; it has been proposed that it can be considered as a therapeutic agent to decrease the severity of diabetic nephropathy.

Diabetic nephropathy is a result of enormous oxidative stress and is mainly due to uncontrolled and/or chronicity of diabetes. This needs to be addressed by estimating stress parameters and proteins that undergo glycosylation for example, glycosylated hemoglobin, fructosamine etc.

I am carrying out a study titled "Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India".

Participant Selection: Healthyage and gender matched normal population residing with the diabetic patients attending the General Medicine OPD and in- patients admitted in General Medicine ward in RL. Jalappa Hospital and Research Centre, aged 25- 60 years are proposed to be recruited with their written consent form.

Procedure: In this regard, I will ask you few questions about your personal, past and family history. I also need to collect 6ml of blood and 10mL of spot urine sample for investigations. You are at liberty to ask any questions. An honest answer to my questions shall help us in better understanding the pathological process and may help in quality patient care. I assure you this will not take much of your precious time and also the investigations you undergo are not charged.

The information obtained from you shall be maintained strictly confidential unless otherwise compelled by law.The entire information, investigative report and other detail obtained from you is used only for research. However, during the course of study, if any issues need to be addressed and which are found accidentally, they will be intimated to you with a proper guidance for further management with standard patient care and would be referred to higher centers, for those treatment modalities which are not available in our Hospital.

If you agree and cooperate with me in carrying out the study using your information and sample you will not be burdened financially and you are also at liberty to withdraw from the study at any point of time

giving substantial reason. Your withdrawal and/or non acceptance to participate in this study will not affect the treatment or the rapport with your physician.

Further I also request you that the left out sample (secondary sample) shall be stored with proper precautions and used for analysis if required. I also assure you that the publications from the present study in the present or future shall be done without disclosing your identity and without your prior permission.

Feel free for any clarification pertaining to this study with the Principal investigator and Supervisor,

Mrs. R. Sai Deepika: 9036413299 (Principal Investigator)
Dr Shashidhar K.N: 09845248742 (Supervisor)
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16	Serum HDL (mg/dL)	<40
17	Serum Albumin (mg/dL)	4.0- 5.0

ರೋಗಿಯ ಮಾಹಿತಿ ಪತ್ರ

ಶೀರ್ಷಿಕೆ: ಎಸ್ಟಿಮೇಷನ್ ಆಫ್ ಪ್ಲೂರೈಡ್ ಅಂಡ್ ಸಿರ್ಟುಯಿನ್ 1 ಇನ್ ಪೇಷಂಟ್ಸ್ ವಿಥ್ ಡಯಾಬಿಟಿಕ್ ನೆಫ್ರೊಪಥಿ ಇನ್ ಕೋಲಾರ್ ಜಿಲ್ಲೆ, ಕರ್ನಾಟಕ, ಭಾರತ.

ಪ್ರಧಾನ ಸಂಶೋಧಕರು: ಶ್ರೀಮತಿ. ಆರ್. ಸಾಯಿ ದೀಪಿಕಾ

ಅವಧಿ: ಸಂಶೋಧನೆ ಸುಮಾರು 3 ವರ್ಷಗಳ ಕಾಲ ನಡೆಯುತ್ತದೆ.

ಪ್ರಬಂಧಕರ ವ್ಯಾಖ್ಯೆ: ಶ್ರೀಮತಿ. ಆರ್.ಸಾಯಿ ದೀಪಿಕಾ ಆದ ನಾನು ಪಿ. ಹೆಚ್. ಡಿ ಉನ್ನತ ವ್ಯಾಸಂಗವನ್ನು ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾ ವಿದ್ಯಾಲಯ, ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ಉನ್ನತ ಶಿಕ್ಷಣ ಹಾಗೂ ಸಹ ಪ್ರಾಯೋಗಿಕ ಸಂಸ್ಥೆ, ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ವಿಭಾಗದಲ್ಲಿ ಮಾಡುತ್ತಿದ್ದೇನೆ,

ಕೋಲಾರ ಜಿಲ್ಲೆಯಲ್ಲಿ ಮಣ್ಣಿನಮತ್ತು ಕುಡಿಯುವ ನೀರಿನಲ್ಲಿ ಫ್ಲೋರೈಡ್ ಅಂಶವು ವರ್ಷದಿಂದ ವರ್ಷಕ್ಕೆ ಉಲ್ಬಣಗೊಳ್ಳುತ್ತಿದೆ, ಈ ಜನಸಂಖ್ಯೆಯ ಬಹುಪಾಲು ಜನ ಕೃಷಿಕರು ಮತ್ತು ಜಾನುವಾರುಗಳ ಮೇಲೆ ಅವಲಂಬಿತವಾಗಿರುವ ಕಾರಣದಿಂದಾಗಿ, ತಮ್ಮ ಜೀವನೋಪಾಯಕ್ಕಾಗಿ ಮಳೆ ನೀರು ಆರ್ಥಿಕ ಸ್ಥಿತಿಯೊಂದಿಗೆ ಅನಕ್ಷರತೆ, ಬಡತನ, ಅಜ್ಞಾನ ಇತ್ಯಾದಿಗಳಿಂದ ಬಳಲುತ್ತಿದ್ದು, ಇದನ್ನು ತಿಳಿಗೊಳಿಸಲು ಒಂದು ಅಧ್ಯಯನವನ್ನು ಮುಂದುವರಿಸಲು ಉದ್ದೇಶಿಸಿ ದ್ದೇನೆ.

ಡಯಾಬಿಟಿಸ್ (ಸಕ್ಕರೆ / ಸಿಹಿ ಮೂತ್ರ ರೋಗ) ಮತ್ತು ಅದರ ಸಂಬಂಧಿತ ಮೈಕ್ರೊವ್ಯಾಸ್ಕುಲರ್ (ಅತೀ ಸಣ್ಣರಕ್ತ ನಾಳಗಳು) ತೊಡಕುಗಳ ಮೇಲೆ ಪ್ರಭಾವ ಬೀರುವ ಫ್ಲೋರೈಡ್ ಅನ್ನೂ ಯಾವುದೇ ಸಂಯೋಜನೆಯ ಡಯಾಬಿಟಿಕ್ ನೆಫ್ರೋಪತಿ ಎಂದು ಕರೆಯಲಾಗುತ್ತದೆ. ಇದು ಮಧುಮೇಹದ ಆರಂಭಿಕ ತೊಡಕುಗಳಲ್ಲಿ ಒಂದಾಗಿದ್ದು ಮತ್ತು ಪ್ರಾಥಮಿಕ ಹಂತದಲ್ಲಿ ಉದ್ದೇಶಿಸಬೇಕಾಗಿರುತ್ತದೆ.ಈ ಅಧ್ಯಯನವು ತಮ್ಮ ಅನುಮತಿ ಹಾಗೂ ಸಹಕಾರದಿಂದ ಸ್ಥಳೀಯ ಜನಸಂಖ್ಯೆಗೆ ಉತ್ತಮ ಗುಣಮಟ್ಟದ ಜೀವನ ನಡೆಸಲು ನೆರವಾಗಬಹುದು.

