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Telomere Shortening and Its Association with Oxidative Stress and Telomere Maintenance Genes in Schizophrenia

(Pemendekan Telomer dan Hubungannya dengan Tekanan Oksidatif dan Gen Penyelenggaraan Telomer dalam Skizofrenia)

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ABSTRACT

Schizophrenia is known to be associated with premature mortality. Oxidative stress and telomere maintenance gene polymorphisms are assumed to be the possible trait markers. Hence, the present study aimed to explore the relationship between leukocyte telomere length (LTL) with oxidative stress status and single nucleotide polymorphisms (SNPs) of *TERT*, *TERC*, and *MYNN* genes in patients suffering from schizophrenia. A total of 150 schizophrenia patients and 139 healthy volunteers were recruited. The participants had their LTL measured and *TERT* rs33954691, *TERC* rs2293607, and *MYNN* rs10936599 were genotyped. Oxidative stress index (OSI) based on the ratio of total oxidant status (TOS) to total antioxidant capacity (TAC) was calculated. We found that patients had significantly decreased relative LTL (p = 0.006), but there were no significant effects of oxidative stress level (p = 0.496 for TOS; p = 0.216 for TAC; p = 0.905 for OSI) on relative LTL in schizophrenia. We also confirmed the association between rs33954691 CC (p = 0.007), rs2293607 AG (p = 0.042), and rs10936599 CC (p = 0.001) genotypes and shortened relative LTL in patients with schizophrenia. Thus, the findings suggested the presence of accelerated biological aging in patients with schizophrenia, despite their chronological ages and oxidative stress status. In conclusion, gene polymorphisms may have varying effects on LTL. To further understand the telomere shortening in schizophrenia, baseline and repeated LTL should be investigated.

Keywords: Leukocyte telomere length; oxidative stress; schizophrenia; single nucleotide polymorphism

ABSTRAK

Skizofrenia diketahui dikaitkan dengan kematian pramatang. Tekanan oksidatif dan polimorfisme gen penyelenggaraan telomer dianggap sebagai penanda ciri yang berpotensi. Oleh itu, penyelidikan ini bertujuan untuk meneroka hubungan antara panjang telomer leukosit (LTL) dengan tekanan oksidatif dan polimorfisme nukleotida tunggal (SNP) dalam gen *TERT*, *TERC* dan *MYNN* dalam kalangan pesakit skizofrenia. Seramai 150 pesakit skizofrenia dan 139 sukarelawan sihat telah direkrut. Panjang relatif LTL peserta diukur, manakala genotip *TERT* rs33954691, *TERC* rs2293607 dan *MYNN* rs10936599 telah dianalisis. Indeks tekanan oksidatif (OSI) dikira berdasarkan nisbah jumlah status oksidan (TOS) kepada kapasiti antioksidan total (TAC). Kami mendapati bahawa pesakit mempunyai LTL relatif yang berkurangan secara signifikan (p = 0.006), tetapi tiada kesan signifikan tahap tekanan oksidatif terhadap LTL relatif dalam skizofrenia (p = 0.496 untuk TOS; p = 0.216 untuk TAC; p = 0.905 untuk OSI). Kami juga mengesahkan hubungan antara genotip rs33954691 CC (p = 0.007), rs2293607 AG (p = 0.042) dan rs10936599 CC (p = 0.001) dengan pemendekan LTL relatif dalam pesakit skizofrenia. Oleh itu, penemuan ini mencadangkan adanya penuaan biologi yang dipercepatkan dalam pesakit skizofrenia, tanpa mengira usia kronologi mereka dan tekanan oksidatif. Kesimpulannya, polimorfisme gen mungkin mempunyai kesan yang berbeza terhadap LTL. Untuk memahami dengan lebih lanjut pemendekan telomer dalam skizofrenia, kajian asas dan pengukuran berulang terhadap LTL harus dilakukan.

