Analogous telomeres shortening and different metabolic profile: hypertension versus hypertension/type 2 diabetes mellitus comorbidity
Dhuha M.B. AlDehaini, Suzanne A. Al-Bustan, Zainab Hasan Abdulla Malalla, Muhalab E. Ali, Mai Sater and Hayder A. Giha

Background Eukaryotes chromosomal ends are capped and protected by telomeres, which are noncoding DNA repeats synthesized by telomerase enzyme. The telomerase enzyme is a nucleoprotein encoded by TERC and TERT genes. Naturally, the length of the telomeres shortens with each cell cycle but the shortening is fastened in certain age-related diseases like hypertension (HTN) and type 2 diabetes mellitus (T2DM).

Materials and methods Blood samples (n=171) were obtained from Kuwaiti subjects with HTN, and HTN/T2DM comorbidity (HTN-DM) and healthy subjects. The leukocyte telomere length (LTL) was measured by SYBR green quantitative hPCR, and plasma telomerase enzyme was measured by ELISA, in addition, three single nucleotide polymorphisms (SNPs) in telomere-related genes; TERC rs12696304GC, TERT rs2736100CA, and ACYP2 rs6713088GC were genotyped by real-time PCR.

Results Marked LTL shortening in subjects with HTN and HTN-DM compared to healthy subjects, P=0.043 and P<0.001, respectively, was noticed. On the contrary, the plasma telomerase enzyme levels and minor allele frequencies and genotypes of the tested SNPs were comparable between the study groups, except for TERT (CA) genotype which was over-represented in HTN (P=0.037). Furthermore, the comparisons between HTN and HTN-DM revealed significantly higher total cholesterol (P=0.015) and LDL-C (P=0.008) in HTN, while higher insulin levels (P<0.001), HOMA-IR (P<0.001), and BMI (P=0.004) were observed in HTN-DM.

Conclusion This study showed comparable LTL shortening in HTN and HTN-DM, irrespective of plasma telomerase enzyme levels or tested TERC, TERT, and ACYP2 gene polymorphisms, although HTN and HTN-DM differed in several metabolic markers. More studies are required to affirm these observations.

Keywords: biochemical profile, hypertension, Kuwait, single nucleotide polymorphism, telomerase, telomeres, type 2 diabetes mellitus

Introduction Hypertension (HTN), namely the essential type, is considered as an aging disease and is linked with cardiac diseases including, coronary heart disease (CHD), cerebrovascular diseases (CVD), and so on [1]. Furthermore, HTN is known to be associated with type 2 diabetes mellitus (T2DM), metabolic syndrome, and other age-related disorders [2]. Telomeres length shortening is one of the molecular markers of aging [3,4], which is known to be associated with T2DM, as well as with HTN complications, for example, aortic dissection [5], abdominal aortic aneurysms [6], atherosclerosis [7], familial hypercholesterolemia [8], coronary artery disease [9], and cognitive aging [10]. The relationship between the telomeres length and HTN is still controversial [11].

Telomeres are nucleoproteins that protect chromosomal ends and maintain genomic stability; they are known to be shortened following replication in each cell cycle, and thus with increasing age [4]. The telomeres length shortening is recognized in several chronic diseases [5]; however, the association of the telomeres length with HTN was not investigated before independent of HTN complications like CHD [12] and CVD [13]. A meta-analysis study concluded that telomeres may be shorter in hypertensive compared to nonhypertensive subjects but larger studies are needed for confirmation [11]. In contrast, a Mendelian randomization study showed no causal effect for telomeres length on ischemic stroke and its subtypes [14]. A review report stated that telomeres and telomerase enzyme activity are important players in occurrence and development of HTN in both humans and animals [15].
The telomerase enzyme is responsible for telomeres synthesis and maintenance [16,17]. It is composed of two components, RNA component (TERC) which acts as a template for the telomere synthesis and a protein component, the telomere reverse transcriptase (TERT) [17]. Normally, the plasma telomerase enzyme activity is scanty [18]; however, it increases exceptionally in certain disorders including different types of cancer [3]. Polymorphisms of the TERC, TERT, and another gene ACYP2 were found to be associated with telomeres shortening [19,20]; but the number of such studies are limited and were carried out in different populations.