ನಮ್ಮ ಅಧ್ಯಯನವು ಸಿರ್ಟುಯಿನ್1 ಎಂಬ ಏಂಜೆಮ್ ಅನ್ನು ಕೇಂದ್ರೀಕರಿಸಲಾಗಿದೆ. ಇದರ ಪ್ರಾಮುಖ್ಯತೆಯನ್ನು ಸಕ್ಕರೆ ಕಾಯಿಲೆಯಿಂದ ಉದ್ಭವಿಸುವ ಮೂತ್ರಪಿಂಡದ ಅಂತಿಮ ಸಣ್ಣ ರಕ್ತ ನಾಳಗಳ ತೊಂದರೆ ಅಂದರೆ "ಡಯಾಬೆಟಿಕ್ ನೆಫ್ರೋಪತಿ"ಗೆ ಹೊಂದಾಣಿಕೆ ಮಾಡಲಾಗಿದ್ದು, ಇದರ ಅಳತೆ ಅಥವಾ ಸಾಂದ್ರತೆ ಏನಾದರೂ ಇದ್ದರೆ ನಿಮ್ಮ ರಕ್ತ ಪರೀಕ್ಷೆಯ ಗುಣಮಟ್ಟವನ್ನು (ಅಣುವನ್ನು) ಹಾಗೂ ಖಾಯಿಲೆ ಪೀಡಿತರ ರಕ್ತದ ಅಣುವಿನ ಮಟ್ಟವನ್ನು ಹೊಂದಿಸಿ ಇದರ ಪ್ರಾಮುಖ್ಯತೆಯನ್ನು ದೃಢಪಡಿಸಲಾಗುತ್ತದೆ. ಈ ಅಣುವನ್ನು ಸಕ್ಕರೆ ಕಾಯಿಲೆಯ ಇತ್ತೀಚಿನ ಹಾಗೂ ಮುಂದುವರಿದ ಪರೀಕ್ಷೆಗಳನ್ನು ಸಹ ಮಾಡಿ ಹೊಂದಾಣಿಕೆ ಮಾಡಲಾಗುತ್ತೆ ಹಾಗೂ ಪ್ಲೂರೈಡ್ ಅಂಶವನ್ನು ಸಹ ಪರೀಕ್ಷೆ ಮಾಡಲಾಗುತ್ತದೆ.

ಕೋಲಾರದ ಈ ಜನಸಂಖ್ಯೆ ಯಲ್ಲಿನ ಯಾರೂ ಸಹ ಮಾಡದ ಪ್ರಥಮ ಪ್ರಯೋಗ ಮತ್ತು ಪ್ರಬಂಧ ವಿಧವೆಂದರೆ ಅಣು ಸಿರ್ಟುಯಿನ್-1 ಅನ್ನು ಮತ್ತು ಡಯಾಬಿಟಿಕ್ ನೆಫ್ರೊಪತಿ ಹಾಗೂ ಇತರೆ ದೈಹಿಕ ಮತ್ತು ರೋಗ ಶಾಸ್ತ್ರೀಯ ಪರಿಸ್ಥಿಗಳ ಅಡಿಯಲ್ಲಿ ವ್ಯಕ್ತ ಪಡಿಸಲಾದ ಹೊಸ ಮಾಹಿತಿಯನ್ನು. ಹಾನಿಯ ತಡೆಗಟ್ಟುವಲ್ಲಿ ಸಿರ್ಟುಯಿನ್- 1 ಸಹಾಯ ಮಾಡುತ್ತದೆ; ಡಯಾಬಿಟಿಕ್ ನೆಫ್ರೋಪತಿಯ ತೀವ್ರತೆಯನ್ನು ಕಡಿಮೆ ಮಾಡಲು ಇದು ಚಿಕಿತ್ಸಕ ಗುರಿಯಾಗಿ ಪರಿಗಣಿಸ ಬಹುದು ಎಂದು ಸೂಚಿಸಲಾಗಿದೆ. ಇದರಿಂದಾಗಿ ನಾನು ಪರೀಕ್ಷಿಸುವ ಸರ್ಟುಯಿನ್- 1 ರ ಪರೀಕ್ಷೆಯಿಂದ ಮುಂದೆಂದಾದರು ನಮ್ಮ ಜಿಲ್ಲೆಯ ಜನರಿಗೆ ಅನುಕೂಲವಾಗಲಿದೆ ಎಂಬ ನಂಬಿಕೆ ನನ್ನಲ್ಲಿದೆ. ಮಧುಮೇಹ ನೆಫ್ರೋಪತಿಗೆ ಅಗಾಧವಾದ ಆಕ್ಸಿಡೇಟಿವ್ ಒತ್ತಡ ಒಳಗೊಂಡಿರುವ ಮತ್ತು ಅನಿಯಂತ್ರಿತ ಅಥವಾ ಡಯಾಬಿಟಿಸ್ ನಿರಂತರತೆ ಅಂದಾಜು ಉದಾಹರಣೆಗೆ, ಗ್ಲೈಕೊಸಿಲೇಟೆಡ್ ಹಿಮೋಗ್ಲೋಬಿನ್, ಫ್ರಕ್ಟೊಸ್ ಅಮೈನ್ ಇತ್ಯಾದಿ ಗಳನ್ನು

ಗ್ಲೈಕೋಸಿಲೇಶನ್ ಒಳಪಡುತ್ತವೆ. ಆಕ್ಸಿಡೇಟಿವ್ ಒತ್ತಡ ಪರಿಣಾಮಗಳು ಮತ್ತು ಪ್ರೋಟಿನ್ ಅಂದಾಜು ಮೂಲದ ಸರಿಪಡಿಸುವ ಅಗತ್ಯ ವಿರುವ ಕಾರಣದಿಂದಾಗಿ ಉಂಟಾಗುವ ಕಾರಣದಿಂದ ರಕ್ತ ಮತ್ತು ಮೂತ್ರ ದಂತಹ ಸೂಕ್ತ ವಾದ ಜೈವಿಕ ಮಾದರಿಯಲ್ಲಿ ಇರುತ್ತದೆ.

ಪರೀಕ್ಷಾ ವಿಧಾನ: ಈ ವಿಷಯದಲ್ಲಿ, ನಿಮ್ಮ ವೈಯಕ್ತಿಕ, ಹಿಂದಿನ ಮತ್ತು ಕೌಟಂಬಿಕ ಇತಿಹಾಸದ ಬಗ್ಗೆ ನಾನು ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುತ್ತೇನೆ. ನನ್ನ ಅಧ್ಯಯನದ ಉದ್ದೇಶಕ್ಕಾಗಿ ನಾನು ತಮ್ಮ ೮ಮಿಲಿ ರಕ್ತ ಮತ್ತು ಮೂತ್ರವನ್ನು (10 ಮಿಲಿ) ಸಂಗ್ರಹಿಸಬೇಕಾಗಿದೆ. ನಿಮಗೆ ಬೇಕಾದ ಪ್ರಶ್ನೆಗಳಿಗೆ ನಾನು ಉತ್ತರಿಸಲು ಸಿದ್ಧನಿರುತ್ತೇನೆ ಹಾಗೂ ನೀವು ಸ್ವತಂತ್ರರಾಗಿರುತ್ತೀರಿ.ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನಿಮ್ಮ ಪ್ರಾಮಾಣಿಕ ಉತ್ತರವು ರೋಗಿಗಳ ಆರೈಕೆಯನ್ನು ಚೆನ್ನಾಗಿ ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ನಮಗೆ ಸಹಾಯ ಮಾಡುತ್ತದೆ.ನಾನು ಈ ನಿಮ್ಮ ಅಮೂಲ್ಯ ಸಮಯ ಮತ್ತು ನಿಮಗೆ ಯಾವುದೇ ಖರ್ಚು ವೆಚ್ಚ ವಿಧಿಸಲಾಗುವುದಿಲ್ಲ. ಪಡೆದು ಮಾಹಿತಿಯನ್ನು ಕಟ್ಟುನಿಟ್ಟಾಗಿ ಗೋಪ್ಯವಾಗಿಡುತ್ತೇನೆ. ಸಂಪೂರ್ಣ ಮಾಹಿತಿ, ನಿಮ್ಮಿಂದ ಪಡೆದ ತನಿಖಾ ವರದಿ ಮತ್ತು ಅದರ ವಿವರಗಳನ್ನು ಸಂಶೋಧನೆಗೆ ಮತ್ತು ರೋಗ ನಿರ್ಣಯ ಮತ್ತು / ಅಥವಾ ನಿರ್ವಹಣೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಒಂದೊಮ್ಮೆ ಎನಾದರೂ, ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ, ಯಾವುದೇ ಪ್ಯಾರಾಮೀಟರ್ಗಳು ಅಥವಾ ಸಮಸ್ಯೆಗಳನ್ನು ಬಗೆಹರಿಸಬೇಕಾದರೆ ಮತ್ತು ಆಕಸ್ಮಿಕವಾಗಿ ಕಂಡುಬರುವ ಅಚಾನಕ ಪ್ರತಿರೋದ ಅಥವ ದುಷ್ಪರಿಣಾಮ ಕಂಡುಬಂದಲ್ಲಿ ಹಾಗೂ ಯಾವುದೇ ತಮಗೆ ಹೆಚ್ಚುವರಿ ವೆಚ್ಚ ಆಗದ ಹಾಗೆ ಹೆಚ್ಚಿನ ನಿರ್ವಹಣೆಯ ಸರಿಯಾದ ಮಾರ್ಗ ದರ್ಶನವನ್ನು ನಿಮಗೆ ತಿಳಿಸಲಾಗುವುದು.