Kata kunci: Panjang telomer leukosit; polimorfisme nukleotida tunggal; skizofrenia; tekanan oksidatif

INTRODUCTION

All-cause mortality in schizophrenia is around three times higher than in the general population (Ali et al. 2022). The frequent aging-related medical conditions in schizophrenia suggest a link between this disorder and accelerated biological aging, implying biological age surpasses chronological age (Lindqvist et al. 2015). Telomere length (TL) serves as a biological aging marker, with telomeres composed of TTAGGG repeats bound by shelterin proteins at chromosome ends to protect it from degradation and fusion. A progressive shortening of telomere at each cell division causes loss of its protective capacity. Telomerase maintains TL by adding TTAGGG repeats, but its expression gradually decreases in the developmental stage and diminishes in somatic cells. When TL falls below a critical threshold, it leads to genomic instability, cellular senescence, and apoptosis (Zhang et al. 2018).

The exact pathways underlying the telomere shortening in schizophrenia are poorly understood. It can be directly related to the pathophysiology of schizophrenia or as a result of cumulative exposure to chronic stress (Schürhoff et al. 2021). The free radical or oxidative stress of aging suggests that aging may result from damage to cells by oxygen-related free radicals (Okusaga 2014). Telomeres are highly susceptible to oxidative stress due to their high guanine content, leading to 8-oxoguanine formation and potential further oxidation. Oxidative stress causes single-strand breaks and telomere erosion. Inefficient repair of telomeric damage results in cellular physiological changes, senescence or apoptosis (Lin & Epel 2022). Thus far, the definitive role of oxidative stress in schizophrenia remains ambiguous. Many studies have measured various oxidant and antioxidant markers in schizophrenia patients, but mixed findings were reported (Rambaud, Marzo & Chaumette 2022). Measuring individual oxidative stress markers can be costly, time-consuming, and labor-intensive. As both oxidant and antioxidant effects are additive, respectively, total oxidant status (TOS) and total antioxidant capacity (TAC) are measured to reflect the overall picture of oxidative and antioxidative status (Erel 2005, 2004). Meanwhile, oxidative stress index (OSI), the ratio of TOS to TAC, indicates the oxidative and antioxidative status concomitantly, and thus, the overall oxidative stress level in the body (Katerji, Filippova & Duerksen-Hughes 2019).

Besides, telomerase consists of the reverse transcriptase (TERT) and the essential RNA component (TERC) that provides the template for the synthesis of telomeric repeats (Armanios & Blackburn 2012). Mutations in *TERT* or *TERC* genes can potentially impair the telomerase activity, and result in telomere shortening and dysfunction (Bertuch 2016). The rs33954691 (C > T) located in exon 14 of the *TERT* gene with a synonymous change of histidine at codon 1013 (Scarabino et al. 2017), has been reported to be associated with longevity (Atzmon et al. 2010). Meanwhile, rs2293607 (A > G) in the promoter region of the *TERC* gene was linked with TL based on a dominant genetic model for the variant allele (Do et al. 2015). The A allele of rs2293607 was associated with longer telomere (Jones et al. 2012), whereas its G allele was associated with shorter LTL (Njajou et al. 2010). *MYNN* gene encodes myoneurin which regulates the gene expression. The rs10936599 (C > T), situated the upstream of *TERC* gene and the second exon of the *MYNN* gene was significantly associated with TL (Do et al. 2015; Jones et al. 2012), in which the association could be due to the linkage disequilibrium with rs2293607 of *TERC* gene (Do et al. 2015).

To the best of our knowledge, the information regarding the shortening of LTL in the Malaysian population with schizophrenia is limited. Hence, our primary objective was to analyze the relationship of the LTL to schizophrenia. We also investigate the effects of oxidative stress level reflected by TOS, TAC, and OSI, and single nucleotide polymorphisms (SNPs) of *TERT*, *TERC*, and *MYNN* on LTL.

MATERIALS AND METHODS

RECRUITMENT OF PARTICIPANTS

The present study was approved by the Medical Research and Ethics Committee (MREC) of the Ministry of Health Malaysia [NMRR-20-3224-57836 (IIR)] and the Scientific and Ethical Review Committee (SERC) of Universiti Tunku Abdul Rahman (U/SERC/308/2023). Informed consent was obtained from each participant. In total, 289 participants including 150 patients with schizophrenia and 139 healthy individuals were recruited.

The recruitment of patients was conducted in the outpatient clinic of the Department of Psychiatry and Mental Health at Hospital Kuala Lumpur, Malaysia. The patients who met the criteria of schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) were recruited. The exclusion criteria included the presence of comorbidity or major illness, other mental illnesses, history of substance abuse, and being unable to provide consent. Healthy controls including university staff, hospital staff, blood donors, and communities were recruited in Klang Valley, Malaysia during the same period. They were without having personal or family history of mental illness, comorbidity or major illness, and history of substance abuse. Their venous blood was collected by certified medical officer or nurse. Buffy coat and serum were obtained by centrifuging at 3,000 rpm for 10 min.

