Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-022-02577-y

REPRODUCTIVE PHYSIOLOGY AND DISEASE

The effect of metformin treatment on leukocyte telomere length in patients with polycystic ovary syndrome: a prospective case–control study

Özlem Kayacık Günday[1] · Müjgan Özdemir Erdoğan[2] · Ayşen Pehlivan[2] · Mehmet Yılmazer[1]

Received: 21 April 2022 / Accepted: 12 July 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract

Purpose The study aimed to investigate the effect of metformin treatment on leukocyte telomere length (LTL) and the relationship of LTL with C-reactive protein (CRP), homocysteine, albumin, complete blood count, and HOMA-IR values in patients with polycystic ovary syndrome (PCOS).

Material and method A prospective case–control study consisting of 30 women with PCOS and 30 healthy women without PCOS was performed. The relationship between clinical and laboratory parameters and LTL was analyzed. PCOS patients were treated with metformin (850 mg/day) for three months. Before treatment (BT) and after treatment (AT), each patient's LTL was evaluated and compared with the control group.

Results In the comparison between PCOS and control groups, the difference was significant for LTL, age, body mass index (BMI), and CRP ($p=0.002$; $p<0.001$; $p=0.001$; $p=0.01$, respectively). In PCOS patients, the difference between BT and AT, LTL was not statistically significant (BT: 6.06 ± 2.12; AT: 6.30 ± 1.93; $p=0.623$; 95% C.I: -1.22–0.74); however, the difference for weight was significant (BT: 83.78 ± 15.31; AT: 80.62 ± 15.40; $p=0.02$; 95% CI: 1.34–4.99). The logistic regression model established by BMI (group 1: 21–24, group 2: 24–29, group 3: 29–34, group 4: > 34), age, and RDW, which predicted the PCOS group by affecting the LTL level, was statistically significant ($p<0.001$/PPV = 96.3%; NPV = 88.5%). Each unit reduction in telomere length increased women's probability of PCOS by 0.4 times ($p=0.013$; OR = 0.419, 95% CI: 0.211–0.835).

Conclusion Although statistically insignificant, LTL increased after metformin use in PCOS patients, and the mean weight loss reduction was statistically significant. Telomere shortening increased the likelihood of PCOS 0.4 times.

Keywords Telomere · Polycystic ovary syndrome · Metformin · Peripheral blood leukocytes

Introduction

Telomeres are sequential non-coding TTAGGG nucleotide repeats located at the ends of chromosomes and primarily function to protect genomic DNA from damage during replication [1]. Telomerase, a ribonucleoprotein reverse transcriptase found in regenerative tissues, repairs and stabilizes short or dysfunctional telomeres by adding nucleotides to the ends of chromosomes. Nevertheless, somatic cells do not contain enough telomerase to maintain telomere length indefinitely. In human somatic cells, the opposite strand during DNA replication cannot be fully replicated due to the end replication problem. Therefore, telomere length shortens by about 50–200 nucleotides with each cell division. Shortening of telomere length to

✉ Özlem Kayacık Günday
kayacikozlem@yahoo.com.tr

Müjgan Özdemir Erdoğan
mozdemir1977@gmail.com

Ayşen Pehlivan
aysen.27.06@hotmail.com

Mehmet Yılmazer
drmehmetyilmazer@yahoo.com

[1] Department of Obstetrics and Gynecology, Faculty of Medicine, Afyonkarahisar University of Health Sciences, Afyonkarahisar, Turkey

[2] Department of Genetics, Faculty of Medicine, Afyonkarahisar University of Health Sciences, Afyonkarahisar, Turkey

Published online: 21 July 2022

the critical limit (known as the Hayflick limit) causes cell cycle arrest and cellular senescence in cells [2, 3].

Telomere length is affected by epigenetic regulation and genetic factors [4], and factors causing inflammation and oxidative stress accelerate telomere shortening [5]. As a result, the risk of age-related chronic diseases increases [6], including type 2 diabetes mellitus (DM). Short telomeres are known to be associated with type 2 DM [7].

Polycystic ovary syndrome (PCOS) is a heterogeneous, endocrine, and metabolic disease that may cause a prediabetic state [8]. In addition to causing infertility in clinical practice, PCOS exposes patients to metabolic syndrome, insulin resistance [9], impaired glucose tolerance and/or type 2 DM [10], cardiovascular disease (CVD), and cancer risk in the long term [11]. Insulin resistance occurs in approximately 50–70% of women with PCOS [12]. A systematic review suggested that PCOS patients have increased oxidative stress markers compared to controls and that oxidative stress may be involved in the pathophysiology of PCOS [13]. Shorter telomeres can be expected in PCOS patients compared to healthy individuals, as oxidative stress has been associated with the shortening of telomeres. In addition, studies regarding the variations in telomere lengths in the human genome have reported shorter telomere lengths in PCOS patients and negative correlations between inflammatory markers and telomere length in PCOS patients [14, 15]. Also, telomere shortening may impair the insulin secretion signaling pathway, mitochondrial function, and Ca + 2 metabolisms in vivo. These are all critical pathophysiological factors for PCOS [16]. Metformin (1,1-dimethylbiguanide hydrochloride) is an oral antihyperglycemic drug [17]. Metabolic features such as insulin resistance and hyperinsulinemia observed in PCOS are similar to type 2 DM. Therefore, insulin-sensitizing agents, particularly metformin, are a treatment option for PCOS [18]. Metformin treatment has been demonstrated to reduce insulin resistance and hyperinsulinemia, menstrual irregularity [18], anovulation, and clinical symptoms of hyperandrogenism. It exerts these effects by increasing insulin sensitivity and decreasing ovarian androgen production [19], hepatic sex hormone-binding globulin production, and ovarian estrogen secretion [20]. The general mechanism of action of metformin is the activation of adenosine monophosphate active kinase (AMPK) [21]. Thus, by inhibiting the production of reactive oxygen radicals, it protects arachidonic acid and iron-induced oxidative stress and positively affects cellular resistance against aging [22]. Besides, metformin may reduce body weight, although it has modest efficacy in managing PCOS and PCOS-related obesity [23]. The benefits of metformin for obesity, a chronic inflammatory condition, and its protection against oxidative stress may positively affect TL in PCOS patients. This study aimed to investigate whether the use of metformin in PCOS patients has a positive effect on leukocyte telomere length (LTL).