ಈ ಸಂಶೋಧನೆ ಯಿಂದಾಗಲಿ, ಪ್ರಬಂಧದಲ್ಲಾಗಲಿ ಅಥವ ಪರೀಕ್ಷೆಯಲ್ಲಾಗಲಿ ಯಾವುದೇ ದುರುದ್ದೇಶವಿಲ್ಲ. ನಿಮ್ಮ ಎಲ್ಲಾ ವಿವರಗಳನ್ನು ಸಂಪೂರ್ಣವಾಗಿ ಗೌಪ್ಯವಾಗಿ ಇಡುತ್ತೇನೆ ಹಾಗೂ ಕಾಪಾಡುತ್ತೇನೆ ಎಂದು ದೃಡಪಡಿಸುತ್ತೇನೆ. ತಮಗಾಗಲಿ ಅಥವ ತಮ್ಮ ವಿಶ್ವಾಸಿಗಳಿಗಾಗಲಿ ಎಂದಿಗಾದರೂ ಯಾವ ರೀತಿಯಲ್ಲಿ ಅನುಮಾನಗಳಿದ್ದರೂ ನಾನು ಖುದ್ದಾಗಿ ಬಗೆಹರಿಸುತ್ತೇನೆ. ತಮಗೇನಾದರೂ ಈ ಪರೀಕ್ಷೆಗೆ ಸಮ್ಮತಿ ಇಲ್ಲ ಎಂದು ಅನ್ನಿಸಿದ ಪಕ್ಷದಲ್ಲಿ ತಾವು ಯಾವುದೇ ಷರತ್ತುಗಳಿಲ್ಲದೆ ಸೂಕ್ತ ಮಾಹಿತಿಯನ್ನು ನೀಡಿ ಹಿಂದಿರುಗಬಹುದು. ನನ್ನಿಂದ ಅಥವ ನನ್ನ ತಂಡದಿಂದ ಯಾವುದೇ ಆಕ್ಷೇಪಣೆಗಳಿರುವುದಿಲ್ಲ.

ರಕ್ುದ ಗುಕ ೋಸ್, ಮ ತ್ರಪಿಂಡದ ಕಾರ್ಯ ಪರೋಕ್ಷ ಗ್ಳು ಮತ್ತು ಫ್ಲುರ ೈಡ್ ಮಟ್ಟವನತು ರಕ್ುದಲ್ಲು ಲೆಂದಾಜತ ಮಾಡಲತ ಸತಮಾರತ

೮ ಮಿಲ್ಲ ರಕ್ುವನತು ತ ಗ ದತಕ ಳಳಲಾಗ್ತತ್ುದ .ನಿಮಮ ಪರಿಸ್ಥಿತಿರ್ ಅವಶ್ಯಕ್ತ ಗ ಅನತಗ್ತಣವಾಗಿ ರಕ್ುದಲ್ಲುನ ಸಕ್ಕರ ಲೆಂದಾಜತಗ್ಳು ಮತ್ತು ಮ ತ್ರಪಿಂಡ ಕಾರ್ಯದ ಲೆಂದಾಜತಗ್ಳನತು ಕ ೈಗ ಳಳಲಾಗ್ತವುದತ.ಮೋಲ್ಲನದದಲುದ ,ಒಟ್ಾಟರ ಉತ್ಕರ್ಯಣ ನಿರ ೋಧಕ್ಗ್ಳನತು ಲೆಂದಾಜತ ಮಾಡತಲಾಗ್ತವುದತ, ಉದಾಹರಣ ಗ ಗ ುೈಕ ಸ ೈಲ ೋರ್ನ್ ೆ ಒಳಗಾಗ್ತವ ಆಕ್ಸಿಡ ೋಟಿವ್ ಒತ್ುಡ ನಿರ್ಾೆಂಕ್ಳು ಮತ್ತು ಪ್ರೋಟೋನೆಳು, ಗ ುೈಕ ಸ ೈಲ ೋಟ್ ಡ್ ಹ ಮೋಗ ುೋಬಿನ್, ಫ್ರಕ ಟೋಸಾಯಮೈನ್ ಕ್ ಡ ರಕ್ುದಲ್ಲು ಮಾಡಲಪಡತತ್ುವ . ಜ ತ ಗ 20 ಮಿಲ್ಲ ಮ ತ್ರವನತು ಸಹ ಆಸಪತ ರಲ್ಲು ಸಿಂಗ್ರಹಿಸ್ಥ ಪರೋಕ್ಷಿಸಲಾಗ್ತವುದತ.ಮೋಲ್ಲನ ಪರೋಕ್ಷ ಗ್ಳಿಗ ಯಾವುದ ೋ ಹ ಚ್ಚುವರ ವ ಚ್ುವನತು ವಿಧಿಸಲಾಗ್ತವುದಿಲು.ಮೂತ್ರ ಪಿಂಡ ವೈಫಲ್ಯದ ಅಂತಿಮ ಮಟ್ಟದ ರೋಗಿಗಳಿಗೆ ನಮ್ಮ ಆಸ್ಪತ್ರೆಯ ವೈದ್ಯಪರಿಣಿತರು ಗುಣಮಟ್ಟದ ರಕ್ತ ಶೋಧನೆ ಪರಿಕ್ರಮಗಳನ್ನು ಸುಧಾರಿಸಬಹುದಾದಂತಹ ವೆಚ್ಚದಲ್ಲಿ ನೀಡಲಾಗುತ್ತದೆ ಹಾಗೂ ಮುಂದುವರೆದ ಚಿಕಿತ್ಸೆಯ ವಿವರಗಳನ್ನು ನೀಡಿ ತಿಳಿಯಪಡಿಸಿಲಾಗುವುದು.

ಅಗತ್ಯವಿರುವ ಸೂಕ್ತ ರಕ್ತದ ಮಾದರಿಯನ್ನು ತಮ್ಮಿಂದ ಪಡೆಯಲು ನನಗೆ ತಾವು ಅನುಮತಿಸಿ ಮತ್ತು ನಿಮ್ಮಿಂದ ಮೂತ್ರವನ್ನು ಸಂಗ್ರಹಿಸಿ ಭವಿಷ್ಯದ ತನಿಖೆಗಳಿಗೆ ದ್ವಿತೀಯ ಮಾದರಿಯನ್ನು ಸಂರಕ್ಷಿಸಲು ನಾನು ತಮ್ಮಲ್ಲಿ ವಿನಂತಿಸುತ್ತೇನೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂ ಪ್ರೇರಿತವಾಗಿರುತ್ತದೆ.
ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ನಿರ್ಬಂಧವಿಲ್ಲ ನೀವು ನಮ್ಮ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ, ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈ ಅಧಯ್ಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನೀವು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದರೆ ಮಾತ್ರ ನೀವು ಸಹಿ ಮಾಡ ಬೇಕಾಗುತ್ತದೆ.ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನ ದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಸ್ವಾತಂತ್ರ ವಾಗಿರುತ್ತೀರಿ ನಿಮ್ಮ ಹಿಂತೆಗದುಕೊಳ್ಳವಿಕೆಯು ವೈದ್ಯಕೀಯ ಸೇವೆಯಲ್ಲಿ ಯಾವುದೇ ರೀತಿಯ ಪ್ರರಣೆಯಿಂದ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ ಎಂದು ನಾವು ಭರವಸೆನೀಡುತ್ತೇವೆ.

