Leukocyte telomere length is not shortened in methamphetamine dependence or methamphetamine-induced psychosis but is increased following traumatic events.

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Abstract

Background: Methamphetamine (MA) is one of the most common drugs of abuse in Thailand. MA use may cause neurotoxicity, immune-inflammatory and oxidative stress responses and, consequently, MA-induced psychosis (MIP).

Aims: This study aims to examine the effects of MA use and dependence and MA withdrawal symptoms on the telomere to single copy gene (T/S) ratio and whether shortening of the latter is associated with MIP.

Methods: This study included 185 MA-abuse, 118 MA-dependent, and 67 MIP patients, diagnosed using DSM-IV-TR criteria. The Semi-structured Assessment for Drug Dependence and Alcoholism (SSADDA) questionnaire was employed to collect clinical and MA-related data. MIP was confirmed using the Methamphetamine Experience Questionnaire (MEQ). The leukocyte telomere length was measured in all participants using real-time polymerase chain reaction measuring the Telomere/Single gene ratio (T/S ratio).

Results: There were no significant associations between the T/S ratio and severity of MA-use, MIP, MA withdrawal symptoms including depression and psychomotor retardation. MIP was significantly predicted by alcohol dependence, antisocial personality disorder, and MA-use severity. There were significant and positive associations between the T/S ratio and previous traumatic events and life-threatening accidents. The T/S ratio was not affected by comorbid alcohol and nicotine dependence. Alcohol and nicotine dependence, antisocial personality, and severity of MA use increased risk of MA withdrawal symptoms.

Conclusion: MIP and MA-use severity do not affect leukocyte telomere length, but telomere length may be affected by previous traumatic events and life-threatening accidents.

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Keywords: methamphetamine, psychosis, schizophrenia, withdrawal, depression, neuro-immune, oxidative stress, telomere length

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Introduction

Methamphetamine (MA) is one of the most commonly used drugs in Southeast Asia. According to Thai epidemiological data, there is an epidemic of MA use since 2009 and approximately 80 percent of drug users uses MA as their primary drug of abuse [1]. MA use can lead to several medical and psychiatric conditions such as increased risk of HIV transmission through shared needles [2, 3], cardiovascular disease [4], psychosis [5, 6] depression and cognitive impairment in long-term users [6, 7]. Recently, we discovered that use of MA may induce a specific pattern in long interspersed nuclear element-1 (LINE-1) methylation [8], namely higher percentage of mCuC and lower percentage of uCmC compared to controls. Moreover, previous studies showed that aberrations in LINE-1 methylation are accompanied by shortening of telomere length [9, 10].

Telomere is the structure at the tip of chromosome which functions as a DNA stabiliser [11, 12]. In humans, telomere shortening may be induced in conditions of systemic inflammation, chronic oxidative stress, and DNA damage [13-15]. Moreover, telomere shortening is also found in physical diseases related to aging such as several types of cancer [16], degenerative diseases [17] and vascular dementia [18]; and psychiatric disorders, including schizophrenia [19, 20], bipolar disorder [21, 22], major depressive disorder [23-25], anxiety disorders [26, 27], life stress [28] and childhood maltreatment [29]. In substance abuse, it was found that telomere shortening is significantly associated with cigarette smoking [30-36] and weakly with alcohol abuse [37, 38].

In humans, MA use induces a specific LINE-1 partial methylation profile and this process may participate in the pathophysiology of MA-induced psychosis (MIP) [39]. Moreover, animal and cell line models show that MA can induce neurotoxicity by increasing the levels of proinflammatory biomarkers such as interleukin-6 (IL-6) [40, 41], IL-8 [41] and tumor necrosis

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factor (TNF)-α [40, 42]. In humans, some of these cytokines, namely IL-6 and TNF-α, may be associated with telomere shortening [43]. By inference, it may be hypothesized that MA use could be associated with telomere shortening through effects on LINE-1 methylation, and inflammatory and oxidative stress pathways [39]. There is only one study which reported the effects of drugs of abuse on telomere length with abuse of diazepam and heroin significantly shortening telomere length in peripheral leukocyte [44]. However, this study did not examine possible associations between MA use, telomere length and MIP or comorbidities with other drugs of abuse.

Hence, the aims of the current study are to examine a) whether telomere length in peripheral leukocytes is increased in patients with MIP and MA use; and b) whether life stressors are accompanied by a shortened telomere length.