Material and method

The presented prospective case–control study was performed in Afyonkarahisar, Turkey, Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Obstetrics and Gynecology and Genetic Diseases. The study was approved by Afyonkarahisar Health Sciences University Clinical Research Ethics Committee Medical Ethics Committee (2020/8; number: 2020/ 340). The procedures comply with the provisions of the Declaration of Helsinki and written informed consent was obtained from all patients. All participants were local Turkish women.

Participants were recruited consecutively between September 2020 and December 2021. The inclusion criteria for the PCOS group were the PCOS-Rotterdam diagnostic criteria recommended by the European Society of Human Reproduction and Embryology, and the diagnosis was made by the presence of at least two of the following criteria: oligoovulation and/or anovulation, clinical and/or biochemical manifestations of hyperandrogenism, and polycystic ovaries [24]. The cases with hyperandrogenemia and other etiologies causing ovulatory dysfunction (congenital adrenal hyperplasia, 21-hydroxylase deficiency, androgen-secreting tumors, Cushing's syndrome, thyroid disease, hyperprolactinemia) were excluded.

The study group consisted of 30 PCOS patients between the ages of 18 and 40 who were eligible to receive metformin therapy (insulin resistance: HOMA-IR > 2.6; waist circumference > 80 cm) [25, 26]. The control group was formed by 30 healthy women between the ages of 18–40, who were healthy, fertile, had regular cycles (28–35 days), had ovaries with a normal appearance in transvaginal ultrasonography, applied to the obstetrics clinic for other reasons, did not use any hormonal drugs, and did not have systemic, inflammatory, infectious diseases, and pregnancy and waist circumference of > 80 cm. Chronic kidney and/or liver disease, clinical cardiovascular disease (CVD), DM, malignancy, pregnancy, thyroid diseases, hyperprolactinemia, lactation, smoking, pelvic infection and other infectious diseases, and refusal to participate were the exclusion criteria for the study. None of the included patients had had hormonal medication, including oral contraceptives, for at least 6 months before the study. The patients' height, weight, waist, and abdominal circumferences were measured and recorded.

Peripheral venous blood samples were collected from all participants between 9 and 11 a.m. on the third day of the menstrual cycle, and homocysteine (Cobas, photometric), C-reactive protein (CRP) (Cobas-immunoturbidimetric test), albumin (Cobas, photometric analysis)

and fasting blood glucose (Cobas, photometric), insulin (Cobas, electrochemiluminescence) for the diagnosis of PCOS patients with insulin resistance, glycosylated hemoglobin (HbA1c) (turbidimetric inhibition immunoassay (TINIA)), and hormone profile on day 3 of the cycle (serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estradiol (E2), progesterone, total testosterone (TT) and dehydroepiandrosterone sulfate (DHEA-S), and 17 hydroxyprogesterone (17 OH-P)) levels (Cobas, 8000e602, electrochemiluminescence test) were measured. The lipid profile consisted of serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels (Cobas, photometric, immunological, and colorimetric assay).

For the analysis of LTL data, 2 ml peripheral blood samples were obtained in ethylenediaminetetraacetic acid (EDTA) tubes. The blood samples were stored at − 20 °C until the study started. Throughout the study, no lifestyle changes were made, and subjects were instructed to follow their normal diet and physical activity and not take any other medication.

Genomic DNA was extracted from whole venous blood leukocytes using the EZ1 DNA Blood 200 µl kit (Qiagen). The concentrations and purity of the obtained DNA were measured using the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). ScienCell's Absolute Human Telomere Length Measurement qPCR Assay (AHTLQ), designed to measure telomere length directly, was used. The qPCR program specified in the kit was installed on the rotor-gene-Q (Qiagen) device. All study groups were analyzed in 2 replicates. The telomere primer set, which recognizes and amplifies the telomere sequences included in the kit, and the single-copy reference (SCR) primer set, which acts as a reference for data normalization, were used. QuantiTect SYBR Green PCR Master Mix was used as the qPCR master mix component, which is not included in the kit. Two qPCR reactions were prepared for reference genomic DNA and each genomic sample, one with the telomere primer stock solution and the other with the SCR primer stock solution. BT values obtained after qPCR and telomere length measurement were calculated manually using the "Comparative ΔΔCq (Quantification Cycle Value) Method" written in the AHTLQ Kit. In conclusion, the average telomere length of the target genomic DNA sample was measured.

The primary outcome measure for this study was the comparison of telomere length and inflammation markers between PCOS and control groups. The secondary outcome was to investigate whether the 3-month metformin treatment applied to PCOS patients significantly affected telomere length.