ನಾವು ತಮಗೆ ಈ ಮೂಲಕ ತಿಳಿಯಪಡಿಸುವುದೇನಂದರೆ ತಮ್ಮ ರಕ್ತ ಅಥವಾ ಮೂತ್ರ ಮಾದರಿಯನ್ನು ಕೂಡಿ ಕರಿಸಿ ಮುಂದೆಂದಾರೂ ಪರೀಕ್ಷೆ ಮಾಡಬೇಕಾದ್ದಲ್ಲಿ ಅದನ್ನು ಉಪಯೋಗಿಸಲು ಸಮ್ಮತಿ ಪಡೆಯುತ್ತೇವೆ.

ನಾವೂ ಪ್ರಕಟಿಸಬಹುದಾದ ಮುಂದೆಂದಾದರೂ ಪ್ರಬಂಧವನ್ನು ಮಂಡಿಸುವಾಗ ತಮ್ಮ ನಿಖರತೆಯ ಗೌಪ್ಯವನ್ನು ಕಾಪಾಡುತ್ತೇವೆ.

ನಾವು ತಮಗೆ ಈ ಮೂಲಕ ದೃಢಪಡಿಸುವುದೇನೆಂದರೆ ಈ ನಮ್ಮ ಅಧ್ಯಯನದಿಂದ ತಮಗೆ ಯಾವುದೇ ರೀತಿಯ ಆರ್ಥಿಕ ಹೊರೆಯಾಗದು ಮತ್ತು ಏನಾದರೂ ಪ್ರಮುಖವಾದ ಅಂಶಗಳು ಅಚಾನಕ ಆಗಿ ಕಂಡು ಬಂದಲ್ಲಿ ತಮಗೆ ಸೂಕ್ತ ಮಾಹಿತಿಯನ್ನು ನೀಡುತ್ತೇವೆ.

ಪ್ರಧಾನ ಸಂಶೋಧಕ ಮತ್ತು ಮೇಲ್ವಿಚಾರಕ ನೊಂದಿಗಿನ ಈ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂದಿಸಿದ ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕೆ ಮುಕ್ತವಾಗಿರಿ

ಪ್ರಧಾನ ಪ್ರಬಂಧಕರು: ಶ್ರೀಮತಿ ಆರ್.ಸಾಯಿದೀಪಿಕಾ: 9036413299
ಮೇಲ್ವಿಚಾರಕರು: ಡಾ॥ ಕೆ.ಎನ್.ಶಶಿಧರ್: 9845248742
ಸಹ ಮೇಲ್ವಿಚಾರಕರು: ಡಾ॥ ರವೀಶಾ. ಎ: 9448448353

ಮಾಹಿತಿ ಪತ್ರ

ಶೀರ್ಷಿಕೆ: ಎಸ್ಟಿಮೇಷನ್ ಆಫ್ ಪ್ಲೂರೈಡ್ ಅಂಡ್ ಸಿರ್ಟಂಯನ್ 1 ಇನ್ ಪೇಷಂಟ್ಸ್ ವಿಥ್ ಡಯಾಬಿಟಿಕ್ ನೆಪ್ರೊಪಥಿ ಇನ್ ಕೋಲಾರ್ ಜಿಲ್ಲೆ, ಕರ್ನಾಟಕ, ಭಾರತ.

ಪ್ರಧಾನ ಸಂಶೋಧಕರು: ಶ್ರೀಮತಿ. ಆರ್. ಸಾಂಖ ದೀಪಿಕಾ

ಅವಧಿ: ಸಂಶೋಧನೆ ಸುಮಾರು 3 ವರ್ಷಗಳ ಕಾಲ ನಡೆಯುತ್ತದೆ.

ಪ್ರಬಂಧಕರ ವ್ಯಾಖ್ಯೆ: ಶ್ರೀಮತಿ. ಆರ್.ಸಾಂಖ ದೀಪಿಕಾ ಆದ ನಾನು ಪಿ. ಹೆಚ್. ಡಿ ಉನ್ನತ ವ್ಯಾಸಂಗವನ್ನು ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾ ವಿದ್ಯಾಲಯ, ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ಉನ್ನತ ಶಿಕ್ಷಣ ಹಾಗೂ ಸಹ ಪ್ರಾಯೋಗಿಕ ಸಂಸ್ಥೆ, ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ವಿಭಾಗದಲ್ಲಿ ಮಾಡುತ್ತಿದ್ದೇನೆ,

ಕೋಲಾರ ಜಿಲ್ಲೆಯಲ್ಲಿ ಮಣ್ಣಿನಮತ್ತು ಕುಡಿಯುವ ನೀರಿನಲ್ಲಿ ಫ್ಲೋರೈಡ್ ಅಂಶವು ವರ್ಷದಿಂದ ವರ್ಷಕ್ಕೆ ಉಲ್ಬಣಗೊಳ್ಳುತ್ತಿದೆ, ಈ ಜನಸಂಖ್ಯೆಯ ಬಹುಪಾಲು ಜನ ಕೃಷಿಕರು ಮತ್ತು ಜಾನುವಾರುಗಳ ಮೇಲೆ ಅವಲಂಬಿತವಾಗಿರುವ ಕಾರಣದಿಂದಾಗಿ, ತಮ್ಮ ಜೀವನೋಪಾಯಕ್ಕಾಗಿ ಮಳೆ ನೀರು ಆರ್ಥಿಕ ಸ್ಥಿತಿಯೊಂದಿಗೆ ಅನಕ್ಷರತೆ, ಬಡತನ, ಅಜ್ಞಾನ ಇತ್ಯಾದಿಗಳಿಂದ ಬಳಲುತ್ತಿದ್ದು, ಇದನ್ನು ತಿಳಿಗೊಳಿಸಲು ಒಂದು ಅಧ್ಯಯನವನ್ನು ಮುಂದುವರಿಸಲು ಉದ್ದೇಶಿಸಿ ದ್ದೇನೆ. ಡಯಾಬಿಟಿಸ್ (ಸಕ್ಕರೆ / ಸಿಹಿ ಮೂತ್ರ ರೋಗ) ಮತ್ತು ಅದರ ಸಂಬಂಧಿತ ಮೈಕ್ರೊವ್ಯಾಸ್ಕುಲರ್ (ಅತೀ ಸಣ್ಣರಕ್ತ ನಾಳಗಳು) ತೊಡಕುಗಳ ಮೇಲೆ ಪ್ರಭಾವ ಬೀರುವ ಫ್ಲೋರೈಡ್ ಅನ್ನೂ ಯಾವುದೇ ಸಂಯೋಜನೆಯ ಡಯಾಬಿಟಿಕ್ ನೆಪ್ರೋಪತಿ ಎಂದು ಕರೆಯಲಾಗುತ್ತದೆ. ಇದು ಮಧುಮೇಹದ ಆರಂಭಿಕ ತೊಡಕುಗಳಲ್ಲಿ ಒಂದಾಗಿದ್ದು ಮತ್ತು ಪ್ರಾಥಮಿಕ ಹಂತದಲ್ಲಿ ಉದ್ದೇಶಿಸಬೇಕಾಗಿರುತ್ತದೆ.ಈ ಅಧ್ಯಯನವು ತಮ್ಮ ಅನುಮತಿ ಹಾಗೂ ಸಹಕಾರದಿಂದ ಸ್ಥಳೀಯ ಜನಸಂಖ್ಯೆಗೆ ಉತ್ತಮ ಗುಣಮಟ್ಟದ ಜೀವನ ನಡೆಸಲು ನೆರವಾಗಬಹುದು.

ಡಯಾಬಿಟಿಕ್ನೆಫ್ರೋಪತಿಅಗಾಧವಾದಆಕ್ಸಿಡೇಟಿವ್ಒತ್ತಡದಿಂದಾಗಿಮತ್ತುಅನಿಯಂತ್ರಿತಮತ್ತು /
ಅಥವಾಮಧುಮೇಹದದೀರ್ಘಕಾಲದಕಾರಣದಿಂದಾಗಿ.ಗ್ಲೈಕೊಸೈಲೇಟೆಡ್ಹೆಮೊಗ್ಲೋಬಿನ್,
ಫ್ರಕ್ಟೋಸ್ಯಾಮೈನ್ಇತ್ಯಾದಿಗಳಿಗೆಗ್ಲೈಕೊಸೈಲೇಷನ್ಒಳಗಾಗುವಒತ್ತಡದನಿಯತಾಂಕಗಳನ್ನುಮತ್ತುಪ್ರೋಟೀನ್ಗಳನ್ನುಅಂದಾಜು ಮಾಡುವುದರಮೂಲಕಇದನ್ನುಗಮನಿಸಬೇಕು.