Hypertension is a common disorder, and frequently it is associated with T2DM (HTN/T2DM comorbidity), a common causal factor could be insulin resistance [21,22]. The insulin resistance alone is unlikely to explain this relationship, thus further molecular and biochemical studies are worth to be considered. In this study, in addition to analysis of the telomeres system pathway components – the telomeres length, telomerase enzyme level, and single nucleotide polymorphisms (SNPs) of three telomeres related genes – some metabolic parameters were analyzed in HTN and HTN coupled with T2DM.

Materials and methods
This study is part of a prospective project about telomeres in T2DM, which was described in more detail somewhere else [23]. Here, we only briefly outline what was relevant to the objectives of this article. The project was carried out in Kuwait hospitals and health centers. The study subjects were: Kuwaiti Arabs, aged between 39 and 84 years, included both males and females and they were divided into three groups; patients with HTN only, HTN associated with T2DM (HTN-DM), and apparently healthy control subjects (Table 1).

Blood sample collection
Fasting (10–12h) blood samples (~10mL) were collected from each study subject into EDTA vacutainers and plain tubes, separated by centrifugation, divided into buffy coat, plasma and serum, and stored frozen at −20°C, until used.

Biochemical parameters measurement
Glycemic and lipid profile parameters were measured in the hospitals using automated biochemical analyzers; TOSOH G8 High-Performance Liquid Chromatography Analyzer (TOSOH Bioscience, California, USA) and UniCel1 DxC Synchron 800 analyzer (Beckman Corporation, Brea, California, USA).

ELISA
A standard ELISA assay was used for measurement of serum insulin and plasma telomerase enzyme by following the company protocols provided with each kit. A human insulin solid-phase sandwich ELISA, Cat. No: KAQ1251A, was used for insulin measurement while a quantitative sandwich ELISA kit, Human Telomerase (telomerase enzyme) ELISA Kit Cat. No: MBS021959 was used for measurement of telomerase enzyme, as mentioned before [23].

DNA extraction
The nuclear DNA was extracted from total blood by QIAamp DNA Blood Mini Kit.

Single nucleotide polymorphism genotyping by real-time PCR analysis
The genotyping was carried out in two steps; cycling (PCR amplification) followed by endpoint detection of fluorescent signals. The allelic discrimination was achieved by selective annealing of TaqMan MGB probes, using both Applied Biosystem StepOne Plus and 7500 real-time PCR systems. Three SNPs in three different

<table>
<thead>
<tr>
<th>Variable</th>
<th>HTN (n=30) M: 17, 13</th>
<th>Healthy (n=63) M: 24, 39</th>
<th>HTN-DM (n=78) M: 31, 47</th>
<th>HTN versus Healthy</th>
<th>HTN versus HTN-DM</th>
<th>Health versus HTN-DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Median</td>
<td>54.5, 49.0–61.0</td>
<td>51.0, 48.3–56.0</td>
<td>59.5, 53.0–66.0</td>
<td>0.109</td>
<td>0.107 T</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>56.2 ± 9.2</td>
<td>59.3 ± 8.7</td>
<td>0.413</td>
<td>0.044</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.0, 24.6–32.5</td>
<td>28.4, 25.7–32.5</td>
<td>31.4, 27.8–37.2</td>
<td>0.015</td>
<td>0.008 T</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T Chol (mmol/L)</td>
<td>5.07±1.49</td>
<td>5.12±0.99</td>
<td>4.34±1.26</td>
<td>0.841 T</td>
<td>0.015 T</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.10±1.31</td>
<td>3.21±0.91</td>
<td>2.39±1.143</td>
<td>0.651 T</td>
<td>0.008 T</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDLC</td>
<td>1.20, 1.03–1.42</td>
<td>1.41, 1.16–1.61</td>
<td>2.30, 1.53–3.29</td>
<td>0.008</td>
<td>0.297 T</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (mmol/L) Mean</td>
<td>1.22±0.26</td>
<td>1.18±0.32</td>
<td>1.18±0.32</td>
<td>&lt;0.001</td>
<td>0.801</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.54, 1.19–2.15</td>
<td>0.98, 0.76–1.23</td>
<td>1.52, 1.11–2.17</td>
<td>&lt;0.001</td>
<td>0.801</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>13.7, 10.8–17.7</td>
<td>11.8, 8.9–15.1</td>
<td>36.8, 25.9–56.2</td>
<td>0.165</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.7, 2.8–4.3</td>
<td>3.1, 2.1–3.8</td>
<td>12.6, 9.9–18.4</td>
<td>0.113</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

