Association of Serum Telomerase Activity in Type 2 Diabetes Mellitus Patients with or without Microalbuminuria

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ABSTRACT

BACKGROUND

Telomerase is an enzyme which helps in maintaining the length of the telomeres. Various studies have associated the telomere length and serum telomerase activity with ageing, insulin resistance and obesity. The metabolic disease type 2 diabetes mellitus is also associated with premature ageing, obesity, insulin resistance. Urine microalbuminuria is one of the frequently used markers to assess renal involvement in non-insulin dependent type 2 diabetes patients. Hence, this study was conducted to estimate the association of serum telomerase activity, a marker of aging and its association with urine microalbuminuria in non-insulin dependent type 2 diabetes patients.

METHODS

The study included 180 non-insulin dependent type 2 diabetes patients divided into two groups – Group I without microalbuminuria; Group II with microalbuminuria and 90 age and sex matched healthy volunteers as control population of the study. The sample size was determined at conveniences. Fasting blood sugar, lipid profile, serum urea, creatinine was estimated by autoanalyser, glycated haemoglobin by high performance liquid chromatography (HPLC) and serum telomerase activity were measured by enzyme-linked immunosorbent assay (ELISA) method. Urinary microalbuminuria was measured by immunoturbidimetry. The data was compared by one-way analysis of variance (ANOVA) and post hoc Tukey's honestly significant difference (HSD) test, linear regression analysis and Pearson correlation analysis.

RESULTS

Non-insulin dependent type 2 diabetes patients with microalbuminuria had significantly lower telomerase activity and higher glycated haemoglobin, dyslipidaemia, increased serum urea and creatinine levels as compared to the group I diabetes mellitus patients and healthy volunteers. The serum telomerase level exhibited a negative correlation with urinary microalbuminuria, glycated hemoglobin and serum triglyceride and BMI.

CONCLUSIONS

Low Serum Telomerase is associated with microalbuminuria, obesity and poor glycaemic control in non-insulin dependent type 2 diabetes mellitus patients, indicating the possible existence of a common clinical profile and premature aging among diabetic patients with chronic kidney disease. Early screening for microalbuminuria and serum telomerase level may assist management of positive cases and reduce the load of chronic renal disease and coronary artery disease in type 2 diabetes patients. Validation of our observations may be done by extending the study in larger population thereby facilitating the understanding of T2DM pathophysiology and its complications.

KEYWORDS

Diabetes Mellitus, Glycated Haemoglobin, Microalbuminuria, Telomerase

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BACKGROUND

Telomeres are found at the ends of chromosomes and are composed of repetitive nucleotide sequence of TTAGGG. The telomeres prevent chromosomal shortening, incomplete replication, protect the chromosomes from fusion with other adjacent chromosomes, deterioration, thus maintaining the regulatory and protein coding sequences in the genome.^{1,2} The telomere shortening is maintained by the enzyme telomerase, whose main components are a reverse transcriptase and an RNA template. Telomerase is responsible for ageing and is not active in mature cells.^{1,2} It is active in immature undifferentiated cells, few mature lymphocytes and during embryogenesis. Various factors such as age, chronic diseases such as diabetes, hypertension, cancer, chronic obstructive lung disease and renal disease have been associated with shortening of telomeres.³⁻⁵ Telomere length is also affected by oxidative and nitrosate stress.^{5,6} Non-insulin dependent type 2 diabetes mellitus (T2DM) is associated with increased body mass Index, insulin resistance, physical inactivity & oxidative stress.⁶⁻¹⁴ Recent research has shown that T2DM and telomere have a bilateral relationship in animal studies.¹⁴⁻¹⁷ These facts have intensified research to evaluate telomerase activity in chronic diseases and whether it can be used therapeutically.¹⁸⁻²³ Many T2DM patients suffer from micro and macro vascular complications such as neuropathy, cardiovascular & coronary artery disease, retinopathy, nephropathy and peripheral vascular disease such as diabetic foot ulcer.21-23

Hence, this study was conducted to estimate the telomerase activity and find its association with microalbuminuria in T2DM Patients.

METHODS

This case control study included 270 individuals, out of which 180 were T2DM patients (Group I - included 90 number of T2DM patients without microalbuminuria; Group II included an equal number of T2DM patients with microalbuminuria) and 90 individuals were age and gender matched healthy volunteers who were controls. This study was conducted between September 2018 to October 2019. The sample size was as per convenient sample size due to constraints of budget and time. The study was conducted in the Molecular Diagnostic Laboratory of the Department of Biochemistry in association with the Department of Medicine, SCB Medical College, Cuttack, Odisha. The study was approved by the institute ethical committee, regd. No. ECR / 84 / Inst / OR / 2013, ICE / IRB No. 697 / 28.9.18. The inclusion criterion was as follows: All T2DM patients within the age of 25 to 65years with confirmed diabetes mellitus as per International Diabetes Federation/ American Diabetes Association (IDF / ADA) criteria were included.²⁴ The duration of disease in majority of the patients was within 7 years. T2DM patients with the history of myocardial disease, neuropathy, stroke, malignancy or other comorbid conditions were excluded. Patients on steroid therapy were excluded. The height, weight, waist circumference was measured, and body mass index was calculated in units of Kg / $m^2.^{25}$