Statistical analysis

The distribution of continuous variables was expressed as mean ± standard deviation values, and categorical variables were expressed as percentages or numbers (*n*). Comparison of continuous variables between groups was performed with Student's *t*-test or Mann–Whitney *U*-test, depending on the normality of the distribution. The paired-samples *t*-test or Wilcoxon test was applied according to data distribution to compare dependent variables. After Pearson or Spearman correlation analysis according to normality analysis for the relationship of continuous variables with each other, regression analysis was performed for the parameters with a significant relationship, and it was evaluated whether the established model was statistically significant. Statistical analysis was performed using the Statistical Program for Social Sciences version 20.0 (SPSS, Chicago, IL, USA). The level of significance was $p \leq 0.05$ for all statistical tests.

Results

PCOS patients' mean age (mean ± SD) was 24.94 ± 5.4 years, body mass index (BMI) was 32.10 ± 5.8 kg/m^2, waist circumference was 102.11 ± 13.3 cm, hip circumference was 118.78 ± 13.4 cm., and the control group patients' mean age was 33.67 ± 5.05 years, BMI was 27.26 ± 4.08 kg/m^2, and waist circumference was 89.91 ± 9.17 cm. In the comparison between PCOS and control groups, the difference was significant for LTL, age, BMI, CRP, and waist circumference ($p = 0.002$, $p < 0.001$, $p = 0.001$, $p = 0.01$, and $p = 0.009$, respectively).

The difference in glucose, homocysteine, albumin levels, and complete blood count parameters between PCOS and the control group was not statistically significant (Table 1).

Though PCOS patients lose weight after metformin therapy (AT) for 3 months compared to that before therapy (BT) (80.62 ± 15.40 vs 83.78 ± 15.31, $p = 0.02$; 95% CI: 1.34–4.99), LTL in PCOS patients did not differ between AT and BT (6.30 ± 1.93 vs 6.06 ± 2.12, $p = 0.623$; 95% C.I: − 1.22–0.74) (Table 2).

While no correlation was observed between baseline LTL level with age and BMI in PCOS patients ($p > 0.05$), there was a significant negative correlation with RDW and a significant positive correlation with HDL level ($r = -0.468$, $p = 0.014$; $r = 0.477$, $p = 0.011$, respectively) (Table 3). Logistic regression analysis was performed to evaluate the relationship between LTL level and PCOS. BMI (group 1: 21–24, group 2: 24–29, group 3: 29–34, group 4: > 34), age, and RDW were independent variables. Age and LTL were negative predictive markers ($p < 0.05$). The predictive power of BMI and RDW was not statistically significant ($p > 0.05$) (Table 4). The positive predictive value of

Table 1 Comparison of PCOS and control group leukocyte telomere length (LTL) and inflammatory parameters

Parameter[c]	PCOS	Control	*p*
LTL (kb)	6.06 ± 2.12	8.01 ± 2.51	**0.002**[a]
Age (years)	24.93 ± 5.36	33.68 ± 5.048	**< 0.001**[a]
BMI (kg/ m^2)	32.10 ± 5.78	27.26 ± 4.075	**0.001**[a]
Glucose (mmol/L)	92.91 ± 10.28	92.87 ± 11.25	0.553[b]
CRP (mg/L)	1.10 ± 1.88	0.36 ± 0.64	**0.01**[b]
Homocysteine (μmol/L)	10.91 ± 2.82	10.47 ± 2.56	0.774[b]
Albumin (g/dl)	5.09 ± 1.87	4.53 ± 0.19	0.127[a]
WBC (10^3/μL)	7.89 ± 2.33	7.70 ± 1.76	0.952[b]
Platelets(10^3/μL)	284.70 ± 70.00	297.63 + 68.33	0.50[a]
MPV(fL)	10.35 ± 1.15	10.49 ± 1.02	0.645[a]
RDW(%)	41.89 ± 4.37	43.06 ± 3.77	0.258[b]
Neutrophil (10^9/L)	4.91 ± 1.96	6.87 ± 10.1	0.337[b]
Lymphocyte (10^9/L)	2.31 ± 0.61	2.0804 ± 0.68	0.200[a]
Wc (cm)	102.11 ± 13.28	89.91 ± 9.17	**0.009**[a]

[a]Independent samples *t*-test

[b]Mann-Whitney *U*

[c]Data are presented as mean ± SD, median (interquartile range)

$p < 0.05$ was considered statistically significant

the model to predict PCOS was 96.3%, and the negative predictive value was 88.5%, being statistically significant ($p < 0.001$). Accordingly, each unit reduction in telomere length increased the probability of women having PCOS by 0.4 times (*p*: 0.013; OR: 0.419, 95% C.I: 0.211–0.835) (Table 5).

Discussion

In our study, although LTL increased after three months of metformin treatment (6.06 ± 2.12 vs. 6.30 ± 1.93) in PCOS patients, the difference was not statistically significant ($p > 0.05$). However, patients had a mean significant weight loss of 3.1 kg ($p = 0.02$; 95% CI: 1.34–4.99). Many studies have reported a relationship between telomere length and DM, and telomere shortening contributes to the pathogenesis of DM [7, 27–29]. Zhao et al. reported that short leukocyte telomeres might predict type 2 DM risk in the native North American population [30]. Liu et al., in their study investigating the effect of antidiabetic agents on telomere length, revealed longer telomere ratios in 388 types 2 DM patients, in the groups using metformin and insulin, compared to those who did not receive any treatment and used acarbose for more than three months [31]. The study of De Zegher et al., in which they investigated the effects of oral contraceptives and insulin-sensitizing drugs on LTL for 18 months in hyperinsulinemic and hyperandrogenemic adolescents, reported a two-fold increase in LTL after the use of metformin (850 mg/day). However, they did not observe any effect after the use of oral contraceptives [32]. In a placebo-controlled study in which 19 prediabetic patients were administered metformin for two months, metformin significantly improved metabolic parameters and insulin sensitivity, and monocyte telomere length increased after treatment [33]. All these findings show that antidiabetic agents have blood sugar regulation and anti-aging effects by increasing LTL [31]. In a retrospective study involving over 180,000 participants, metformin users lived 15% longer than nondiabetic controls when comparing metformin, a sulfonylurea, and a control group [34]. PCOS also produces a prediabetic state in most patients, increasing the risk of type 2 DM [10]. Moreover, in PCOS treatment, metformin has been used for many years [18]. Therefore, our study supports the hypothesis that the positive effect of metformin on LTL and lifespan in type 2 DM patients could also be observed in PCOS patients.