F, Females; HTN, hypertension; HTN-DM, hypertension associated with type 2 diabetes mellitus; M, males; T, T-test.

*For one variable, both mean and median are measured based on normality of data distribution.
genes related to telomeres length, the TERC rs12696304 C/G, TERT rs2736100 C/A, and ACYP2 rs6713088 C/G, were genotyped as described in the protocols provided with the kits.

**Absolute telomere length measurement by real-time PCR**

Human Telomere Length Quantification (AHTLQ) kit, containing SYBR Green master mix, was used for measurement of the absolute average telomere length in human genome, as previously described [23,24]. The telomeres lengths of the targeted samples were calculated compared to the length of a known telomere of a reference genomic DNA.

**Research ethics**

This study was ethically approved by the research and ethics committees of the College of Medicine and Medical Sciences, Arabian Gulf University, approval letter (E28-PI-01/20) and Kuwait Ministry of Health, approval letter number (2015/242). Informed consent was obtained from each study subject.

**Statistical analysis**

Sigma Stat software was used for data analysis. T-test and Chi-square test were used for analysis of normally distributed data, otherwise Mann–Whitney rank-sum test and Kruskal–Wallis one-way analysis of variance was used. Pearson product–moment correlation coefficient was used for correlation analysis. The statistical significance was set to be $P < 0.05$.

**Results**

**Patients and controls characteristics**

As seen in Table 1, a total of 171 subjects (72 male and 99 female) were included in this study. The sex distribution within the three study groups was not equal. The ages of healthy controls (51.0, 48.3–56.0 years) and patients with HTN (54.5, 49.0–61.0 years) were comparable, $P = 0.109$, and both were significantly smaller than patients with HTN-DM (59.5, 53.0–66.0 years), $P < 0.001$.

The BMI of patients with HTN (28.0, 24.6–32.5 kg/m$^2$) was comparable to that of the healthy subjects (28.4, 25.7–32.5 kg/m$^2$), $P = 0.764$, and both were significantly lower than the BMI of patients with HTN-DM (31.4, 27.8–37.2 kg/m$^2$), $P = 0.004$.

**The lipid profile of the study groups**

Analysis of the lipid profile showed that patients with HTN-DM had significantly lower plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels (4.34 ± 1.26 and 2.39 ± 1.14 mmol/L) compared to patients with HTN (5.07 ± 1.49 and 3.10 ± 1.31 mmol/L) and healthy subjects (5.12 ± 0.99 and 3.21 ± 0.91 mmol/L), $P$ values, 0.015 to <0.001, respectively (Table 1). In contrast, the healthy subjects had significantly lower triacylglycerol (0.98, 0.73–1.23 mmol/L) compared to patients with HTN (1.54, 1.19–2.15 mmol/L) or HTN-DM (1.52, 1.11–2.17 mmol/L), $P < 0.001$. The high-density lipoprotein cholesterol (HDL-C) was significantly lower in patients with HTN (1.20, 1.03–1.42 mmol/L) and HTN-DM (1.11, 0.92–1.31 mmol/L) compared to healthy subjects (1.41, 1.16–1.61 mmol/L), $P = 0.008$ and $P < 0.001$, respectively. However, the level of HDL-C was comparable between patients with HTN and HTN-DM, $P = 0.297$, (Table 1).