Fasting venous samples were collected in clot-activator, fluoride and ethylenediaminetetraacetic acid (EDTA) tubes. Blood sugar (hexokinase method), serum lipid profile (total cholesterol by cholesterol oxidase method; triglyceride by glycerol-3-phosphate-oxidase / peroxidase method; low density lipoprotein-cholesterol and high density lipoprotein cholesterol were measured by selective inhibition method), serum urea and creatinine was estimated by commercial kits adapted to autoanalyser to assess the renal function. Estimated glomerular filtration rate (eGFR) was calculated using Cockroft–Gault formula: creatinine clearance = {((140 - age) x weight) / (72 x serum creatinine)} x 0.85 (if female).²⁶ Glycated haemoglobin was estimated by HPLC method to estimate the glycaemic control of the study population in the past six weeks. Serum telomerase was estimated by Biocodon kits, Kanas, USA by Quantitative Sandwich Elisa method. Urinary microalbuminuria was measured by immunoturbidimetric method.

Statistical Analysis

All the data has been represented as mean \pm standard deviation. The data was compared by one-way ANOVA with post hoc Tuckey HSD test, linear regression analysis and Pearson correlation analysis using Statistical Package for the Social Sciences (SPSS) version 21 software. Differences were considered significant for P < 0.05.

RESULTS

The demography details and the comparison of biochemical parameters is depicted in Table-I. A significant difference at P value < 0.05 was observed in biochemical parameters between the groups. Serum telomerase activity was significantly lower in the T2DM patients with renal involvement as compared to the diabetic mellitus patients without complications and healthy volunteers. The prognostic factors such as body mass index (BMI), waist circumference, glycated haemoglobin, serum creatinine and eGFR was lower in the T2DM patients with microalbuminuria. Table 2 shows the Pearson correlation of glycated haemoglobin, eGFR and urinary microalbuminuria with serum telomerase level. A significant positive correlation was observed between serum telomerase activity with eGFR, urinary microalbuminuria and Glycated haemoglobin. Figure 1 depicts the graphical representation of the tables 1 and 2.

All data was represented as mean \pm SD. The data was compared by one-way ANOVA followed by post hoc Tuckey HSD test. A P-value of < 0.05 was considered significant. A: depicts P-value < 0.05 between group I & III; B: depicts P value < 0.05 between group III & II; C: depicts P value < 0.05 between group I & II. A significant correlation was present between the prognostic markers and serum telomerase activity. The linear regression analysis of serum telomerase level with glycated haemoglobin and microalbuminuria revealed that serum telomerase activity significant association with increase in level an

microalbuminuria and glycated haemoglobin (r = 0.766; P < 0.05).

Characteristics	Group I- T2DM Patients without Microalbuminuria (N-90)	Group II-T2DM Patients with Microalbuminuria (N-90)	Age and Gender Matched Healthy Volunteers as Controls (N-90)	
Age (years)	49 ± 20.2	49.8 ± 21	45 ± 21.2	
Gender (M / F)	58 / 32	61 / 29	56 / 34	
(years)	9.29	9.31	0	
Waist circumference (cm)	113.8 ± 9.79	109 ± 9.12	87 ± 2.4 ^{a, b}	
Body mass index (Kg / m2)	29.9 ± 0.78	31.87 ± 0.72	$24.1 \pm 0.12^{a, b}$	
History of smoking (% of individuals in a group)	58	64	28	
Fasting blood glucose (FBS) mg %	138 ± 14.2	159.4 ± 16.4 c	$101 \pm 1.2^{a, b}$	
Serum total cholesterol (TC) mg %	166.2 ± 4.1	189.2 ± 3.9 c	148.4 ± 1.24 ^{a, b}	
Serum triglyceride (TG) mg %	188.4 ± 6.2	212.4 ± 8.4	$144.2 \pm 1.08^{a, b}$	
Serum HD-Cholesterol (HDL-C) mg %	42.1 ± 0.12	36.6 ± 0.97°	$54.9 \pm 1.2^{a, b}$	
Serum LDL-Cholesterol (LDL-C) mg %	118.4 ± 6.2	143.8 ± 5.9°	$96.7 \pm 8.2^{a,b}$	
Glycated haemoglobin (HbA1c) %	7.2 ± 0. 21	8.3 ± 4.59	$5.6 \pm 1.2^{a, b}$	
Serum urea mg %	28 ± 1.23	32 ± 2.13	$18.4 \pm 2.31^{a,b}$	
Serum creatinine mg %	1.8 ± 0.14	2.8 ± 0.33	0.8 ± 2.48 ^{a, b}	
Estimated glomerular filtration rate (eGFR) mL / min / 1.73 m ²	76.9 ± 5.34	54.4 ± 3.6	96 ± 2.48 ^{a, b}	
Serum telomerase activity (AU)	883 ± 10. 2	772.4 ± 9.8 c	$1608 \pm 149.5^{a, b}$	
Table 1. Comparison of Demographic and Biochemical				
Characteristics among the Different Groups				
Prognostic Parameters R2 P-Value				
		-		