ಇದರೊಂದಿಗೆ, ನಾನು "ಎಸ್ಟಿಮೇಷನ್ ಆಫ್ ಪ್ಲೂರೈಡ್ ಅಂಡ್ ಸಿರ್ಟಂಯನ್ 1 ಇನ್ ಪೇಷಂಟ್ಸ್ ವಿಥ್ ಡಯಾಬಿಟಿಕ್ ನೆಪ್ರೊಪಥಿ ಇನ್ ಕೋಲಾರ್ ಜಿಲ್ಲೆ, ಕರ್ನಾಟಕ, ಭಾರತ "ಎಂಬ ಶೀರ್ಷಿಕೆಯ ಅಧ್ಯಯನವನ್ನು ನಡೆಸುತ್ತಿದ್ದೇನೆ ಎಂದು ತಿಳಿಸಲು ನಾನು ಬಯಸುತ್ತೇನೆ.

ಪರೀಕ್ಷಾರ್ತಿಗಳ ಆಯ್ಕೆ:ಆರ್.ಎಲ್. ಜಾಲಪ್ಪ ಹಾಸ್ಪಿಟಲ್ ಮತ್ತು ರಿಸರ್ಚ್ ಸೆಂಟರ್ನ ಜೆನರಲ್ ಮೆಡಿಸಿನ್ ವಿಭಾಗದ ಹೊರ ರೋಗಿಗಳ ಮತ್ತು ಒಳರೋಗಿಗಳ ವಾರ್ಡನಲ್ಲಿ ವಯಸ್ಸಿನ ವಯೋಮಿತಿ 25-60 ವರ್ಷ ಒಳಗಿನ ಇರತಕ್ಕ ಸಕ್ಕರೆ ಕಾಯಿಲೆಯ ರೋಗಿಗಳು ಹೊಸ ನೇಮಕವಾದರು ಭರ್ತಿಯಾದ ಸಮ್ಮತಿ ಪತ್ರವನ್ನು ತುಂಬುತಕ್ಕದ್ದು.

ಈಅಧ್ಯಯನದಲ್ಲಿನಸಂಶೋಧನೆಗಳುಈತೊಡಕುಗಳನ್ನುಉತ್ತಮವಾಗಿಅರ್ಥಮಾಡಿಕೊಳ್ಳಲುಸಹಾಯಮಾಡುತ್ತದೆಮತ್ತುಅದರೊಂದಿಗೆಸಂಬಂಧಿಸಿದಹಾನಿಗಳನ್ನುಒಳಗೊಳ್ಳಲುಉತ್ತಮಚಿಕಿತ್ಸಕಮಧ್ಯಸ್ಥಿಕೆಗಳಿಗೆಕಾರಣವಾಗಬಹುದು: ವಯಸ್ಸಾದ,

ಮತ್ತುಮಧುಮೇಹನಫ್ರೋಪತಿಗಳಂತಹಶಾರೀರಿಕಮತ್ತುರೋಗಶಾಸ್ತ್ರೀಯಪರಿಸ್ಥಿತಿಗಳಅಡಿಯಲ್ಲಿವ್ಯಕ್ತಪಡಿಸಿದಹೊಸಅಣುವಾಗಿದೆ ಸಿರ್ಟಂಯುನ್ 1.
ಪರೀಕ್ಷಾ ವಿಧಾನ: ಈ ವಿಷಯದಲ್ಲಿ, ನಿಮ್ಮ ವೈಯಕ್ತಿಕ, ಹಿಂದಿನ ಮತ್ತು ಕೌಟಂಬಿಕ ಇತಿಹಾಸದ ಬಗ್ಗೆ ನಾನು ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುತ್ತೇನೆ. ನನ್ನ ಅಧ್ಯಯನದ ಉದ್ದೇಶಕ್ಕಾಗಿ ನಾನು ತಮ್ಮ 6 ಮಿಲಿ ರಕ್ತ ಮತ್ತು ಮೂತ್ರವನ್ನು (10 ಮಿಲಿ) ಸಂಗ್ರಹಿಸಬೇಕಾಗಿದೆ. ನಿಮಗೆ ಬೇಕಾದ ಪ್ರಶ್ನೆಗಳಿಗೆ ನಾನು ಉತ್ತರಿಸಲು ಸಿದ್ಧನಿರುತ್ತೇನೆ ಹಾಗೂ ನೀವು ಸ್ವತಂತ್ರರಾಗಿರುತ್ತೀರಿ.ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನಿಮ್ಮ ಪ್ರಾಮಾಣಿಕ ಉತ್ತರವು ರೋಗಿಗಳ ಆರೈಕೆಯನ್ನು ಚೆನ್ನಾಗಿ ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ನಮಗೆ ಸಹಾಯ ಮಾಡುತ್ತದೆ.ನಾನು ಈ ನಿಮ್ಮ ಅಮೂಲ್ಯ ಸಮಯ ಮತ್ತು ನಿಮಗೆ ಯಾವುದೇ ಖರ್ಚು ವೆಚ್ಚ ವಿಧಿಸಲಾಗುವುದಿಲ್ಲ.

ರಕ್ತದಗ್ಲುಕೋಸ್, ಮೂತ್ರಪಿಂಡದಕಾರ್ಯಪರೀಕ್ಷೆಗಳುಮತ್ತುಫ್ಲೂರೈಡ್ಮಟ್ಟವನ್ನುರಕ್ತದಲ್ಲಿಅಂದಾಜುಮಾಡಲುಸುಮಾರು 6
ಮಿಲಿರಕ್ತದನಿರೋಧಕಮುನ್ನೆಚ್ಚರಿಕೆಗಳನ್ನುತೆಗೆದುಕೊಳ್ಳಲಾಗುತ್ತದೆ.ನಿಮ್ಮಸ್ಥಿತಿಯಅವಶ್ಯಕತೆಗೆಅನುಗುಣವಾಗಿರಕ್ತದಲ್ಲಿನಸಕ್ಕರೆಅಂದಾಜುಗಳುಮತ್ತುಮೂತ್ರಪಿಂಡಕಾರ್ಯದಅಂದಾಜುಗಳನ್ನುಕೈಗೊಳ್ಳಲಾಗುವುದು.ಮೇಲಿನದ್ದಲ್ಲದೆ,
ಒಟ್ಟಾರೆಉತ್ಕರ್ಷಣನಿರೋಧಕಗಳನ್ನುಅಂದಾಜುಮಾಡುವುದು,
ಉದಾಹರಣೆಗೆಗ್ಲೈಕೊಸೈಲೇಷನ್ಗೆಒಳಗಾಗುವಆಕ್ಸಿಡೇಟಿವ್ಒತ್ತಡನಿಯತಾಂಕಗಳುಮತ್ತುಪ್ರೋಟೀನ್ಗಳು,
ಗ್ಲೈಕೊಸೈಲೇಟೆಡ್ಹೆಮೋಗ್ಲೋಬಿನ್ (ಅಾಛಂ೧ಛ), ಫ್ರಕ್ಟೋಸ್ಯಾಮೈನ್ಮೂಡರಕ್ತ / ಮೂತ್ರದಲ್ಲಿಯೂಮಾಡಲ್ಪಡುತ್ತವೆ. ಜೊತೆಗೆ 10 ಮಿಲಿಮೂತ್ರವನ್ನುಸಹಆಸ್ಪತ್ರೆಯಲ್ಲಿಸಂಗ್ರಹಿಸಿಪರೀಕ್ಷಿಸಲಾಗುವುದು.ಮೇಲಿನಪರೀಕ್ಷೆಗಳಿಗೆಯಾವುದೇಹೆಚ್ಚುವರಿವೆಚ್ಚವನ್ನುವಿಧಿಸಲಾಗುವುದಿಲ್ಲ.