**Fasting plasma insulin levels and homeostatic model assessment of insulin resistance (HOMA-IR) profiles of the study groups**

Both fasting plasma insulin levels and HOMA-IR index were significantly markedly higher in patients with HTN-DM (36.8, 25.9–56.2 µIU/mL and 12.8, 9.9–18.4, respectively) compared to patients with HTN (13.7, 10.8–17.7 µIU/mL and 3.7, 2.8–4.3, respectively) and the healthy control subjects (11.8, 8.9–15.1 µIU/ml and 3.1, 2.1–3.8, respectively), $P < 0.001$. Limiting the comparison between the HTN and healthy subjects, the differences between the two parameters were NS, $P = 0.165$ and $P = 0.113$, respectively (Table 1).

**Comparison of the mean leukocyte telomere length between the study groups**

As shown in Fig. 1a, the mean leukocyte telomere length (LTL) was significantly different between the three study groups: HTN, healthy subjects, and HTN-DM, $P < 0.001$. Limiting the comparison between patients with HTN ($n = 28$) and healthy subjects ($n = 59$), the LTL remained significantly shorter in the former group (8.956, 3.517–16.405 kb) compared with the latter (12.311, 7.136–26.486 kb), $P = 0.033$, Mann–Whitney rank sum test, but it was comparable to the LTL in patients with HTN-DM ($n = 72$), (6.554, 2.517–11.218 kb), $P = 0.068$, Mann–Whitney rank-sum test. The mean LTL was also markedly shorter in patients with HTN-DM compared to healthy subjects, $P < 0.001$ (Fig. 1a). All patients with HTN taken together (including HTN-DM) ($n = 100$) compared with nonhypertensive subjects had significantly shorter LTL, 7.842, 2.728–12.041 versus 12.311, 7.136–26.486 kb, $P < 0.001$, Mann–Whitney rank sum test (data not shown).

**Plasma telomerase concentrations among the study groups**

Although the concentration of the telomerase enzyme was relatively high in patients with HTN (5.35, 3.10–8.21 U/L), compared to levels in healthy subjects (4.16, 1.13–8.23 U/L), or patients with HTN-DM (3.07, 0.00–5.72 U/L), the differences were NS, $P = 0.115$ (Fig. 1b). Limiting the comparison between patients with HTN and HTN-DM, still the difference was NS, $P = 0.065$, Mann–Whitney rank-sum test. Furthermore, there were
no correlations between plasma telomerase enzyme levels and mean LTL, whether each study group analyzed separately (data not shown) or all study subjects taken together ($n=115$), $CC=0.0126$, $P=0.894$ (Fig. 1c).

The $TERC$, $TERT$, and $ACYP2$ alleles/genotypes distribution among the study groups

As seen in Table 2, the minor allele frequency (MAF) and genotypes of the three tested SNPs, $TERC$ rs12696304 C/G, $TERT$ rs2736100 C/A, and $ACYP2$ rs6713088 C/G, were not significantly different between patients with HTN and healthy controls or patients with HTN-DM. The only exception was the significantly higher prevalence of $TERT$ genotype CA in patients with HTN compared to healthy subjects (53.3 versus 28.6%), $P=0.037$. However, there was a general trend that patients with HTN-DM consistently had relatively higher MAF for the three SNPs, but below the significance level.

Discussion

Telomeres shortening may be acquired due to notorious environmental factors, or inherited due to genetic/epigenetic faults or a combination of both factors as happens in aging and chronic age-related diseases [25,26]. The association of HTN with LTL shortening is still controversial [11]. In this study, we demonstrated a significant LTL shortening in Kuwaiti Arab patients with HTN and with HTN coupled with T2DM (HTN-DM), while other investigations showed significant metabolic differences between HTN, HTN-DM, and healthy subjects (Table 1).

Regardless of the type of HTN (primary or secondary), this study showed a significant reduction in mean LTL in patients with HTN compared to healthy subjects. Using the same data set, we recently demonstrated a similar shortening in the LTL in patients with T2DM [23]. Interestingly, patients with both HTN and T2DM (HTN-DM) had similar LTL shortening comparable to patients with only one of the two disorders, that is, there was no additive effect from the combination of the two diseases. To our knowledge, no previous study compared the LTL between patients with HTN and HTN-DM, although T2DM alone [27,28] and HTN alone [15,29] were inconsistently reported to be associated with LTL shortening. Nevertheless, one Ukrainian study was reported an influence for lipid metabolism control on LTL in subjects with both HTN and T2DM [30].