MEASUREMENT OF LTL

DNA was extracted from all participants' peripheral blood leukocytes using QIAamp DNA Blood Mini Kit (51106, Qiagen, Hilden, Mettmann, Germany). A

real-time quantitative polymerase chain reaction (qPCR) was performed to measure the relative LTL using each set of primers for telomere (target gene) and *36B4* (single copy gene) described by Cawthon (2002) and 2X SensiFAST SYBR Lo-ROX mix (BIO-94020, Bioline, London, England). Relative LTL was calculated using the formula of $2^{-\Delta\Delta Ct}$, in which $\Delta\Delta Ct = [Ct_{telomere} - Ct_{36B4}]$ of sample - $[Ct_{telomere} - Ct_{36B4}]$ of reference DNA from the HEK293T cell line (CRL-3216, ATCC, Manassas, Virginia, United States).

MEASUREMENT OF OXIDATIVE STRESS LEVEL

Serum TOS was assessed using TOS Colorimetric Assay Kit (E-BC-K802-M, Elabscience Biotechnology Co., Ltd., Houston, Texas, United States). The TOS level was measured using a spectrophotometer at 590 nm. The results were expressed in µmol H_2O_2 Equivalent/L. Serum TAC was measured using OxiSelect™ TAC Assay Kit (STA-360, Cell Biolabs Inc., San Diego, California, United States) at 490 nm. Serum TAC was expressed in mM uric acid equivalents (UAE). OSI was calculated as the ratio of TOS to TAC using the formula of OSI (arbitrary unit) = TOS (µmol H_2O_2 Equiv./L) / TAC (mM UAE).

GENOTYPING

Whole blood DNA was extracted using QIAamp DNA Blood Mini Kit. SNPs in *TERT* (rs33954691), *TERC* (rs2293607), and *MYNN* (rs10936599) genes were analyzed using PCR-restriction fragment length polymorphism (RFLP) (Scarabino et al. 2017).

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) version 29 (SPSS Inc., Chicago, Illinois, United States) was used to analyze the data. Both descriptive and analytical statistics were conducted. Data were presented in the median and interquartile range (IQR). Mann-Whitney U Test was conducted to compare non-normally distributed variables. Meanwhile, Kruskal-Wallis test was utilized for comparing continuous variables across multiple groups. To adjust the effects of covariates including gender, age, ethnicity, BMI, and smoking, Quade test was conducted. Spearman's correlation analysis was employed to evaluate the association between continuous variables. Hardy-Weinberg equilibrium (HWE) was calculated using Chi-square or Fisher's exact tests for each SNP in both patient and control groups. The association between genotype and allele frequencies of each SNP and schizophrenia was analyzed using Chi-square test. To determine odds ratios (ORs) and 95% confidence intervals (CIs), a logistic regression analysis was performed. Linkage disequilibrium analysis was conducted using SHEsis Online Version (http://analysis.bio-x.cn/myAnalysis.php) (Shi & He 2005). A result was regarded as statistically significant if the *p*-values were lower than 0.05.

RESULTS

DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

The patient group [median (IQR) age = 42.6 (17.6) years old] comprised 63.3% Malay, 24.0% Chinese, and 12.7% Indian. The recorded ethnicity of the control group [median (IQR) age = 36.5 (11.1) years old] included Malay (48.2%), Chinese (39.6%), and Indian (12.2%). The median (IQR) BMIs of patients with schizophrenia and healthy controls were 26.08 (7.36) and 25.28 (7.41) kg/m^2, respectively. Among patients, 35.3% of them were smokers, 42.0% were working, and 10.7% had a family history of schizophrenia. The median (IQR) age of diagnosis with schizophrenia was 27.0 (11.3) years old. On the other hand, 7.9% of the controls were smokers and 82.7% were employed. All patients were on antipsychotic treatment. The median (IQR) chlorpromazine equivalent dose was 359.3 (421.0) mg/day. Among them, 18.0% were taking typical antipsychotics, 53.3% were taking atypical antipsychotics, and 28.7% were taking combined antipsychotics (both typical and atypical antipsychotics).