	K2	P-value		
Glycated hemoglobina	- 0.894	< 0.05		
Estimated glomerular filtration rate	0.882	< 0.05		
Urinary microalbuminuria	- 0.856	< 0.05		
Table 2. Pearson Correlation Analysis between Prognostic				
Parameters and Serum Telomerase				

DISCUSSION

This study aimed at evaluating the serum telomerase activity in type 2 DM with or without microalbuminuria. We observed hyperglycaemia, poor glycaemic control increases serum triglycerides and decrease in serum telomerase activity in the T2DM patients. The hyperglycaemic and increased serum fatty acids lead to deposition of fatty acids in nonadipose tissues and disrupt the homeostasis of oxidative stress pathway. It also activates the PKC pathway thereby causing phosphorylation, inhibition of insulin signalling pathway.^{27,28} Our study observed increase in truncal obesity as the waist circumference is comparatively higher in the T2DM patients. An increase in adipose tissue stimulates various inflammatory cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor alpha which causes activation of NF-kß and c-Jun N-terminal kinase and increase the insulin resistance, obesity and metabolic syndrome thereby resulting in chronic inflammation and oxidative stress. In our study, we found an increased in serum triglyceride level which was associated inversely with serum telomerase level.

The BMI and waist circumference of the patient population was also inversely correlated with the serum

telomerase level. This shows truncal obesity adversely affects the balance of telomerase function. This observation is in congruence with previous studies by Daubenmier J. et al and Hamel et al.^{27,28} The enzyme telomerase is also known as terminal transferase is a ribonucleoprotein complex and helps to maintain the length of the telomeric part of the chromosome.²⁶ The telomeric shortening occurs with age and hence the telomerase activity is reduced or absent in mature somatic cells which results in the death of the cells. The telomerase activity is highest during gestation and in immature cells.^{28,26}

In chronic diseases such as non-insulin dependent type 2 diabetes mellitus, chronic renal disease there occurs increased production and secretion of proinflammatory cytokines like interferon γ , interleukin 6 and tumour necrosis factor alpha etc. This leads to a dysfunction in the telomerase activity. The inflammation and oxidative stress alter the telomerase activity, decrease the telomere length affect the development and functioning of both the alpha and beta islet cells leading to T2DM. Inflammation and reduced telomerase activity also causes endothelial cell dysfunction leading to renal impairment, Figure-1. Our study shows that in diabetic patients along with glycaemic control, the presence of increased triglycerides and truncal obesity contribute to decrease in the serum telomerase level.

The study used microalbuminuria as a marker for detecting target organ damage by T2DM. Microalbuminuria is an early marker of renal glomerular dysfunction. In T2DM case microalbuminuria is most often used to ascertain the involvement of target organ damage. In this study, we observed an inverse relation of serum telomerase activity with microalbuminuria and glycated haemoglobin. This implies that an impaired glycaemic control is associated with target organ damage and decreased telomerase function, but we were unable to explain whether this was the cause or consequence.



Various studies have associated the telomere length and serum telomerase activity with ageing, insulin resistance and

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obesity. We observed a decrease in serum telomerase activity in the T2DM patients, which further decreased in the T2DM patients with nephropathy. This decrease is irrespective of gender and age. Studies in the past have suggested that telomerase level decreases with age but we observed that the level of decrease is more in association with the glycated haemoglobin. The increase in glycaemic control of the T2DM patients had an adverse impact on the serum telomerase level in our study and it was in agreement with the study by Vasran et al.²⁹ Studies have suggested a decrease in telomerase activity in the obesity.³⁰ We too observed an increase in the body mass index in the T2DM patients who had a decrease in telomerase activity. This suggests that obesity in T2DM leads to a pro-inflammatory status, increasing the release of pro-inflammatory cytokines, increasing oxidative stress and deranging the telomerasetelomere system implicating a higher susceptibility of end organ damage.²⁷

CONCLUSIONS

The observations of our study indicate that serum telomerase activity plays an important role in T2DM disease progression and target organ damage; hence, it might be considered as a perspective therapeutic agent. T2DM patients with poor glycemic control have increased serum triglyceride, truncal obesity, microalbuminuria and decreased serum telomerase activity, thereby making the patients susceptible to both cardiovascular and peripheral vascular diseases. Therefore, estimation of serum telomerase level in these patients will help prevent these future complications in the T2DM patients.

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

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