The LTL in HTN, HTN-DM or healthy subjects was not associated with plasma telomerase enzyme levels and the levels of the telomerase were comparable between the three study groups (Fig. 1b), debating previous reports related the LTL shortening to changes in the enzyme activity [15,31]. Furthermore, the mean LTL was not correlated with the plasma telomerase enzyme
Table 2 The distribution of minor allele frequency and genotypes of the TERC, TERT, and ACYP2 single nucleotide polymorphisms in patients with hypertension, hypertension associated with type 2 diabetes mellitus, and healthy subjects

<table>
<thead>
<tr>
<th>SNPs</th>
<th>HTN (n=30)</th>
<th>Healthy (n=63, 63, 62)</th>
<th>HTN-DM (n=72, 73, 74)</th>
<th>HTN versus health</th>
<th>HTN versus DL-HTN</th>
<th>Health versus DL-HTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2736100 C/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAF</td>
<td>0.333 (20/60)</td>
<td>0.286 (36/126)</td>
<td>0.361 (52/144)</td>
<td>0.624</td>
<td>0.828</td>
<td>0.235</td>
</tr>
<tr>
<td>Rare homo</td>
<td>6.7% [2]</td>
<td>14.3% (9)</td>
<td>15.3% (11)</td>
<td>0.493 F</td>
<td>0.388 F</td>
<td>0.935</td>
</tr>
<tr>
<td>Hetero</td>
<td>53.3% (16)</td>
<td>28.6% (18)</td>
<td>41.7% (30)</td>
<td>0.037</td>
<td>0.389</td>
<td>0.160</td>
</tr>
<tr>
<td>Common homo</td>
<td>40.0% (12)</td>
<td>57.1% (36)</td>
<td>43.0% (31)</td>
<td>0.185</td>
<td>0.948</td>
<td>0.144</td>
</tr>
<tr>
<td>rs12696304 C/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAF</td>
<td>0.417 (25/60)</td>
<td>0.349 (44/126)</td>
<td>0.418 (61/144)</td>
<td>0.467</td>
<td>0.888</td>
<td>0.301</td>
</tr>
<tr>
<td>Rare homo</td>
<td>23.3% (7)</td>
<td>11.1% (7)</td>
<td>13.7% (10)</td>
<td>0.135 F</td>
<td>0.366 F</td>
<td>0.845</td>
</tr>
<tr>
<td>Hetero</td>
<td>36.7% (11)</td>
<td>47.6% (30)</td>
<td>52.7% (41)</td>
<td>0.441</td>
<td>0.114</td>
<td>0.411</td>
</tr>
<tr>
<td>Common homo</td>
<td>40.0% (12)</td>
<td>41.3% (28)</td>
<td>30.1% (22)</td>
<td>0.913</td>
<td>0.461</td>
<td>0.240</td>
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<tr>
<td>rs6713088 C/G</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MAF</td>
<td>0.433 (26/60)</td>
<td>0.395 (49/124)</td>
<td>0.493 (73/148)</td>
<td>0.738</td>
<td>0.528</td>
<td>0.134</td>
</tr>
<tr>
<td>Rare homo</td>
<td>23.3% (7)</td>
<td>19.4% (12)</td>
<td>23.0% (17)</td>
<td>0.867</td>
<td>0.828</td>
<td>0.762</td>
</tr>
<tr>
<td>Hetero</td>
<td>40.0% (12)</td>
<td>40.3% (25)</td>
<td>52.7% (39)</td>
<td>0.844</td>
<td>0.338</td>
<td>0.205</td>
</tr>
<tr>
<td>Common homo</td>
<td>36.7% (11)</td>
<td>40.3% (25)</td>
<td>24.3% (18)</td>
<td>0.913</td>
<td>0.303</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Numbers between brackets in the heading row stand for the number of samples analyzed for each SNP, respectively.
F, Fisher’s exact test; Hetero, heterozygous genotype; Homo, homozygous genotype; HTN, hypertension; HTN-DM, hypertension associated with type 2 diabetes mellitus; MAF, minor allele frequency; \( \chi^2 \), Chi-square test. The heterozygous rs2736100 C/A, was significantly over represented in HTN compared to healthy subjects. Bold indicates significant of \( P \) value.

