Original Article

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Oxidative Stress, DNA Damage and Serum Vitamin D Levels in Prediabetes

Internal Medicine Section

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ABSTRACT

Introduction: Prediabetes or intermediate hyperglycaemia is a high risk condition with blood glucose levels higher than the normal glycaemic values but lower than the Diabetes Mellitus (DM) diagnostic value. Individuals in prediabetic state have increased likelihood of development of Cardiovascular Diseases (CVD), endothelial dysfunction and obesity. Increased oxidative stress leads to pathogenesis of DM. Early assessment of DNA damage and vitamin D helps in timely management of the condition.

Aim: To assess the association of oxidative stress and serum vitamin D levels in prediabetic individuals.

Materials and Methods: This cross-sectional study was conducted in the Departments of Medicine and Biochemistry, PGIMER and Dr. Ram Manohar Lohia Hospital, New Delhi, India. A total of 60 prediabetic cases and 30 age and sex matched healthy controls were included in the study. Serum and urinary levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (a marker of oxidative stress) and serum levels of vitamin D were compared between prediabetic subjects and healthy controls. An 8-OHdG was measured by sandwich Enzyme-linked Immunosorbent Assay (ELISA) and serum vitamin D levels were measured by enhanced chemiluminescence. Continuous variable was assessed using t-test and nominal variables were compared using chi-square test. The p-value <0.05 was considered significant.

Results: Serum levels of 8-OHdG was significantly higher in prediabetic subjects than controls (826.4±583 pg/mL vs. 584.567±375.205 pg/mL, p-value=0.042). Similarly, urinary levels of 8-OHdG were significantly higher in prediabetic subjects than controls (571.975±421.7 pg/mL vs. 374.9±291.877 pg/mL, p-value=0.024). Serum levels of vitamin D were significantly lower in prediabetic subjects than controls (18.3±14.9 ng/mL vs. 26.7±15.75 ng/mL, p-value=0.016).

Conclusion: Oxidative stress, as confirmed by the biomarker, 8-OHdG, was significantly increased while serum levels of vitamin D were significantly lowered in prediabetes. Therefore, the study recommends the use of vitamin D supplementation as preventive intervention for management of dysglycaemia.

Keywords: 8-hydroxy-2'-deoxyguanosine, Biomarker, Dysglycaemia, Hyperglycaemia

INTRODUCTION

Diabetes Mellitus (DM) refers to a group of metabolic disorders with hyperglycaemia due to genetic or environmental factors. Long-term presence of uncontrolled elevated blood glucose in DM leads to end-organ damage of kidney, retina, nerves and heart [1]. Recent studies suggest the development of microvascular and macrovascular complications such as peripheral neuropathy and Left Ventricular Diastolic Dysfunction (LVDD) in prediabetic stage [2,3]. Literature suggests the role of oxidative stress and decreased levels of vitamin D in the pathogenesis of various complications of diabetes including diabetic neuropathy [4-6]. An 8-OHdG is a major by-product of oxidative DNA damage formed by enzymatic cleavage of ROS induced 8-hydroxylation of the guanine base in mitochondria and nuclear DNA [7,8]. The oxidative stress can be measured by estimation of serum and urinary 8-OHdG levels.

Vitamin D deficiency has been known to be associated with higher Insulin Resistance (IR) and high risk of development of diabetes [9]. Low serum vitamin D levels were found to be associated with high blood glucose levels in Asian Indian prediabetic women [10].

Based on these findings, the present study was aimed to assess the association of oxidative stress (measured by novel biomarker called 8-OHdG) and serum vitamin D levels in the prediabetic individuals.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Departments of Medicine and Biochemistry at PGIMER and Dr. Ram Manohar Lohia Hospital, New Delhi, India, from November 2016 to March 2018. Institutional Ethical Approval (Reg. No.: TP (MD/MS) (38/2016)/IEC/PGIMER/RMLH) and informed consent was obtained before commencement of the study.

For this pilot study, convenient samplling was done, and 60 prediabetic patients either admitted in Inpatient Department (IPD) or visiting the Outpatient Department (OPD) were recruited as per American Diabetes Association (ADA) guidelines [11]. Apparently healthy 30 age and sex-matched individuals were recruited as controls.

Inclusion Criteria

Individuals aged 35-60 years, with fasting plasma glucose between 100 to 125 mg/dL or 2-hours postprandial plasma glucose between 140 to 199 mg/dL {were included in the study only after reconfirming with standard 2-hours OGTT (after 75 gm of glucose solution ingestion)} or HbA1c=5.7-6.4% were recruited as cases. Individuals with fasting plasma glucose below 100 mg/dL or 2-hour postprandial plasma glucose between below 140 mg/dL or HbA1c less than or equal to 5.6 were recruited as controls.