DIFFERENCE IN RELATIVE LTL BETWEEN PATIENTS WITH SCHIZOPHRENIA AND HEALTHY CONTROLS

There was a significantly decreased relative LTL in patients compared to healthy controls (U = 7920.00, $p < 0.001$) (Figure 1). The significant difference was unaltered after controlling for covariates including gender, age, ethnicity, BMI, and smoking [F (1, 287) = 7.606, $p = 0.006$].

AGE EFFECT ON RELATIVE LTL

There was no significant correlation between age and relative LTL in patients [r (144) = -0.018, p = 0.832]. In healthy controls, a significant negative correlation existed between age and relative LTL [r (133) = -0.177, $p = 0.040$].

DIFFERENCE IN OXIDATIVE STRESS LEVEL BETWEEN PATIENTS WITH SCHIZOPHRENIA AND HEALTHY CONTROLS

Median (IQR) OSI was significantly higher in patients with schizophrenia [66.91 (60.14)] compared to healthy controls [51.23 (66.41)] [F (1, 287) = 4.868, p = 0.028] (Table 1). No significant differences in TOS [F (1, 287) = 1.282, p = 0.259] and TAC [F (1, 287) = 3.683, p = 0.056] were observed between patients and healthy controls.

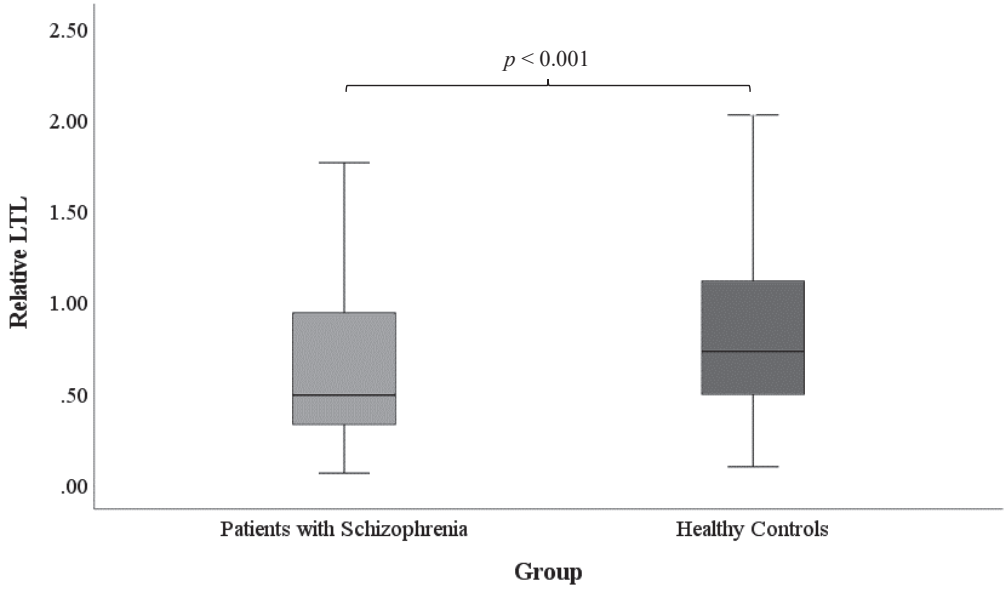

FIGURE 1. Boxplot illustrating relative LTL in patients with schizophrenia and healthy controls. The median (IQR) relative LTL of patients was 0.49 (0.62) whereas that of controls was 0.73 (0.63)

TABLE 1. Oxidative stress level [median (IQR)] between patients with schizophrenia and healthy controls

Oxidative stress level	Patients with schizophrenia (n = 150)	Healthy controls (n = 139)	Mann-Whitney U test	Quade test*
TOS (μmol H_2O_2 Equiv./L)	27.93 (28.75)	25.00 (28.58)	$U = 9132.50$, $p = 0.069$	$F(1, 287) = 1.282$, $p = 0.259$
TAC (mM UAE)	0.42 (0.13)	0.43 (0.22)	$U = 9653.00$, $p = 0.277$	$F(1, 287) = 3.683$, $p = 0.056$
OSI (arbitrary unit)	66.91 (60.14)	51.23 (66.41)	$U = 8489.50$, $p = 0.006$	$F(1, 287) = 4.868$, $p = 0.028$

