Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

ASSOCIATION OF TELOMERASE ACTIVITY WITH THE PROGNOSIS OF DIABETES MELLITUS IN SAUDI POPULATION

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Received – May 09, 2017; Revision – June 17, 2017; Accepted – June 29, 2017
Available Online – June 30, 2017

DOI: http://dx.doi.org/10.18006/2017.5(3).277.281

KEYWORDS

Type 2 diabetes

PCR-ELISA

Telomerase activity

Type I diabetes

Gestational diabetes

ABSTRACT

Telomeres are important for chromosome stability and they are replenished by telomerase enzyme. Impaired telomerase activity has a strong association with ageing and diabetes. Research data have shown that telomerase activity plays a vital role in pathogenesis of type I and type II diabetes by influencing pancreatic cell regeneration. This study was aimed to assess telomerase activity in three diabetic groups (type I, type II and gestational diabetes mellitus (GDM)) and compare it with a population with normal blood glucose levels to establish a correlation between reduced telomerase activity and prognosis of diabetes in the Saudi population. This study was conducted on 200 Saudi diabetic and non-diabetic participants (20-60 years). Peripheral mononuclear cells (PMNCs) were extracted from the blood samples and the activity of telomerase in the PMNCs was detected by the TRAP-ELISA assay. Results of study revealed that there was no telomerase activity in control healthy group, moderate activity in type I, some activity in GDM, and significantly high activity in type II group. This might indicate that telomerase activity has a strong correlation to the prognosis of type II diabetes. However, the molecular mechanism still needs to be investigated.
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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

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1 Introduction

At each end of eukaryotic chromosomes, there are regions of repetitive nucleotide sequences known as telomeres. These regions protect the chromosomes from being fused with neighboring chromosomes. The average telomere length in human chromosomes ranges from 11 kb to less than 4 kb from birth to older ages, respectively (Arai et al., 2015). Each time the cell is divided, the length of telomere decrease (Shay, 2016). However, these shortened ends are replenished by specific enzyme known as telomerase (Greider & Blackburn, 1985; Greider & Blackburn, 1989; Parks & Stone, 2017). Telomerase is a ribonucleoprotein enzyme that composed of reverse transcriptase enzyme and a long non-coding RNA required for telomere synthesis (Blackburn, 1991). The high expression of telomerase is most notably in germline cells and many cancerous cells (Shay, 2016; Armstrong & Tomita, 2017). Telomerase is a marker for several physiological processes such as cellular proliferation activity, ageing process (senescence), and cell-death (apoptosis). Moreover, it helps in the diagnosis of human tumors and regeneration of damaged tissue particularly liver and heart (Salpea & Humphries, 2010).

In recent years, several studies have been conducted to correlate telomeres shortening and telomerase activity with several metabolic diseases notably cardiovascular diseases, cancer and diabetes (Adaikalakoteswari et al., 2007; Salpea & Humphries, 2010; Elks & Scott, 2014). Diabetes mellitus (DM) considered one of the prevalent metabolic disorders. According to the latest World Health Organization (WHO) report, the rate of diabetes in Saudi Arabia is high (it ranks the second highest rate in the Middle East, and the seventh worldwide) (Al Dawish et al., 2016). Studies have shown that diabetic patients are apparently having shorter telomeres in their chromosomes (Tamura et al., 2016). It is not clear if the shortened telomeres have a role in diabetes pathogenesis (Adaikalakoteswari et al., 2007). However, it is well-known that telomeres are important in the progression and complications of diabetes including nephropathy (Verzola et al., 2008) and microalbuminuria (Tntolouris et al., 2007). Therefore, more investigations are needed to determine the relation of telomeres shortening and telomerase activity with diabetes pathogenesis.

The detection of the telomerase activity in tissues under normal physiological or pathological conditions is important step for tissue homeostasis. Several techniques are available to detect telomerase activity such as telomeric repeat amplification protocol (TRAP) assay, the fluorescent TRAP (F-TRAP) assay, stretch PCR assay, and transcription mediated amplification with the hybridization protection (TMA-HPA) assay (Durusoy & Üztűrk, 2001). These assays can either directly measure telomerase products, or indirectly measure the amplification signals produced from DNA that yields from telomerase (Wei et al., 1997; Skvortsov et al., 2011). Telomeric repeat amplification protocol (TRAP) is one of the oldest techniques used to measure the activity of telomerase. This technique has number of limitations that lead to the development of other modified techniques such as TRAP-ELISA. TRAP-ELISA assay is a non-radioisotopic technique that combines PCR-ELISA with TRAP to determine the telomerase activity, quantitatively and qualitatively, in a wide range of human tissues (Wei et al., 1997). Therefore, the current study was performed to estimate the telomerase activity in three types of diabetes mellitus namely type I diabetes mellitus (T1DM), type II diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM). People diagnosed clinically with this disease were assessed for telomerase activity by the TRAP-ELISA assay. Then the telomerase activity was compared to a group of control subjects with normal blood glucose levels to investigate the role of telomerase activity in the maintenance of normal blood glucose levels and impaired glucose metabolism in the Saudi population.