Exclusion Criteria

Individuals with history of diabetes, atherosclerotic diseases, malignancy, chronic kidney disease or autoimmune diseases like SLE were excluded from the study.

Biochemical Analysis

Levels of serum 8-OHdG, urine 8-OHdG and serum vitamin D levels were measured for both cases and control. The spot urine samples and fasting serum samples for 8-Hydroxy-2′-

deoxyguanosine (8-OHdG) and vitamin D were collected and centrifuged at 3000 rpm for 10 minutes. For estimation of urine and serum 8-OHDG, the supernatant was immediately aliquoted and stored at -20°C till batch analysed by sandwich ELISA on EVOLIS twin plus analyser by Biorad (Sincere Biotech Co.; Catalogue No: E13650037). Serum vitamin D levels were measured by Enhanced Chemiluminescence on ECiQ, by Orthoclinical Diagnostics.

STATISTICAL ANALYSIS

Data were represented as Mean±SD. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 22.0. Mean value of continuous variable was compared using Independent Unpaired t-test and nominal variables were compared using chi-Square test. The p-value <0.05 was considered statistically significant.

RESULTS

Demographic and biochemical characteristics of prediabetic cases and controls are shown in [Table/Fig-1].

Variables	Prediabetic cases (n=60)		Controls (n=30)		p-value
	Min.–Max.	Mean±SD	Min.–Max.	Mean±SD	
Age (years)	35-60	48.68±7.78	36-60	46.67±7.99	0.254
Serum Vitamin-D (ng/mL)	8-95.3	18.3±14.9	8-63.8	26.7±15.75	0.016*
Serum 8-OHdG (pg/mL)	105.0-2882.4	826.40±583	134.6-1440.5	584.56±375.2	0.042*
Urine 8-OHdG (pg/mL)	82.0-1974.9	571.97±421.72	59.5-923.8	374.9±291.87	0.024*

[Table/Fig-1]: Demographic and biochemical characteristics of prediabetic cases and controls.
8-OHdG- 8-hydroxy-2'-deoxyguanosine. Unpaired t-test and Chi-Square test, *p-value <0.05 statistically significant

DISCUSSION

The Diabetes Atlas of the International Diabetes Federation reported that the number of people with diabetes in India ("called the Diabetic capital of the world") in 2006 was around 40.9 million, and the prevalence rate is estimated to rise to 69.9 million by the year 2025 [12].

Recent studies suggest the role of oxidative stress in the development of macrovascular and microvascular complications. Nishikawa T et al., reported high urinary 8-OHdG levels depicting high oxidative stress in T2DM patients linked to higher risk of macrovascular complications like atherosclerotic disease [13]. Another study reported that diabetes is a state of increased oxidative stress and increased DNA damage leads to mutations that increase the risk of carcinogenesis and incidence of colon, breast and pancreatic cancer [14]. Kayama Y et al., reported that higher oxidative stress is associated with the entity called diabetic cardiomyopathy that initially presents with diastolic dysfunction, progresses to systolic dysfunction and over heart failure with time [15]. Lee WC et al., showed the beneficial effects of vitamin D analogs supplementation in improving oxidative stress status in diabetics patients [5]. Vitamin D plays a cardinal role in calcium and phosphate metabolism and its deficiency may be associated with various disorders in the body including malignancy, cardiovascular disease, and Type 2 Diabetes Mellitus (T2DM) [16]. Although the exact mechanism remains debatable, the lowered vitamin D levels in T2DM are known to effect β-cells of pancreas [17]. Direct association of lower vitamin D levels and insulin resistance have also been observed [18]. A previous study concluded that subjects with vitamin D deficiency had higher risk of development of DM and its sufficiency is protective against the onset of diabetes [10]. In the present study, with the assistance of novel biochemical marker called 8-OHdG, authors established the role of oxidative stress in prediabetes. Similar results were reported by Shin C et al., where diabetic patients had significantly higher serum 8-OHdG levels and levels were even higher in T2DM patients with advanced microvascular complications [19]. Al-Aubaidy HA et al., also reported that elevated levels of serum 8-OHdG levels in prediabetic group than control group indicating increased oxidative stress even with modest elevation of blood glucose [20].

LIMITATION

The sample size was relatively small and demographic and regional variations were not taken into consideration for assessment of biochemical parameters. Further studies with larger sample size are required.

CONCLUSION

Deoxyribonucleic Acid (DNA) damage was found to be prevalent in prediabetic cases with low vitamin D levels. Vitamin D supplementation is recommended for prevention of progression to diabetic state and development of macrovascular and microvasscular complications.

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