*Adjusted for gender, age, ethnicity, BMI, and smoking

TABLE 2. Spearman's correlation between relative LTL with oxidative stress level in patients with schizophrenia and healthy controls

Group	Correlation analysis	Oxidative stress level		
		TOS	TAC	OSI
Patients with schizophrenia	Spearman's correlation	$r(148) = -0.064$, $p = 0.433$	$r(148) = -0.136$, $p = 0.097$	$r(148) = -0.001$, $p = 0.993$
	Partial correlation*	$r(143) = -0.057$, $p = 0.496$	$r(143) = -0.103$, $p = 0.216$	$r(143) = -0.010$, $p = 0.905$
Healthy controls	Spearman's correlation	**$r(137) = 0.392$, $p < 0.001$**	$r(137) = 0.143$, $p = 0.093$	**$r(137) = 0.313$, $p < 0.001$**
	Partial correlation*	**$r(132) = 0.368$, $p < 0.001$**	$r(132) = 0.084$, $p = 0.334$	**$r(132) = 0.317$, $p < 0.001$**

*Adjusted for gender, age, ethnicity, BMI, and smoking

RELATIONSHIP BETWEEN RELATIVE LTL AND OXIDATIVE STRESS LEVEL

As shown in Table 2, significant positive correlations were observed between relative LTL and TOS [r (132) = 0.368, $p < 0.001$] as well as between relative LTL and OSI [r (132) = 0.317, $p < 0.001$] in the healthy control group. Meanwhile, relative LTL was not correlated with oxidative stress level in patients with schizophrenia ($p > 0.05$).

TERT, *TERC*, AND *MYNN* SNPs IN SCHIZOPHRENIA

All cases and controls were in HWE (Cases: $p > 0.05$ for rs33954691, $p = 0.734$ for rs2293607, $p = 0.338$ for rs10936599; Controls: $p > 0.05$ for rs33954691, $p = 0.671$ for rs2293607, $p = 0.748$ for rs10936599). Significant associations were observed between both rs2293607($\chi2 = 6.230$, $p = 0.044$ for genotype; $\chi2 = 4.899$, $p = 0.027$ for allele) and rs10936599 ($\chi2 = 8.232$, $p = 0.016$ for genotype; $\chi2 = 5.725$, $p = 0.017$ for allele) with schizophrenia (Table 3). No significant associations between rs33954691 and schizophrenia were found ($p > 0.05$ for both genotype and allele).

In rs2293607 (Table 3), AG genotype [Adjusted OR (95% CI) = 2.137 (1.013 - 4.509), $p = 0.046$] and GG genotype [Adjusted OR (95% CI) = 2.593 (1.170 - 5.747), $p = 0.019$] significantly augmented the risk of schizophrenia compared to those with AA genotype. G allele carriers had approximately 1.45 times higher risk of developing schizophrenia compared to A allele carriers [OR (95% CI) = 1.454 (1.043 - 2.025), $p = 0.027$].

Meanwhile, as illustrated in Table 3, only the CT genotype of rs10936599 significantly increased the risk of schizophrenia compared to CC genotype carriers [Adjusted OR (95% CI) = 2.585 (1.475 - 4.528), $p < 0.001$]. A higher risk of emerging schizophrenia was found in individuals with T allele than C allele carriers with OR (95% CI) of 1.582 (1.085 - 2.307) ($p = 0.017$).

LINKAGE DISEQUILIBRIUM ANALYSIS OF *TERC* AND *MYNN* SNPs

Moderate degree of linkage disequilibrium was observed in rs2293607-rs10936599 (D' = 0.647, $r^2 = 0.107$).

RELATIONSHIP BETWEEN *TERT*, *TERC*, AND *MYNN* SNPs AND RELATIVE LTL

As demonstrated in Table 4, rs10936599 CC genotype carriers had significantly ($p = 0.005$) increased relative LTL compared to individuals with CT genotype in the control group only. For rs33954691 and rs2293607, no significant differences in relative LTL were observed ($p > 0.05$) in this group. On the other hand, all three SNPs were not significantly associated with differences in relative LTL among patients ($p > 0.05$). Between groups (Table 4), patients with rs33954691 CC genotype ($p = 0.007$), rs2293607 AG genotype ($p = 0.042$), and rs10936599 CC genotype ($p = 0.001$) had significantly shorter relative LTL than controls.