All of the PCOS patients included in our study had abdominal adiposis (waist circumference > 80 cm), and although weight loss was significant after metformin, the 3-month period may have been insufficient for its effect on TL to occur. Rosa et al. stated that LTL was not correlated with HbA1c in the long term (6 and 12 months) and that short-term metabolic control was more determinant for LTL in type 2 DM, and suggested that this may be due to the rapid response of LTL to changes in the metabolic environment [28]. Although the duration of treatment was 3 months in our study, more studies are needed to reveal the effect of different treatment durations in PCOS patients.

In our study, no significant correlation was observed between LTL and age in PCOS and control groups. However, PCOS patients were significantly younger than the control group, and age was a significant negative predictor for PCOS in the regression analysis. In the literature, regular aging itself has been associated with telomere shortening [5]. Although PCOS patients were younger, LTL was

Table 2 Comparison of leukocyte telomere length (LTL) before and after metformin treatment in PCOS patients (paired samples test)

Parameter[a]	Before treatment (*n*: 30)	After treatment (*n*: 30)	*p*	95% C.I	*t*
LTL (kb)	6.06 ± 2.12	6.30 ± 1.93	0.623	− 1.22–0.74	− 0.49
Weight (kg)	83.78 ± 15.31	80.62 ± 15.40	**0.002**	1.34–4.99	3.59

[a]Data are presented as mean ± SD

Table 3 Correlation analysis of clinical characteristics and laboratory parameters with the LTL

Variable	PCOS		Control	
	r	*p*	*r*	*p*
CRP (mg/L)	−0.115[b]	0.545	0.142[a]	0.481
Homocysteine (µmol/L)	−0.030[b]	0.878	0.071[b]	0.730
Age (years)	−0.092[a]	0.627	−0.103[b]	0.601
Glucose (mmol/L)	−0.067[b]	0.725	−0.257[b]	0.225
HbA1c (%)	0.091[a]	0.637	−0.396[a]	0.202
HOMA-IR	−0.091[a]	0.632	0.041[a]	0.904
Insuline (mIU/L)	−0.087a	0.649	0.155[a]	0.630
WBC (10^3/µL)	−0.053[b]	0.792	0.115[a]	0.568
BMI (kg/m^2)	−0.314[a]	0.091	−0.046[a]	0.819
RDW (%)	−0.468[b]	**0.014***	0.012[b]	0.954
Wc (cm)	−0.192[a]	0.338	−0.134[a]	0.694
Hc (cm)	−0.193[a]	0.335	−0.275[a]	0.413
Albumin (g/dl)	−0.138[a]	0.476	0.075[a]	0.711
HDL (mg/dL)	0.477[a]	**0.011***	−0.203[b]	0.526

r: [a]Pearson correlation coefficient, *r*: [b]Spearman correlation coefficient

PCOS polycystic ovary syndrome, *LTL* leukocyte telomere length, *Wc* waist circumference, *Hc* hip circumference

*$p < 0.05$ was considered statistically significant

Table 4 Predictive effect of LTL on PCOS (omnibüs tests of model coefficients: $p < 0.001$). Covariates: LTL, age, BMI, RDW

İndependent variable	*p*	OR	95% C.I	*B*
LTL (kb)	**0.013**	0.419	0.211–0.84	−0.87
Age (years)	**0.003**	0.722	0.58–0.9	−3.25

$p < 0.05$ was considered statistically significant

significantly shorter than the control group in our study. We consider that this result is due to the negative impact of PCOS on telomere length.

In all patients, HOMA-IR was > 2.6, consistent with insulin resistance, and there was no significant correlation between LTL with fasting blood glucose, HOMA-IR, BMI, and waist circumference in PCOS and control groups. In contrast, Ma et al. determined the rate of telomere shortening was inversely correlated to HbA1c, age, fasting plasma glucose, and waist circumference [29]. Visceral obesity causes an increased inflammatory response and oxidative stress, resulting in telomere attrition [35]. In one study, LTL was indicated to be negatively correlated with direct measures of abdominal adiposity, as well as traditional measures of adiposity such as BMI and waist circumference (Wc) [36, 37]. Most PCOS patients have visceral obesity. In PCOS patients, the early and long-term insulin sensitivity provided by metformin reduces abdominal, visceral, and hepatic adiposity and low-grade inflammation and reduces endothelial dysfunction and vascular intima-media thickness. Metformin protects endothelial cells from hyperglycemic damage by directly stimulating the expression/activity of Sirtuin-1 (SIRT1), a deacetylase involved in metabolism and longevity [38]. In conclusion, it can have an anti-aging effect, meaning longer telomeres [39]. From this point of view, we consider the possible protective effect of metformin use and the possibility of increased surveillance in terms of long-term complications associated with metabolic syndrome and insulin resistance in PCOS patients.

In the Dallas Heart Study, RDW level, after adjusting for age and race, was negatively correlated with LTL in 3157 subjects over 18 years of age [40]. Moreover, an independent, strong positive correlation was demonstrated between RDW and conventional inflammatory markers [41]. Consistent with this study, although the number of patients was limited, there was a significant negative correlation between RDW and LTL in PCOS patients [41]. RDW may be affected due to chronic inflammation [42] in PCOS patients. When we performed regression analysis to investigate the effect of RDW on LTL, RDW did not have a significant effect on LTL in the model created. Furthermore, there was no significant difference between PCOS and control groups in terms of RDW levels.