ಪಡೆದ ಮಾಹಿತಿಯನ್ನು ಕಟ್ಟುನಿಟ್ಟಾಗಿ ಗೋಪ್ಯವಾಗಿಡುತ್ತೇನೆ. ಸಂಪೂರ್ಣ ಮಾಹಿತಿ, ನಿಮ್ಮಿಂದ ಪಡೆದ ತನಿಖಾ ವರದಿ ಮತ್ತು ಅದರ ವಿವರಗಳನ್ನು ಸಂಶೋಧನೆಗೆ ಮತ್ತು ರೋಗ ನಿರ್ಣಯ ಮತ್ತು / ಅಥವಾ ನಿರ್ವಹಣೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಒಂದೊಮ್ಮೆ ಎನಾದರೂ, ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ, ಯಾವುದೇ ಪ್ಯಾರಾಮೀಟರ್ಗಳು ಅಥವಾ ಸಮಸ್ಯೆಗಳನ್ನು ಬಗೆಹರಿಸಬೇಕಾದರೆ ಮತ್ತು ಆಕಸ್ಮಿಕವಾಗಿ ಕಂಡುಬರುವ ಅಚಾನಕ ಪ್ರತಿರೋದ ಅಥವ ದುಷ್ಪರಿಣಾಮ ಕಂಡುಬಂದಲ್ಲಿ ಹಾಗೂ ಯಾವುದೇ ತಮಗೆ ಹೆಚ್ಚುವರಿ ವೆಚ್ಚ ಆಗದಹಾಗೆ ಹೆಚ್ಚಿನ ನಿರ್ವಹಣೆಯ ಸರಿಯಾದ ಮಾರ್ಗದರ್ಶನವನ್ನು ನಿಮಗೆ ತಿಳಿಸಲಾಗುವುದು.

ಈ ಸಂಶೋಧನೆ ಯಿಂದಾಗಲಿ, ಪ್ರಬಂಧದಲ್ಲಾಗಲಿ ಅಥವ ಪರೀಕ್ಷೆಯಲ್ಲಾಗಲಿ ಯಾವುದೇ ದುರುದ್ದೇಶವಿಲ್ಲ. ನಿಮ್ಮ ಎಲ್ಲಾ ವಿವರಗಳನ್ನು ಸಂಪೂರ್ಣವಾಗಿ ಗೌಪ್ಯವಾಗಿ ಇಡುತ್ತೇನೆ ಹಾಗೂ ಕಾಪಾಡುತ್ತೇನೆ ಎಂದು ದೃಡಪಡಿಸುತ್ತೇನೆ. ತಮಗಾಗಲಿ ಅಥವ ತಮ್ಮ ವಿಶ್ವಾಸಿಗಳಿಗಾಗಲಿ ಎಂದಿಗಾದರೂ ಯಾವ ರೀತಿಯಲ್ಲಿ ಅನುಮಾನಗಳಿದ್ದರೂ ನಾನು ಖುದ್ದಾಗಿ ಬಗೆಹರಿಸುತ್ತೇನೆ. ತಮಗೇನಾದರೂ ಈ ಪರೀಕ್ಷೆಗೆ ಸಮ್ಮತಿ ಇಲ್ಲ ಎಂದು ಅನ್ನಿಸಿದ ಪಕ್ಷದಲ್ಲಿ ತಾವು ಯಾವುದೇ ಷರತ್ತುಗಳಿಲ್ಲದೆ ಸೂಕ್ತ ಮಾಹಿತಿಯನ್ನು ನೀಡಿ ಹಿಂದಿರುಗಬಹುದು. ನನ್ನಿಂದ ಅಥವ ನನ್ನ ತಂಡದಿಂದ ಯಾವುದೇ ಆಕ್ಷೇಪಣೆಗಳಿರುವುದಿಲ್ಲ.

ಅಗತ್ಯವಿರುವ ಸೂಕ್ತ ರಕ್ತದ ಮಾದರಿಯನ್ನು ತಮ್ಮಿಂದ ಪಡೆಯಲು ನನಗೆ ತಾವು ಅನುಮತಿಸಿ ಮತ್ತು ನಿಮ್ಮಿಂದ ಮೂತ್ರವನ್ನು ಸಂಗ್ರಹಿಸಿ ಭವಿಷ್ಯದ ತನಿಖೆಗಳಿಗೆ ದ್ವಿತೀಯ ಮಾದರಿಯನ್ನು ಸಂರಕ್ಷಿಸಲು ನಾನು ತಮ್ಮಲ್ಲಿ ವಿನಂತಿಸುತ್ತೇನೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂ ಪ್ರೇರಿತವಾಗಿರುತ್ತದೆ.ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ನಿರ್ಬಂಧವಿಲ್ಲ ನೀವು ನಮ್ಮ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ, ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈ ಅಧಯ್ಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನೀವು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದರೆ ಮಾತ್ರ ನೀವು ಸಹಿ ಮಾಡ ಬೇಕಾಗುತ್ತದೆ.ನೀವು

ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನ ದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಸ್ವಾತಂತ್ರ ವಾಗಿರುತ್ತೀರಿ ನಿಮ್ಮ ಹಿಂತೆಗದುಕೊಳ್ಳವಿಕೆಯು ವೈದ್ಯಕೀಯ ಸೇವೆಯಲ್ಲಿ ಯಾವುದೇ ರೀತಿಯ ಪ್ರತಿಕೂಲ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ ಎಂದು ನಾವು ಭರವಸೆನೀಡುತ್ತೇವೆ. ನಾವು ತಮಗೆ ಈ ಮೂಲಕ ತಿಳಿಯಪಡಿಸುವುದೇನಂದರೆ ತಮ್ಮ ರಕ್ತ ಅಥವಾ ಮೂತ್ರ ಮಾದರಿಯನ್ನು ಕೂಡಿ ಕರಿಸಿ ಮುಂದೆಂದಾರೂ ಪರೀಕ್ಷೆ ಮಾಡಬೇಕಾದ್ದಲ್ಲಿ ಅದನ್ನು ಉಪಯೋಗಿಸಲು ಸಮ್ಮತಿ ಪಡೆಯುತ್ತೇವೆ.

ನಾವೂ ಪ್ರಕಟಿಸ ಬಹುದಾದ ಮುಂದೆಂದಾದರೂ ಪ್ರಬಂಧವನ್ನು ಮಂಡಿಸುವಾಗತಮ್ಮ ನಿಖರತೆಯ ಗೌಪ್ಯವನ್ನು ಕಾಪಾಡುತ್ತೇವೆ.

ನಾವು ತಮಗೆ ಈ ಮೂಲಕ ದೃಢಪಡಿಸುವುದೇನೆಂದರೆ ಈ ನಮ್ಮ ಅಧ್ಯಯನದಿಂದ ತಮಗೆ ಯಾವುದೇ ರೀತಿಯ ಆರ್ಥಿಕ ಹೊರೆಯಾಗದು ಮತ್ತು ಏನಾದರೂ ಪ್ರಮುಖವಾದ ಅಂಶಗಳು ಅಚಾನಕ ಆಗಿ ಕಂಡು ಬಂದಲ್ಲ ತಮಗೆ ಸೂಕ್ತ ಮಾಹಿತಿಯನ್ನು ನೀಡುತ್ತೇವೆ.

ಪ್ರಧಾನ ಸಂಶೋಧಕ ಮತ್ತು ಮೇಲ್ವಿಚಾರಕ ನೊಂದಿಗಿನ ಈ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂದಿಸಿದ ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕೆ ಮುಕ್ತವಾಗಿರಿ

ಪ್ರಧಾನ ಪ್ರಬಂಧಕರು: ಶ್ರೀಮತಿ ಆರ್.ಸಾಯಿದೀಪಿಕಾ: 9036413299

ಮೇಲ್ವಿಚಾರಕರು: ಡಾ॥ಕೆ.ಎನ್.ಶಶಿಧರ್: 9845248742

ಸಹ ಮೇಲ್ವಿಚಾರಕರು: ಡಾ॥ ರವೀಶಾ. ಎ: 9448448353

WRITTEN CONSENT FORM

(For healthy controls from the same population)

Sl.no:

Title of the study: Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India

I do here by give my written consent for the study titled: Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India

I understand that I remain free to withdraw from this study at any time giving a valid reason. I have accepted to give 6ml of blood and 10mL of spot urine to the principal investigator or any person assigned for this study.