DISCUSSION

Significant shortened relative LTL in this study indicated the presence of accelerated aging in Malaysian patients with schizophrenia. This may explain the higher mortality rate in patients with schizophrenia (0.5%) compared to that of the general population (0.001%) in Malaysia (Teoh et al. 2017). Besides, longer LTL may help in maintaining brain structure and function, including larger grey matter volumes (Topiwala et al. 2023), in which shorter TL in peripheral blood was linked to reduced grey matter volume, and all were related to worse memory performance in schizophrenia (Czepielewski et al. 2018). LTL shortening was also related to cortical thinning (Puhlmann et al. 2019), where the cortex of individuals with schizophrenia exhibits widespread excessive thinning over time, especially in the frontal and temporal regions (Van Haren et al. 2011). In addition to the shortened TL in leukocytes, accelerated aging was also confirmed by the findings of reduced TL in the superior temporal gyrus white matter of patients with schizophrenia (Van Mierlo et al. 2017).

Moreover, LTL was summarized to be negatively correlated with age (Müezzinler, Zaineddin & Brenner 2013). As expected, a significant inverse relationship between relative LTL and age was observed among healthy controls in the present study. However, the negative correlation was not significant in the patient group. A possible explanation comes from a markedly negative correlation between age and TL in the white matter of the superior temporal gyrus of the controls but not in the patients (Van Mierlo et al. 2017). In the first year following the onset of schizophrenia, patients' brains experience accelerated aging, resulting in the brain age exceeding the chronological age (or the brain age gap) by approximately two years after this initial period. The acceleration rate then slows down significantly over the subsequent five years (Schnack et al. 2016). All these may indicate that chronological age is not the major factor in decreasing the telomeres in patients with schizophrenia. Instead, premature aging was found to occur at younger ages of patients compared with the controls of the same age, whereas the LTL shortening was similar in elder patients and controls (Russo et al. 2018). As the present investigation was a cross-sectional design, the shortening rate of LTL with age in schizophrenia could not be concluded.

Alternatively, this study found significantly elevated OSI in outpatients with schizophrenia than in healthy participants, despite the non-significant differences in serum TOS and TAC. A previous longitudinal study found that serum TAC levels at midday and midnight were normalized three months after patients were discharged from hospitalization. The levels were comparable with those of healthy controls, which were higher TAC at midday than that at midnight. This may convey that patients behave as healthy individuals after discharge (Morera-Fumero et al. 2017). Nevertheless, as oxidative stress is a dynamic process, both oxidant and antioxidant

TABLE 3. Distribution, association, and OR of genotypes and alleles of *TERT*, *TERC*, and *MYNN* SNPs

SNP	Variable		Patients with schizophrenia (n = 150)	Healthy controls (n = 139)	χ2	*p*-value	OR (95 % CI), *p*-value	Adjusted OR (95 % CI), *p*-value[†]
TERT								
rs33954691	Genotype	CC	141 (94.0 %)	128 (92.1 %)	0.410	$p = 0.522$	1.000	1.000
		CT	9 (6.0 %)	11 (7.9 %)			0.743 (0.298 - 1.851), $p = 0.523$	1.054 (0.379 - 2.932), $p = 0.920$
		TT	0 (0 %)	0 (0 %)			-	-
	Allele	C	291 (97.0 %)	267 (96.0 %)	0.395	$p = 0.529$	1.000	
		T	9 (3.0 %)	11 (4.0 %)			0.751 (0.306 - 1.840), $p = 0.531$	
	HWE (χ2, *p*-value)		0.110, $p > 0.05$[*]	0.208, $p > 0.05$[*]				
TERC								
rs2293607	Genotype	AA	19 (12.7 %)	33 (23.7 %)	6.230	$p = 0.044$	1.000	1.000
		AG	75 (50.0 %)	64 (46.0 %)			2.035 (1.057 - 3.921), $p = 0.034$	2.137 (1.013 - 4.509), $p = 0.046$
		GG	56 (37.3 %)	42 (30.2 %)			2.316 (1.159 - 4.626), $p = 0.017$	2.593 (1.170 - 5.747), $p = 0.019$
	Allele	A	113 (37.7 %)	130 (46.8 %)	4.899	$p = 0.027$	1.000	
		G	187 (62.3 %)	148 (53.2 %)			1.454 (1.043 - 2.025), $p = 0.027$	
	HWE (χ2, *p*-value)		0.619, $p = 0.734$	0.798, $p = 0.671$				
MYNN								
rs10936599	Genotype	CC	69 (46.0 %)	87 (62.6 %)	8.232	$p = 0.016$	1.000	1.000
		CT	71 (47.3 %)	44 (31.7 %)			2.035 (1.245 - 3.325), $p = 0.005$	2.585 (1.475 - 4.528), $p < 0.001$
		TT	10 (6.7 %)	8 (5.8 %)			1.576 (0.590 - 4.207), $p = 0.364$	1.373 (0.444 - 4.248), $p = 0.582$
	Allele	C	209 (69.7 %)	218 (78.4 %)	5.725	$p = 0.017$	1.000	
		T	91 (30.3 %)	60 (21.6 %)			1.582 (1.085 - 2.307), $p = 0.017$	
	HWE (χ2, *p*-value)		2.167, $p = 0.338$	0.580, $p = 0.748$				