2 Materials and Methods

2.1 Study Subjects and Samples

This study was conducted on 200 Saudi males and females (20-60 years), Who routinely attended the diabetes clinic, Association of Diabetic Patient Friends Jeddah, King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia. The unit of Biochemical Ethics Research committee (UBERC) at King Abdulaziz University approved this study. All subjects gave their written consent for their participation in the study and based on their blood glucose levels, they were classified into four groups namely: normal glucose metabolism, T1DM, T2DM and GDM. The control group consisted of 25 males and 25 females, their mean ages ranged from 37 to 60 years and they had overnight fasting blood glucose levels of <110 mg/dl. The T1DM and T2DM subjects, were (20 males and 30 females) and (25 males and 25 females) in the same age group between 37 to 60 years who had a fasting blood glucose of >110 mg/dl. Regarding the GDM group, samples were collected from 50 pregnant females who only showed elevated blood glucose levels.

2.2 Separation of Peripheral Blood Mononuclear Cells (PBMCs)

From each participant, 5 ml of peripheral blood were collected in tubes contain heparin anticoagulant. Then 2.5 ml of Ficoll was added to each sample and was centrifuged at 2000 rpm for 20 min. After centrifugation, the PBMCs were collected from the Ficoll/plasma interface and they were washed three times in normal saline before they were pelleted by low-speed centrifugation. Finally, the cells (2×10^5/tube) were collected and stored at -80 C for telomerase assay (Yao et al., 2006).

2.3 TRAP Reaction

The telomerase activity in PBMCs was determined by a commercial telomerase TRAP-ELISA kit (TRAPEZE® ELISA Telomerase Detection Kit, Chemicon International, cat. No. S7750) by following manufacturer's instructions. To perform TRAP reaction, 2×10^5 PBMCs were lysed in 200 µl lysis reagent and incubated for 30 min. Following the incubation

period, cells were centrifuged at 16000 rpm for 20 min at 4^{o}C and the supernatant was removed and was kept frozen at -80 ^{o}C. The telomeric repeats were added to a primer (biotin-labeled) for 30 min at 25^{o}C during the first reaction. To induce telomerase inactivation, the mixture was incubated at 94^{o}C for 5 min, then was subjected to 30 PCR cycles as follow: 94^{o}C for 30 s, 50^{o}C for 30 s, and 72^{o}C for 90 s, and final extension at 72^{o}C for 10 min. These PCR products were used for the analysis of telomerase activity and gel electrophoresis alongside two types of controls [the positive control was (immortalized telomerase-expressing human kidney cells) and the negative control cell extract was (heat-treatment of the cell extract for 10 min at 65^{o}C prior to the TRAP reaction)] (Yao et al., 2006).

2.4 Detection of Telomerase Activity

The denatured PCR product was hybridized to a digoxigenin-(DIG)-labeled, telomeric repeat-specific detection probe. Then, via biotin labeled primer, the resulted product was immobilized to a streptavidin coated microtiter plate. The detection was performed using a peroxidase- conjugated antibody against digoxigenin (anti-DIG-POD). Within 30 min of adding the stop reagent, the probe was visualized with peroxidase metabolizing tetramethyl benzidine (TMB) and the absorbance (A) was measured at 450 nm. Telomerase activities were expressed using the following equation = Absorbance of sample - Absorbance of negative control (Yao et al., 2006).

2.5 Statistical Analysis

Comparison of telomerase activity between normal glucose subjects and different categories of diabetes was done using GraphPad Prism version 7.0 to establish a possible correlation between reduced telomerase activity and the prognosis of diabetes in Saudi population. Results were statistically analyzed using one-way analysis of variance (one-way ANOVA) test and *p* values were corrected with Bonferroni's correction post hoc test. *P* values <0.05 were considered statistically significant.

3 Results

3.1 Determination of Telomerase Activity

The absorbance of telomerase activity was calculated and represented as mean ±SEM. Comparisons of mean revealed that patients with T1DM (0.253±0.027), T2DM (0.752±0.054), and GDM (0.192±0.036) had a significant telomerase activity (p<0.05) compared to control group (0.038±0.006). However, patients with T2DM (0.752±0.054) had the highest telomerase activity among the diabetic groups when compared to control group (***p≤0.001).

4 Discussions

Since the discovery of telomerase, several studies have been performed to identify its role and how to measure its activity. Telomerase activity is usually less in most normal somatic cells compared to cancer cells (Kim et al., 1994). Human telomerase activity can be assessed by using several assays such as TRAP assay (Durusoy & Üztűrk, 2001). The deregulation of telomerase expression and activity was found to be linked to several metabolic syndromes and diseases such as diabetes (Cong et al., 2002).