In a prospective cohort study, lower HDL was found to be associated with lower LTL [42]. A significant positive correlation was observed between LTL level and HDL in PCOS patients in our study, but there was no significant correlation in the control group. Cheng et al. determined low HDL levels to be associated with shorter LTL as a component of metabolic syndrome [43]. Dyslipidemia is a very common finding in PCOS patients, characterized by high triglyceride and lower HDL levels, increasing the risk of cardiovascular disease [44]. The mean LTL of PCOS patients included in the study was significantly shorter than the control group (PCOS: 6.06 ± 2.12; control: 8.01 ± 2.51,

Table 5 Logistic regression analysis showing the predictive effect of LTL on PCOS (omnibus tests of model coefficients: $p < 0.001$). In the model, decreased LTL resulted in a higher number of PCOS patients (PPV: 96.3; NPV: 88.5)

Groups	LTL (mean ± SD)	*P* value	Nagelkerke R^2	OR (95% C.I.)
Controls (*n*: 30)	8.01 ± 2.51	**0.013**	0.787	Reference
PCOS (*n*: 30)	6.06 ± 2.12			0.419 (0.211–0.835)

$p = 0.002$). In one of the first studies on the subject, a significant decrease in LTL was noted in nearly 700 PCOS patients compared to healthy controls, and it was reported that the risk of disease increased with short LTL [14]. Wei et al. also revealed shorter LTL in PCOS patients [45]. In another study, Wang et al. measured the level of telomeric repeat-containing RNA (TERRA) and LTL in 40 PCOS patients. Longer LTL was revealed in PCOS patients, while TERRA expression was lower [46]. However, another study with fewer participants (including 150 patients with PCOS and 124 controls) in Brazilian women failed to determine an association between LTL and PCOS [15]. In our study, although the number of participants was less, the LTL length was significantly shorter in the PCOS group, and according to the model we established, each unit reduction in telomere length increased the probability of women having PCOS by 0.4 times ($p = 0.013$; OR = 0.419, 95% CI: 0.211–0.835).

Leukocytes serve as a much better biomarker than somatic cells due to their shorter half-life and presence in all body parts. Therefore, LTL has been used to represent TL in tissues affected by aging [47]. Although some studies used tissues such as granulosa cells in PCOS patients, we also preferred LTL. Even though our study results support the hypothesis that the shorter LTL and telomere biology are affected in PCOS patients, further studies may help to explain the underlying mechanisms.

In our study, only CRP, one of the inflammatory parameters, was significantly higher in PCOS patients ($p < 0.05$). There was no difference with the control group regarding homocysteine, neutrophil, lymphocyte, and WBC levels ($p > 0.05$).

In a meta-analysis, a large increase in circulating markers of oxidative stress such as serum homocysteine, malondialdehyde, asymmetric dimethylarginine level, and superoxide dismutase activity was reported in PCOS patients independent of body weight compared to controls [13]. In another meta-analysis, circulating CRP was reported to be a reliable marker of low-grade chronic inflammation in women with PCOS [48]. There is also a relationship between increased levels of inflammatory markers in the disorder and insulin resistance (IR) [49], and IR and hyperinsulinemia may result in elevated serum homocysteine, which has been identified as an independent risk marker for CVD [50]. Elevated plasma concentrations of homocysteine indicate oxidative stress, inflammation, and increased risk of CVD. Therefore, it is thought that homocysteine may also play a role in telomere shortening [51]. Most studies investigating the chronic low-grade inflammation state in PCOS have usually focused on CRP measurement. CRP is produced from the liver and directly from adipose tissue by stimulating interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α). Evidence suggests that CRP is positively correlated to insulin resistance, body weight, and fat mass, and further studies are required to clarify the precise mechanism by which CRP concentrations increase in women with PCOS [52]. Many studies have determined higher WBC levels in PCOS patients than in the control group [52–54]. Although this situation was tried to be explained by the presence of high amounts of androgen receptors in human leukocytes [55] and hyperandrogenemia in PCOS patients, the distribution of WBC in the study of Tola et al. was homogeneously distributed between PCOS and control groups [56]. The exact mechanism of leukocytosis in PCOS has not been elucidated [52]. In our study, only the CRP level was significantly higher among inflammatory markers. No difference was found for homocysteine. Wang et al., in their metaanalysis, reported that serum concentrations of homocysteine increase with obesity, independent of insulin resistance [57]. The difference might not be significant in our study due to the high mean BMI values in both PCOS and control groups.

In PCOS patients, abdominal adiposity, obesity, and insulin resistance may contribute to oxidative stress, resulting in telomere shortening and affecting endocrine, metabolic, or reproductive-related gene expression. Telomere abnormalities may impair folliculogenesis, a proliferative process, and involve this condition in PCOS etiology [45]. When all this information is evaluated, the question of whether telomere loss is a cause or a result of PCOS in PCOS, the etiology of which is still not fully revealed, should be investigated.