[*]Data were analyzed using Fisher's exact test

[†]Adjusted for gender, age, ethnicity, BMI, and smoking

TABLE 4. Comparison of relative LTL in patients with schizophrenia and healthy controls based on *TERT*, *TERC*, and *MYNN* SNPs

SNP	Genotype	Median (IQR) relative LTL		Mann-Whitney U test	Quade test*
		Patients with schizophrenia (n = 150)	Healthy controls (n = 139)		
TERT					
rs33954691	CC	0.48 (0.59), n = 141	0.74 (0.67), n = 128	$U = 6792.00$, $p < 0.001$	$F(1, 267) = 7.457$, $p = 0.007$
	CT	0.74 (0.92), n = 9	0.66 (0.42), n = 11	$U = 48.00$, $p = 0.909$	$F(1, 18) = 0.014$, $p = 0.908$
	TT	-, n = 0	-, n = 0	-	-
Mann-Whitney U test		$U = 557.50$, $p = 0.542$	$U = 675.00$, $p = 0.821$		
Quade test*		$F(1, 148) = 0.369$, $p = 0.545$	$F(1, 137) = 0.006$, $p = 0.936$		
TERC					
rs2293607	AA	0.45 (0.74), n = 19	0.75 (0.69), n = 33	$U = 220.50$, $p = 0.077$	$F(1, 50) = 0.661$, $p = 0.420$
	AG	0.47 (0.71), n = 75	0.72 (0.59), n = 64	$U = 1833.50$, $p = 0.017$	$F(1, 137) = 4.202$, $p = 0.042$
	GG	0.51 (0.46), n = 56	0.66 (0.71), n = 42	$U = 960.50$, $p = 0.122$	$F(1, 96) = 2.195$, $p = 0.142$
Kruskal-Wallis test		$H(2) = 0.501$, $p = 0.778$	$H(2) = 0.708$, $p = 0.702$		
Quade test*		$F(2, 147) = 0.055$, $p = 0.947$	$F(2, 136) = 0.742$, $p = 0.478$		
MYNN					
rs10936599	CC	0.48 (0.49), n = 69	0.78 (0.92), n = 87	$U = 1790.50$, $p < 0.001$	$F(1, 154) = 11.219$, $p = 0.001$
	CT	0.46 (0.75), n = 71	0.59 (0.81), n = 44	$U = 1538.00$, $p = 0.890$	$F(1, 113) = 0.006$, $p = 0.938$
	TT	0.62 (0.56), n = 10	0.54 (0.42), n = 8	$U = 33.00$, $p = 0.534$	$F(1, 16) = 0.137$, $p = 0.716$
Kruskal-Wallis test		$H(2) = 0.892$, $p = 0.640$	$H(2) = 10.507$, $p = 0.005$†		
Quade test*		$F(2, 147) = 0.295$, $p = 0.745$	$F(2, 136) = 4.585$, $p = 0.012$‡		

*Adjusted for gender, age, ethnicity, BMI, and smoking

†Mann-Whitney U test. CC vs. CT: $U = 1296.00$, $p = 0.003$; CC vs. TT: $U = 221.50$, $p = 0.090$; CT vs. TT: $U = 171.50$, $p = 0.909$

‡Quade test. CC vs. CT: $p = 0.005$; CC vs. TT: $p = 0.140$; CT vs. TT: $p = 0.958$

capacities should be considered simultaneously. The increased OSI indicated the shift of the equilibrium to the oxidative side, thus, demonstrating the presence of oxidative stress in the investigated population (Sánchez-Rodríguez & Mendoza-Núñez 2019).