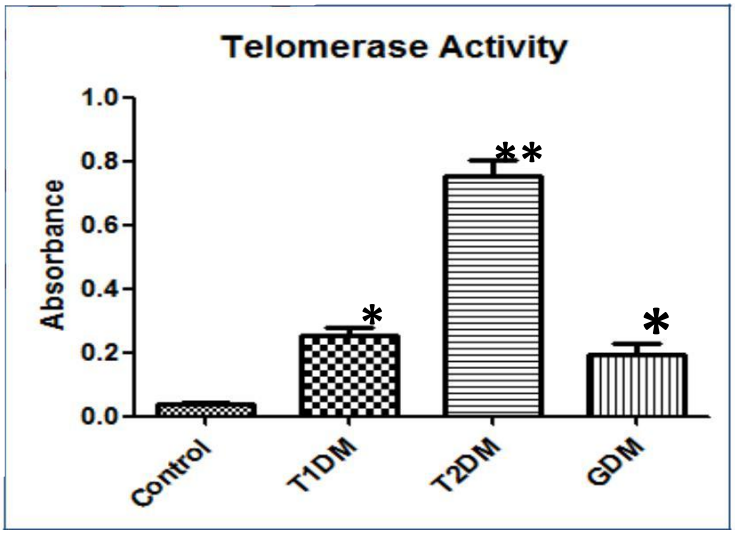

Figure 2 The absorbance of telomerase activity in control subjects versus different diabetic patient groups. The telomerase activity, obtained from TRAP-ELISA assay, was analyzed by one-way ANOVA test followed by Bonferroni's correction. Data were represented as mean±SEM and P values were obtained by comparing the means of each diabetic group vs. control group (*p<0.05 and ***p≤0.001).

In the current study, telomerase activity was evaluated in three different diabetic groups (T1DM, T2DM, and GDM) and compared to control non-diabetic group in order to estimate the possible role of telomerase in the prognosis of diabetes. As shown by the absorbance of telomerase activity with TRAP-ELISA assay, it was reported that there was no telomerase activity in control healthy group while moderate activity was reported in T1DM, some activity in GDM, and significantly high activity in T2DM group, which might indicate that telomerase activity has a role in the prognosis of T2DM. However, the exact mechanisms behind this strong link need more investigation. One of the postulated mechanisms that could explain the possible correlation is the production of senescence in premature pancreatic β-cell because of short telomeres, which in turn reduced β-cell mass and subsequently inhibit insulin secretion and cause glucose intolerance (Arai et al., 2015). Further, Oxidative stress damages telomeres, and diabetics usually have short telomeres and abnormal telomerase activity (Serra et al., 2000). In an *in vivo* study on mice, telomerase was found to play a role in maintaining glucose homeostasis (Kuhlow et al., 2010). Conversely, high levels of glucose elevate oxidative stress rate, therefore, impaired telomerase function and resulted in shortened telomeres (Serra et al., 2000). Findings of Zhao et al. (2014) are in agreement of the results of present study. These researchers studied the correlation between leukocyte telomeres length with the development of T2DM. They found that shortened telomeres are associated with T2DM development independently on risk factors. Their result apparently confirms that threshold effect is related to telomere length and cellular senescence. Another study performed by Rentoukas et al. (2012) to reveal the effect of telomerase in patients with inflammation and impaired endothelial function found that patients with metabolic syndromes had significant telomerase activity in their circulating PBMC. These results suggested that prolonged inflammation could act as a pathway in atherosclerosis resulted from diabetes.

In contrast to findings of this study, a study was conducted on newly diagnosed T2DM patients to determine the effect of Sitagliptin medicine on telomere length and telomerase activity, found that leukocyte telomere length was significantly reduced although that telomerase activity was less influenced. This result indicated that Sitagliptin might protect pancreatic β-cells from damage by elongating the length of its telomere (Ma et al., 2014). Moreover, a previous study performed on cord blood samples from pregnant women with pregestational T1DM, T2DM and gestational diabetes found significant differences between groups telomerase activity (it was high in cord blood from Type 1 and gestational diabetes pregnancies, but not in Type 2 diabetes) (Cross et al., 2010).

It is also important to state that telomerase expression and activity have been found to be upregulated not only in diabetes but also in other diseases that are classified as complications of diabetes. In vascular smooth muscle cells of diabetic patients, telomerase enzyme activity was found highly upregulated. This finding is crucial since high proliferation of these cells contributes to atherosclerosis and vascular disease (Sun et al., 2013).

In conclusion, although telomerase activity has been theoretically linked to cell ageing, damage and tumor phenotype, not much work has been published in relation to the possible association of increased telomerase activity with metabolic syndrome. To the best of our knowledge, our study is the first one that compares activity of telomerase in all three types of diabetes and we have found significantly high activity in type II diabetic patients as compared with type I and gestational diabetes. Hence, we recommend using these interesting findings as a starting point to pursue further research in this area with a higher sample size.

Acknowledgement

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. (G-564-130-37). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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