Our study determined no correlation between TT, DHEA-S, androstenedione, and LTL in PCOS patients ($p > 0.05$). Results regarding the relationship between androgen levels and telomere length are conflicting in the literature. Wang et al. found a positive correlation between LTL and testosterone [46]. However, in the study of Li et al., LTL was not associated with testosterone levels, as in our study [58]. In human primary hematopoietic cells, androgens have been reported to increase telomerase activity [59], and sex hormones such as estrogen and androgen have been indicated to be associated with decreased telomere erosion due to higher telomerase expression and/or activity [60]. Another study has revealed that there is a positive correlation between androgen level and telomerase activity in cumulus cells in PCOS patients, and androgens stimulate telomerase [59]. The authors argued that hyperandrogenism in PCOS may compensate for the detrimental effect of PCOS-related metabolic changes and inflammation. In our study, we hypothesize that the decreased telomerase activity associated with metformin's effect of reduction in intraovarian androgen levels in PCOS patients may censor the positive effect of metformin on LTL. However, further studies are required to evaluate the effect of metformin on telomerase activity and to prove this hypothesis.

We emphasized that when prescribing metformin to PCOS patients, its effects on telomere dynamics, not just ovulation induction, should be considered. Due to the

relatively small number of patients included in our study, the results were limited in generalization. It is also suggested that LTL is affected by various factors such as race, heredity [60], and environmental factors [61]. All participants in our study consisted of local Turkish people. Nevertheless, the effect of nutrition, lifestyle, and stress factors specific to each individual cannot be ignored. Despite these limitations, the fact that it was a prospective controlled study adds to its value.

Conclusion

To our knowledge, this was the first prospective study to investigate the effect of metformin treatment on LTL in adult PCOS patients. Long-term comorbidities such as DM, dyslipidemia, obesity, hypertension, metabolic syndrome, endometrial cancer, and CVD occur in both reproductive age and elderly women with PCOS. Oxidative and inflammatory stress caused by all these risks may cause a shortening of life span due to telomere shortening. Therefore, it is necessary to make an appropriate treatment plan for PCOS patients for not only infertility treatment but also long-term risks. There is a need for larger-scale and long-term studies to support metformin use and determine the optimal dose and duration in terms of long-term health risks and positive effects on telomere length in PCOS patients.

Acknowledgements The authors thank patients for their participation in this study, Mustafa Solak from Afyonkarahisar Health Sciences University Genetics Clinic, and Onur Bender from Ankara University Biotechnology Institute for their analysis and scientific advice.

Funding This study was supported by Afyonkarahisar Health Sciences University Scientific Research Projects Unit (project no: 20.011).

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Pfeiffer V, Lingner J. Replication of telomeres and the regulation of telomerase. Cold Spring Harb Perspect Biol. 2013;5:a010405.
2. Jiang H, Ju Z, Rudolph KL. Telomere shortening and ageing. Z Gerontol Geriatr. 2007;40:314–24.
3. Mc Auley MT, Guimera AM, Hodgson D, McDonald N, Mooney KM, Morgan AE, Proctor CJ. Modelling the molecular mechanisms of aging. Biosci Rep. 2017;37(1):BSR20160177. https://doi.org/10.1042/BSR20160177.
4. Kalmbach KH, FontesAntunes DM, Dracxler RC, Knier TW, Seth-Smith ML, Wang F, Liu L, Keefe DL. Telomeres and human reproduction. Fertil Steril. 2013;99:23–9.
5. Zhang J, Rane G, Dai X, Shanmugam MK, Arfuso F, Samy RP, Lai MK, Kappei D, Kumar AP, Sethi G. Ageing and the telomere connection: an intimate relationship with inflammation. Ageing Res Rev. 2016;25:55–69.
6. Kordinas V, Ioannidis A, Chatzipanagiotou S. The telomere/telomerase system in chronic inflammatory diseases. Cause or Effect?. Genes (Basel). 2016;7(9):60. https://doi.org/10.3390/genes7090060.
7. Willeit P, Raschenberger J, Heydon EE, Tsimikas S, Haun M, Mayr A, Weger S, Witztum JL, Butterworth AS, Willeit J, Kronenberg F, Kiechl S. Leucocyte telomere length and risk of type 2 diabetes mellitus: new prospective cohort study and literature-based meta-analysis. PLoS ONE. 2014;9:e112483.
8. Stein IF. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29:181–91.
9. Park KH, Kim JY, Ahn CW, Song YD, Lim SK, Lee HC. Polycystic ovarian syndrome (PCOS) and insulin resistance. Int J Gynaecol Obstet. 2001;74:261–7.
10. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999;22:141–6.
11. Palomba S, Santagni S, Falbo A, La Sala GB. Complications and challenges associated with polycystic ovary syndrome: current perspectives. Int J Womens Health. 2015;7:745–63.
12. Ovalle F, Azziz R. Insulin resistance, polycystic ovary syndrome, and type 2 diabetes mellitus. Fertil Steril. 2002;77:1095–105.
13. Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update. 2013;19:268–88.
14. Li Q, Du J, Feng R, Xu Y, Wang H, Sang Q, Xing Q, Zhao X, Jin L, He L, Wang L. A possible new mechanism in the pathophysiology of polycystic ovary syndrome (PCOS): the discovery that leukocyte telomere length is strongly associated with PCOS. J Clin Endocrinol Metab. 2014;99:E234-240.
15. Pedroso DC, Miranda-Furtado CL, Kogure GS, Meola J, Okuka M, Silva C, Calado RT, Ferriani RA, Keefe DL, dos Reis RM. Inflammatory biomarkers and telomere length in women with polycystic ovary syndrome. Fertil Steril. 2015;103:542-547.e542.
16. Guo N, Parry EM, Li LS, Kembou F, Lauder N, Hussain MA, Berggren PO, Armanios M. Short telomeres compromise β-cell signaling and survival. PLoS ONE. 2011;6:e17858.
17. Patel R, Shah G. Effect of metformin on clinical, metabolic and endocrine outcomes in women with polycystic ovary syndrome: a meta-analysis of randomized controlled trials. Curr Med Res Opin. 2017;33:1545–57.
18. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism. 1994;43:647–54.
19. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab. 2000;85:139–46.
20. Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L. Metformin to restore normal menses in oligo-amenorrheic teenage girls with polycystic ovary syndrome (PCOS). J Adolesc Health. 2001;29:160–9.
21. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001;108:1167–74.