Significant weak positive correlations were found between TOS and OSI with relative LTL, respectively, in healthy controls. Generally, LTL was found to be positively correlated with TAC and reduced GSH, whereas negatively correlated with MDA during aging in the healthy population. It is yet to be identified whether there is a direct or indirect effect of oxidative stress on LTL (Yadav & Maurya 2022). Both oxidative stress and TL vary depending on the sample types. Hence, it may be hard to conclude their relationship if they are measured in different biological samples (Reichert & Stier 2017). Besides, oxidative stress can contribute to telomeric attrition only after subsequent cellular replication. Hence, the sampling intervals may also influence the correlation between oxidative stress and telomere shortening. Meanwhile, there were no significant correlations between relative LTL with serum levels of TOS, TAC, and OSI, respectively, in patients with schizophrenia, though ROS was significantly inversely related to LTL in individuals with type 2 diabetes mellitus and severe mental illnesses including schizophrenia (Sánchez-Ortí et al. 2024).

Both rs2293607 and rs10936599 were significantly associated with schizophrenia in the Malaysian population. The carriers of rs2293607 AG and GG genotypes, and the rs10936599 CT genotype notably increased the risk of schizophrenia. In addition, the rs2293607 G allele and the rs10936599 T allele also had the remarkable higher likelihoods of developing schizophrenia. Although we did not observe any significant relationship between rs33954691 in the *TERT* gene and schizophrenia, another three SNPs (rs2075786, rs4975605, and rs10069690) in this gene were found to be significantly associated with paranoid schizophrenia in the Chinese Han population (Rao et al. 2016).

In the current study, only patients with the rs33954691 CC genotype had significantly shorter relative LTL than healthy individuals. In contrast, the T allele was longevity-related (Atzmon et al. 2010). The rs33954691 may alter a splice site, thus, changing the splicing patterns or efficiency (Wysoczanska et al. 2019). Meanwhile, significantly decreased relative LTLs were observed in patients with the rs2293607 AG genotype. One explanation for these observations is attributed to the fact that rs2293607 could potentially influence *TERC* expression, which in turn may impact LTL. The A allele was associated with upregulated *TERC* and longer telomeres (Jones et al. 2012). Conversely, the G allele was linked with shorter LTL in healthy populations (Njajou et al. 2010). Our results showed that patients with the CC genotype of rs10936599 were having significantly shorter relative LTL compared to controls. The major and minor alleles of rs10936599 may exhibit varying effects on LTL depending on the population and disease context. For instance, in our investigated population, the major allele of rs10936599 was the C allele whereas the T allele was the minor allele. The minor T allele was key to shortening LTL in UK samples with childhood-onset major depressive disorder (Michalek et al. 2017), but the major C allele was linked to shorter LTL in cancer-free controls (Antwi et al. 2020), as well as Pakistan newborns and their parents with diseases including hypertension and diabetes mellitus (Farrukh et al. 2024). The association between this synonymous SNP and TL may also be due to LD with rs2293607 of *TERC* (Do et al. 2015). This explanation is supported by the moderate LD between rs10936599 and rs2293607 in this study.

This study has several limitations. As a cross-sectional study, it cannot determine the cause-and-effect relationship between LTL and schizophrenia. It remains unclear when the shortened relative LTL occurred, as well as what the accelerated rate of telomere attrition is in schizophrenia patients. Additionally, the lack of information on confounding factors including lifestyle and environmental variables may further limit the interpretation of the current results. To further understand the telomere shortening in schizophrenia, this would best be considered in the future to measure baseline and repeated LTL.

CONCLUSIONS

Malaysian outpatients with schizophrenia experienced accelerated biological aging compared to healthy individuals, despite the patients' chronological ages. However, this shortened relative LTL was not related to oxidative stress, but may be influenced by *TERT*, *TERC*, and *MYNN* SNPs.

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