The procedure and consequence has been explained to me in my own understandable language. I have read and understood the purpose of this study and the confidential nature of the information that will be collected and preserved throughout the study as explained to me in the patient information sheet. The information collected will be used only for research.

I permit you to perform the tests as well as preserve the secondary sample for any future investigations.

I have taken the opportunity to ask questions/doubts regarding various aspects of this study and my questions have been answered by the Principal investigator to my satisfaction.

I the undersigned agree to participate in this study and authorize the collection of samples.

I also understand that there is no risk to my life from this study. Participation in this study does not involve any financial burden.

1. Healthy volunteer's name and signature / thumb impression Date:

2. Name and signature of witness Date:

3. Name and signature of interviewer/Investigator: Date:

WRITTEN CONSENT FORM

(For Patients)

Sl.no:

Title of the study: Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India

I do here by give my written consent for the study titled: Estimation of fluoride and sirtuin1 in patients with diabetic nephropathy in Kolar district of Karnataka, India

I understand that I remain free to withdraw from this study at any time giving a valid reason. I have accepted to give 6ml of blood and 10mL of spot urine to the principal investigator or any person assigned for this study.

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I permit you to perform the tests as well as preserve the secondary sample for any future investigations.

I have taken the opportunity to ask questions/doubts regarding various aspects of this study and my questions have been answered by the Principal investigator to my satisfaction.

I the undersigned agree to participate in this study and authorize the collection of samples.

I also understand that there is no risk to my life from this study. Participation in this study does not involve any financial burden.

1. Patient's name and signature / thumb impression Date:

2. Name and signature of witness Date:

3. Name and signature of interviewer/Investigator: Date:

ರೋಗಿಯಸಮ್ಮತಿ ಪತ್ರ

ಅಧ್ಯಾಯನದ ಶೀರ್ಷಿಕೆ: ಎಸ್ಟಿಮೇಷನ್ ಆಫ್ ಫ್ಲೋರೆಡ್ ಅಂಡ್ ಸಿರ್ಟಂಯನ್ 1 ಇನ್ ಪೇಷಂಟ್ಸ್ ವಿಥ್ ಡಯಾಬಿಟಿಕ್ ನೆಪ್ರೊಪಥಿ ಇನ್ ಕೋಲಾರ್ ಜಿಲ್ಲೆ, ಕರ್ನಾಟಕ, ಭಾರತ.

ನಾನು ನನ್ನ ಪೂರ್ಣ ಸಮ್ಮತಿಯನ್ನು ಅಧ್ಯಯನದ ವಿಷಯವಾದ "ಎಸ್ಟಿಮೇಷನ್ ಆಫ್ ಫ್ಲೋರೆಡ್ ಅಂಡ್ ಸಿರ್ಟಂಯನ್ 1 ಇನ್ ಪೇಷಂಟ್ಸ್ ವಿಥ್ ಡಯಾಬಿಟಿಕ್ ನೆಪ್ರೊಪಥಿ ಇನ್ ಕೋಲಾರ್ ಜಿಲ್ಲೆ, ಕರ್ನಾಟಕ, ಭಾರತ" ಎಂಬ ಪ್ರಬಂಧಕ್ಕೆ ಕೊಟ್ಟಿರುತ್ತೇನೆ.

ನನ್ನ ಗ್ರಹಿಕೆಯಿಂದ ಒಂದು ಸೂಕ್ತ ಕಾರಣವನ್ನು ನೀಡಿ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನಾನು ಈ ಅಧ್ಯಯನದಿಂದ ಉದಾರವಾಗಿ ಹಿಂಜರಯ ಬಹುದು ಎಂದು ಖಚಿತ ಪಡಿಸಿರುತ್ತೇನೆ. ನಾನು ಈ ಅಧ್ಯಯನಕ್ಕಾಗಿ ನನ್ನ 6 ಮಿಲಿ ರಕ್ತ ಹಾಗೂ 10ಮಿಲಿ ಸಾಂಧರ್ಭಿಕ ಮೂತ್ರವನ್ನು ಪ್ರದಾನ ತನಿಖೆದಾರ ಅಥವಾ ಇದಕ್ಕೆ ಸಂಬಂಧಪಟ್ಟ ಯಾವುದೇ ವ್ಯಕ್ತಿಗೆ ವಹಿಸಿದವಲಿಗೆ ನೀಡಲು ಒಪ್ಪಿರುತ್ತೇನೆ.

ನನಗೆ ಸ್ವಂತ ಅರ್ಥವಾಗುವ ಭಾಷೆಯಲ್ಲಿ ಇದರ ಕಾರ್ಯ ವಿಧಾನ ಮತ್ತು ಪರಣಾಮವನ್ನು ಪ್ರಧಾನ ಸಂಶೋಧಕರು ವಿವರಿಸಿರುತ್ತಾರೆ. ನಾನು ಅಧ್ಯಯನ ವಿಷಯವನ್ನು ಓದಿ ಮತ್ತು/ ಅಥವ ಅವರ ವಿವರಣೆ ನನಗೆ ಪಡೆದುಕೊಂಡು, ಸದರಿ ಅಧ್ಯಯನ ವಿಷಯದ ಉದ್ದೇಶ ಮತ್ತು ಅದರ ಗೌಪ್ಯ ಸ್ವಭಾವ, ಅದರ ಬಗ್ಗೆ ಮಾಹಿತಿ ಅದನ್ನು ಸಂಗ್ರಹಿಸಿ ಮತ್ತು ಸುರಕ್ಷ ವಾಗಿಡಲು ಈ ಅಧ್ಯಯನದಕಾಲಾವಧಿಯಲ್ಲಿ ಮಾಡಲು ಈ ಪ್ರಕಾರ ಸಮ್ಮತಿಸಿರುತ್ತೇನೆ. ಈ ಮಾಹಿತಿ ಸಂಗ್ರಹಣೆಯನ್ನು ಅಧ್ಯಯನ ಉದ್ದೇಶಕ್ಕಾಗಿ ಮಾತ್ರ ಬಳಸಿತಕ್ಕದ್ದು.

ನಾನು ಪ್ರಶ್ನೆಗಳು/ ಶಂಕೆಗಳು ಕೇಳುವ ಅವಕಾಶವನ್ನು ಈ ಅಧ್ಯಯನದ ವಿಷಯಾಂಶಕ್ಕೆ ಸಂಬಂಧಪಟ್ಟ ಹಾಗೂ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಪ್ರಧಾನ ತನಿಖೆದಾರ ನನಗೆ ತೃಪ್ತಿಯಾಗುವ ರೀತ್ಯಾ ಉತ್ತರಿಸಿರುತ್ತಾರೆ.

ನಾನು ಈ ಕೆಳಗೆ ರುಜು ಮಾಡಿರುವ ಈ ಅಧ್ಯಯನದ ವಿಷಯದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪಿ ಮತ್ತು ನನ್ನ ರಕ್ತದ ಮಾದರಿಗಳನ್ನು ಒಟ್ಟುಗೂಡಿಸಿಡುವುದಕ್ಕೆ ಪ್ರತಿನಿಧಿಸಿರುತ್ತೇನೆ.

ನಾನು ಈ ಅಧ್ಯಯನದಿಂದ ನನ್ನ ಜೀವಕ್ಕೆ ಯಾವುದೇ ರೀತಿ ಹಾನಿ ಉಂಟಾಗದೆಂದು ಅರ್ಥಯಿಸಿ ಕೊಂಡಿರುತ್ತೇನೆ. ಈ ಅಧ್ಯಯನದ ವಿಷಯ ದಿಂದ ಯಾವುದೇ ರೀತಿಯ ಆರ್ಥಿಕ ಹೊರೆ ಹಾಗದೆಂದು ತಿಳಿದಿರುತ್ತೇನೆ.