22. Kim YW, Lee SM, Shin SM, Hwang SJ, Brooks JS, Kang HE, Lee MG, Kim SC, Kim SG. Efficacy of sauchinone as a novel AMPK-activating lignan for preventing iron-induced oxidative stress and liver injury. Free Radic Biol Med. 2009;47:1082–92.
23. Palomba S, Orio F Jr, Falbo A, Manguso F, Russo T, Cascella T, Tolino A, Carmina E, Colao A, Zullo F. Prospective parallel randomized, double-blind, double-dummy controlled clinical trial comparing clomiphene citrate and metformin as the first-line treatment for ovulation induction in nonobese anovulatory women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:4068–74.
24. Revised. consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2003;19(2004):41–7.
25. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120:1640–5.
26. Tripathy D, Carlsson M, Almgren P, Isomaa B, Taskinen MR, Tuomi T, Groop LC. Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study. Diabetes. 2000;49:975–80.
27. Wang J, Dong X, Cao L, Sun Y, Qiu Y, Zhang Y, Cao R, Covasa M, Zhong L. Association between telomere length and diabetes mellitus: a meta-analysis. J Int Med Res. 2016;44:1156–73.
28. Rosa E, Dos Santos RRC, Fernandes LFA, Neves FAR, Coelho MS, Amato AA. Leukocyte telomere length correlates with glucose control in adults with recently diagnosed type 2 diabetes. Diabetes Res Clin Pract. 2018;135:30–6.
29. Ma D, Zhu W, Hu S, Yu X, Yang Y. Association between oxidative stress and telomere length in Type 1 and Type 2 diabetic patients. J Endocrinol Invest. 2013;36:1032–7.
30. Zhao J, Zhu Y, Lin J, Matsuguchi T, Blackburn E, Zhang Y, Cole SA, Best LG, Lee ET, Howard BV. Short leukocyte telomere length predicts risk of diabetes in american indians: the strong heart family study. Diabetes. 2014;63:354–62.
31. Liu J, Ge Y, Wu S, Ma D, Xu W, Zhang Y, Yang Y. Association between antidiabetic agents use and leukocyte telomere shortening rates in patients with type 2 diabetes. Aging (Albany NY). 2019;11:741–55.
32. de Zegher F, Díaz M, Ibáñez L. Association between long telomere length and insulin sensitization in adolescent girls with hyperinsulinemic androgen excess. JAMA Pediatr. 2015;169:787–8.
33. de Kreutzenberg SV, Ceolotto G, Cattelan A, Pagnin E, Mazzucato M, Garagnani P, Borelli V, Bacalini MG, Franceschi C, Fadini GP, Avogaro A. Metformin improves putative longevity effectors in peripheral mononuclear cells from subjects with prediabetes A randomized controlled trial. Nutr Metab Cardiovasc Dis. 2015;25:686–93.
34. Bannister CA, Holden SE, Jenkins-Jones S, Morgan CL, Halcox JP, Schernthaner G, Mukherjee J, Currie CJ. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. Diabetes Obes Metab. 2014;16:1165–73.
35. Gardner JP, Li S, Srinivasan SR, Chen W, Kimura M, Lu X, Berenson GS, Aviv A. Rise in insulin resistance is associated with escalated telomere attrition. Circulation. 2005;111:2171–7.
36. Lee M, Martin H, Firpo MA, Demerath EW. Inverse association between adiposity and telomere length: The Fels Longitudinal Study. Am J Hum Biol. 2011;23:100–6.
37. Cui Y, Gao YT, Cai Q, Qu S, Cai H, Li HL, Wu J, Ji BT, Yang G, Chow WH, Shu XO, Zheng W. Associations of leukocyte telomere length with body anthropometric indices and weight change in Chinese women. Obesity (Silver Spring). 2013;21:2582–8.
38. de Kreutzenberg SV, Ceolotto G, Papparella I, Bortoluzzi A, Semplicini A, Dalla Man C, Cobelli C, Fadini GP, Avogaro A. Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: potential biochemical mechanisms. Diabetes. 2010;59:1006–15.
39. Ibáñez L, Ong KK, López-Bermejo A, Dunger DB, de Zegher F. Hyperinsulinaemic androgen excess in adolescent girls. Nat Rev Endocrinol. 2014;10:499–508.
40. Kozlitina J, Garcia CK. Red blood cell size is inversely associated with leukocyte telomere length in a large multi-ethnic population. PLoS ONE. 2012;7:e51046.
41. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. Arch Pathol Lab Med. 2009;133:628–32.
42. Révész D, Milaneschi Y, Verhoeven JE, Lin J, Penninx BW. Longitudinal associations between metabolic syndrome components and telomere shortening. J Clin Endocrinol Metab. 2015;100:3050–9.
43. Cheng YY, Kao TW, Chang YW, Wu CJ, Peng TC, Wu LW, Yang HF, Liaw FY, Chen WL. Examining the gender difference in the association between metabolic syndrome and the mean leukocyte telomere length. PLoS ONE. 2017;12:e0180687.
44. Zhang J, Fan P, Liu H, Bai H, Wang Y, Zhang F. Apolipoprotein A-I and B levels, dyslipidemia and metabolic syndrome in southwest Chinese women with PCOS. Hum Reprod. 2012;27:2484–93.
45. Wei D, Xie J, Yin B, Hao H, Song X, Liu Q, Zhang C, Sun Y. Significantly lengthened telomere in granulosa cells from women with polycystic ovarian syndrome (PCOS). J Assist Reprod Genet. 2017;34:861–6.
46. Wang C, Shen F, Zhu Y, Fang Y, Lu S. Telomeric repeat-containing RNA (TERRA) related to polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf). 2017;86:552–9.
47. Müezzinler A, Zaineddin AK, Brenner H. A systematic review of leukocyte telomere length and age in adults. Ageing Res Rev. 2013;12:509–19.
48. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. Circulation. 2001;103:1194–7.
49. Muscari A, Antonelli S, Bianchi G, Cavrini G, Dapporto S, Ligabue A, Ludovico C, Magalotti D, Poggiopollini G, Zoli M. Serum C3 is a stronger inflammatory marker of insulin resistance than C-reactive protein, leukocyte count, and erythrocyte sedimentation rate: comparison study in an elderly population. Diabetes Care. 2007;30:2362–8.
50. Lentz SR, Haynes WG. Homocysteine: is it a clinically important cardiovascular risk factor? Cleve Clin J Med. 2004;71:729–34.
51. Richards JB, Valdes AM, Gardner JP, Kato BS, Siva A, Kimura M, Lu X, Brown MJ, Aviv A, Spector TD. Homocysteine levels and leukocyte telomere length. Atherosclerosis. 2008;200:271–7.
52. Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, Duszewska AM, Smolarczyk R, Meczekalski B. Chronic low grade inflammation in pathogenesis of PCOS. Int J Mol Sci. 2021;22(7):3789. https://doi.org/10.3390/ijms22073789.
53. Papalou O, Livadas S, Karachalios A, Tolia N, Kokkoris P, Tripolitakis K, Diamanti-Kandarakis E. White blood cells levels and PCOS: direct and indirect relationship with obesity and insulin resistance, but not with hyperandogenemia. Hormones (Athens). 2015;14:91–100.
54. Covington JD, Tam CS, Pasarica M, Redman LM. Higher circulating leukocytes in women with PCOS is reversed by aerobic exercise. Biochimie. 2016;124:27–33.