concentration whether all study subjects taken together (Fig 1c), or the correlations were limited to each study group separately (data not shown). Together, the above findings are suggesting that the telomeres shortening in HTN or HTN-DM were independent of the plasma telomerase enzyme level, as reported in T2DM before [23], and that HTN is not one of the disorders in which the plasma telomerase enzyme is raised. Worth noting, certain clinical disorders are associated with raised plasma telomerase enzyme level, for example, Alzheimer’s disease [32], sickle cell disease [33], and cancer [34,35]. However, in these studies the human telomerase reverse transcriptase (hTERT) mRNA was detected by real-time quantitative PCR instead of ELISA used for detection of the telomerase enzyme protein in this study.

Finally, the CA genotype of TERT rs2736100 SNP was significantly over-represented in patients with HTN compared to healthy subjects. In contrast, the MAFs and genotypes of TERC rs12696304 C/G and ACYP2 rs6713088 C/G SNPs were comparable between the three study groups. Moreover, none of the alleles or genotypes of the three tested SNPs had an influence in the LTL in this setting as mentioned before [23]. In a Chinese study, the TERC rs12696304 C/G was found to be associated with LTL, while both the SNP and the LTL were not associated with CHD [36]. Another Chinese study showed that the TERT rs2736100 C/A was not associated with CHD [37]. Hypertension is a known risk factor for CHD, otherwise, these SNPs were not tested before in relation to HTN, based on PubMed search.

This study is part of a project in which 64% of patients with T2DM have HTN compared with 34.4% in healthy subjects, \( P<0.001 \). Here, we showed that HTN had no additive or synergistic effect with T2DM in shortening the LTL (Fig. 1a), although individually each condition was previously reported to be associated with LTL shortening [23,38].

The HTN and HTN/T2DM comorbidity were metabolically two distinct disorders as portrayed from the lipid profile, plasma insulin, HOMA-IR, and BMI. The biochemical profile of the patients with HTN-DM was characterized by low total plasma cholesterol and LDL-C and raised fasting plasma insulin, HOMA-IR, and BMI. The low total cholesterol and LDL-C were unexpected in patients with HTN/T2DM comorbidity, however, our undocumented data from the region is supporting this finding. This later finding is important in understanding the metabolic changes in both disorders and in their management, therefore further studies with larger sample sizes are needed. In contrast, the subjects with HTN alone had comparable biochemical profile to the healthy subjects except for the decreased levels of HDL-C and increased levels of triacylglycerol, which were comparable to the HTN-DM profile (Table 1). Obesity, marked by raised HOMA-IR and BMI, was reported before to be associated with LTL shortening especially in hypertensive patients [39,40]. Worth noting, the molecular and metabolic differences between HTN and HTN/T2DM comorbidity are missing from the literature.

In conclusion, although this study was concise we were able to show the association of HTN with LTL shortening among Kuwaiti Arabs. Using the telomeres system pathway as a molecular tool for the distinction between HTN and HTN-T2DM comorbidity, we showed that the LTL shortening seen in HTN was comparable to that in HTN/DM groups. Furthermore, with exception to TERT genotype CA, the telomerase enzyme-related genes (TERC and ACYP2) SNPs and telomerase enzyme activity were comparable between the study groups. In contrast, the metabolic markers (lipid profile, insulin, and HOMA-IR)
were markedly significantly different between HTN and HTN-T2DM comorbidity. Finally, further studies are required to confirm the above observations.

**Acknowledgements**

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The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

In this study, the procedures involving human participants were done in accordance with the ethical standards of the Research Committees of the Arabian Gulf University (Manama, Bahrain) and Kuwait Ministry of Health (Kuwait) and with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from each subject in the study.

The patients gave consent for publication.

**Conflicts of interest**

There are no conflicts of interest.

**References**


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