1. ಅಧ್ಯಯನದ ವಿಷಯ ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆಣನಗುರುತು ದಿನಾಂಕ :

2. ಸಾಕ್ಷಿದಾರನ ಹೆಸರು ಮತ್ತು ಸಹಿ ದಿನಾಂಕ :

3. ಶೋಧನಕಾರ / ತನಿಖೆದಾರನ ಹೆಸರು ಮತ್ತು ಸಹಿ ದಿನಾಂಕ :

ನಿರೋಧಿಯ ಸಮ್ಮತಿ ಪತ್ರ

ಅಧ್ಯಾಯನದ ಶೀರ್ಷಿಕೆ: ಎಸ್ಟಿಮೇಷನ್ ಆಫ್ ಫ್ಲೋರೆಡ್ ಅಂಡ್ ಸಿರ್ಟಂಖನ್ 1 ಇನ್ ಪೇಷಂಟ್ಸ್ ವಿಥ್ ಡಯಾಬಿಟಿಕ್ ನೆಪ್ರೊಪಥಿ ಇನ್ ಕೋಲಾರ್ ಜಿಲ್ಲೆ, ಕರ್ನಾಟಕ, ಭಾರತ.

ನಾನು ನನ್ನ ಪೂರ್ಣ ಸಮ್ಮತಿಯನ್ನು ಅಧ್ಯಯನದ ವಿಷಯವಾದ "ಎಸ್ಟಿಮೇಷನ್ ಆಫ್ ಫ್ಲೋರೆಡ್ ಅಂಡ್ ಸಿರ್ಟಂಖನ್ 1 ಇನ್ ಪೇಷಂಟ್ಸ್ ವಿಥ್ ಡಯಾಬಿಟಿಕ್ ನೆಪ್ರೊಪಥಿ ಇನ್ ಕೋಲಾರ್ ಜಿಲ್ಲೆ, ಕರ್ನಾಟಕ, ಭಾರತ" ಎಂಬ ಪ್ರಬಂಧಕ್ಕೆ ಕೊಟ್ಟಿರುತ್ತೇನೆ.

ನನ್ನ ಗ್ರಹಿಕೆಖಂದ ಒಂದು ಸೂಕ್ತ ಕಾರಣವನ್ನು ನೀಡಿ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನಾನು ಈ ಅಧ್ಯಯನದಿಂದ ಉದಾರವಾಗಿ ಹಿಂಜರಯ ಬಹುದು ಎಂದು ಖಚಿತ ಪಡಿಸಿರುತ್ತೇನೆ. ನಾನು ಈ ಅಧ್ಯಯನಕ್ಕಾಗಿ ನನ್ನ ೬ ಮಿಲಿ ರಕ್ತ ಹಾಗೂ 10ಮಿಲಿ ಸಾಂಧರ್ಬಿಕ ಮೂತ್ರವನ್ನು ಪ್ರದಾನ ತನಿಖೆದಾರ ಅಥವಾ ಇದಕ್ಕೆ ಸಂಬಂಧಪಟ್ಟ ಯಾವುದೇ ವ್ಯಕ್ತಿಗೆ ವಹಿಸಿದವಲಿಗೆ ನೀಡಲು ಒಪ್ಪಿರುತ್ತೇನೆ.

ನನಗೆ ಸ್ವಂತ ಅರ್ಥವಾಗುವ ಭಾಷೆಯಲ್ಲಿ ಇದರ ಕಾರ್ಯ ವಿಧಾನ ಮತ್ತು ಪರಣಾಮವನ್ನು ಪ್ರಧಾನ ಸಂಶೋಧಕರು ವಿವರಿಸಿರುತ್ತಾರೆ. ನಾನು ಅಧ್ಯಯನ ವಿಷಯವನ್ನು ಓದಿ ಮತ್ತು/ ಅಥವ ಅವರ ವಿವರಣೆ ನನಗೆ ಪಡೆದುಕೊಂಡು, ಸದರಿ ಅಧ್ಯಯನ ವಿಷಯದ ಉದ್ದೇಶ ಮತ್ತು ಅದರ ಗೌಪ್ಯ ಸ್ವಭಾವ, ಅದರ ಬಗ್ಗೆ ಮಾಹಿತಿ ಅದನ್ನು ಸಂಗ್ರಹಿಸಿ ಮತ್ತು ಸುರಕ್ಷ ವಾಗಿಡಲು ಈ ಅಧ್ಯಯನದಕಾಲಾವಧಿಯಲ್ಲಿ ಮಾಡಲು ಈ ಪ್ರಕಾರ ಸಮ್ಮತಿಸಿರುತ್ತೇನೆ. ಈ ಮಾಹಿತಿ ಸಂಗ್ರಹಣೆಯನ್ನು ಅಧ್ಯಯನ ಉದ್ದೇಶಕ್ಕಾಗಿ ಮಾತ್ರ ಬಳಸಿತಕ್ಕದ್ದು.

ನಾನು ಪ್ರಶ್ನೆಗಳು/ ಶಂಕೆಗಳು ಕೇಳುವ ಅವಕಾಶವನ್ನು ಈ ಅಧ್ಯಯನದ ವಿಷಯಾಂಶಕ್ಕೆ ಸಂಬಂಧಪಟ್ಟ ಹಾಗೂ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಪ್ರಧಾನ ತನಿಖೆದಾರ ನನಗೆ ತೃಪ್ತಿಯಾಗುವ ರೀತ್ಯಾ ಉತ್ತರಿಸಿರುತ್ತಾರೆ.

ನಾನು ಈ ಕೆಳಗೆ ರುಜು ಮಾಡಿರುವ ಈ ಅಧ್ಯಯನದ ವಿಷಯದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪಿ ಮತ್ತು ನನ್ನ ರಕ್ತದ ಮಾದರಿಗಳನ್ನು ಒಟ್ಟುಗೂಡಿಸಿಡುವುದಕ್ಕೆ ಪ್ರತಿನಿಧಿಸಿರುತ್ತೇನೆ.

ನಾನು ಈ ಅಧ್ಯಯನದಿಂದ ನನ್ನ ಜೀವಕ್ಕೆ ಯಾವುದೇ ರೀತಿ ಹಾನಿ ಉಂಟಾಗದೆಂದು ಅರ್ಥಖಿಸಿ ಕೊಂಡಿರುತ್ತೇನೆ. ಈ ಅಧ್ಯಯನದ ವಿಷಯ ದಿಂದ ಯಾವುದೇ ರೀತಿಯ ಆರ್ಥಿಕ ಹೊರೆ ಹಾಗದೆಂದು ತಿಳಿದಿರುತ್ತೇನೆ.

1. ಅಧ್ಯಯನದ ವಿಷಯ ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆಟಿನಗುರುತು ದಿನಾಂಕ :

2. ಸಾಕ್ಷಿದಾರನ ಹೆಸರು ಮತ್ತು ಸಹಿ ದಿನಾಂಕ :

3. ಶೋಧನಕಾರ / ತನಿಖೆದಾರನ ಹೆಸರು ಮತ್ತು ಸಹಿ ದಿನಾಂಕ :

NEW KNOWLEDGE GENERATED

1. During renal damage fluoride excretion is hindered leading to increased serum fluoride and complications as evinced from our study
2. Increase in sirt1 acted as a protective molecule against fluoride damage
3. Increased CML and F acts as pro- oxidant restricting the effect of sirtuin1 on cellular damage causing further complication such as increased insulin resistance and decreased insulin sensitivity
4. Fructosamine levels are dependent on serum albumin concentration and eventually the serum albumin is decreased in DN and hence there is a fall in fructosamine concentration in DN than in T2DM
5. HOMA- IR and QUICKI can act as better surrogate markers of insulin action in diagnosis of diabetes
6. eGFR equation 2009 is a better indicator and serum Cystatin C is a better standalone marker than creatinine in diagnosis of DN
7. CML decrease during increased sirt1 indicating deacetylation of the protein supression
8. Correlation of insulin resistance and sensitivity with sirt1 is an evidence for curative property of sirt1 in maintaining the metabolism linked with aging