55. Mantalaris A, Panoskaltsis N, Sakai Y, Bourne P, Chang C, Messing EM, Wu JH. Localization of androgen receptor expression in human bone marrow. J Pathol. 2001;193:361–6.
56. Tola EN, Yalcin SE, Dugan N. The predictive effect of inflammatory markers and lipid accumulation product index on clinical symptoms associated with polycystic ovary syndrome in nonobese adolescents and younger aged women. Eur J Obstet Gynecol Reprod Biol. 2017;214:168–72.
57. Wang J, You D, Wang H, Yang Y, Zhang D, Lv J, Luo S, Liao R, Ma L. Association between homocysteine and obesity: a meta-analysis. J Evid Based Med. 2021;14:208–17.
58. Li Y, Deng B, Ouyang N, Yuan P, Zheng L, Wang W. Telomere length is short in PCOS and oral contraceptive does not affect the telomerase activity in granulosa cells of patients with PCOS. J Assist Reprod Genet. 2017;34:849–59.
59. Pedroso DCC, Santana VP, Donaires FS, Picinato MC, Giorgenon RC, Santana BA, Pimentel RN, Keefe DL, Calado RT, Ferriani RA, Furtado CLM, Reis RM. Telomere length and telomerase activity in immature oocytes and cumulus cells of women with polycystic ovary syndrome. Reprod Sci. 2020;27:1293–303.
60. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, Hottenga JJ, Fischer K, Esko T, Surakka I, Broer L, Nyholt DR, Mateo Leach I, Salo P, Hägg S, Matthews MK, Palmen J, Norata GD, O'Reilly PF, Saleheen D, Amin N, Balmforth AJ, Beekman M, de Boer RA, Böhringer S, Braund PS, Burton PR, de Craen AJ, Denniff M, Dong Y, Douroudis K, Dubinina E, Eriksson JG, Garlaschelli K, Guo D, Hartikainen AL, Henders AK, Houwing-Duistermaat JJ, Kananen L, Karssen LC, Kettunen J, Klopp N, Lagou V, van Leeuwen EM, Madden PA, Mägi R, Magnusson PK, Männistö S, McCarthy MI, Medland SE, Mihailov E, Montgomery GW, Oostra BA, Palotie A, Peters A, Pollard H, Pouta A, Prokopenko I, Ripatti S, Salomaa V, Suchiman HE, Valdes AM, Verweij N, Viñuela A, Wang X, Wichmann HE, Widen E, Willemsen G, Wright MJ, Xia K, Xiao X, van Veldhuisen DJ, Catapano AL, Tobin MD, Hall AS, Blakemore AI, van Gilst WH, Zhu H, Erdmann J, Reilly MP, Kathiresan S, Schunkert H, Talmud PJ, Pedersen NL, Perola M, Ouwehand W, Kaprio J, Martin NG, van Duijn CM, Hovatta I, Gieger C, Metspalu A, Boomsma DI, Jarvelin MR, Slagboom PE, Thompson JR, Spector TD, van der Harst P, Samani NJ. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet. 2013;45:422427–427421422.
61. Bekaert S, De Meyer T, Rietzschel ER, De Buyzere ML, De Bacquer D, Langlois M, Segers P, Cooman L, Van Damme P, Cassiman P, Van Criekinge W, Verdonck P, De Backer GG, Gillebert TC, Van Oostveldt P. Telomere length and cardiovascular risk factors in a middle-aged population free of overt cardiovascular disease. Aging Cell. 2007;6:639–47.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.