Patients and Methods

Participants

Subjects and measures have been described in previous study [45]. In brief, we recruited 370 participants of Thai nationality, aged 18-65 years old of both genders. All participants were hospitalized in the Princess Mother National Institute on Drug Abuse Treatment in Thailand for treatment of MA-related problems due to government regulations for drug using individuals. We included only individuals who used MA more than 10 times in their lifetime. Participants were categorized by DSM IV-TR criteria into MA dependence and abuse. The exclusion criteria for both groups were other axis 1 diagnosis including schizophrenia, bipolar disorder, obsessive compulsive disorder, and psycho-organic disorders, neurological diseases including stroke, Parkinson's disease, multiple sclerosis, brain injury, epilepsy, or dementia. The study procedure

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was approved by the Human Ethics Committee of Faculty of Medicine, Chulalongkorn University (Grant number RA 58/069). Written informed consent was obtained from all participants.

Measurements

Data were obtained using the Thai version of Semi-structured Assessment for Drug Dependence and Alcoholism (SSADDA) questionnaire based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [46]. The interviews were conducted by trained clinical psychologists certified to carry out the SSADDA interview. The diagnosis of MA-induced psychosis (MIP) was corroborated by using the Thai version of the Methamphetamine Experience Questionnaire (MEQ). This scale has been used in a previous MA study [45] and shows adequate internal consistency validity ($\kappa = 0.87$).

Based on the MA use features we have computed a total severity index using a z unit weighted composite score of z transformations of duration of illness + number of episodes + daily number of pills + number of days per month of MA use.

Leukocyte Telomere length measurement

Peripheral blood samples were drawn from all participants for extracting DNA in EDTA tubes and, consequently, sent to the Center for Excellence in Molecular Genetics of Cancer and Human Diseases, Faculty of Medicine, Chulalongkorn University. Genomic DNA was directly extracted from blood samples by standard procedures and kept at -20 ºC storage. DNA (4 ng) was processed in triplicates in quantitative real time polymerase chain reaction (PCR) both for telomere (Tel) and single-copy gene (hemoglobin-β, Hgb). PCR was done in Microamp Fast

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Optical 96-well reaction plate using power SYBR GREEN Master PCR Mix© (Invitrogen) according to the manufacturer's instruction. The primers for telomere PCR were Tel1 (5'-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGA GGGT-3') and Tel2 (5'-TCCCGAC TATCCCTATCCCTATCCCTATCCCTATCCCTA-3') while the primers for Hgb PCR were Hgb1 (5'-GCTTCTGACACAAC TGTGTTCACTAGC-3') and Hgb2 (5'-CACCAACTTCATCCAC GTTCACC-3'). The applied condition for PCR was based on procedure by Martinsson et al [47]. The relative gene expression was calculated by using comparative $2^{-\triangle\triangle Ct}$ method, which $\triangle\triangle Ct = \triangle Ct_{sample} - \triangle Ct_{calibrator}$ and $\triangle Ct_{sample} = \triangle Ct_{Tel} - \triangle Ct_{Hgb}$. DNA from MA-dependent and MA-abuse participants were assayed in the same plate. Inter-plate calibration was conducted by average of three control sample. Telomere length in leukocyte was calculated using T/S (telomere/single-copy gene) ratio.

Statistical analysis

We used analysis of variance (ANOVA) to assess differences in continuous variables between diagnostic groups and analysis of contingency tables (χ^2-tests) to assess associations between two sets of categorical variables. Multiple regression analysis was employed to assess the most significant input variables predicting the T/S ratio, including sex, age, alcohol and nicotine dependence, severity of MA dependence and traumatic events and life-threatening accidents in the past. Regression analysis were checked for multicollinearity using VIF and tolerance. We employed binary logistic regression analysis to examine the predictors of MIP or withdrawal symptoms as dependent groups (no MIP or withdrawal as reference groups) and with T/S ratio, alcohol and nicotine dependence, MA severity and antisocial personality as explanatory variables. The T/S ratio was processed in Ln transformation.

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Results

Table 1 shows the socio-demographic, clinical and T/S ratio data in patients with MA abuse, MA dependence and MIP. There were no significant differences in age, sex, and marital status. The T/S ratio was not significantly different between these three groups. The frequencies of unemployment and antisocial personality were significantly different between the three groups and increased from MA abuse to MA dependence and MIP. All MA-associated features (except age at onset and duration of MA use) were significantly higher in patients with MA dependence and MIP than in patients with MA abuse, including tolerance, withdrawal, episodes of heavy use, number of pills per day and days per month of use, severity of dependence and withdrawal, withdrawal symptoms such as depression, agitation, psychomotor retardation and nicotine dependence. In addition, severity of dependence and withdrawal, withdrawal with depressive and agitation symptoms, and alcohol dependence were significantly higher in MIP than in MA abuse patients.

In order to examine the MA-associated predictors of the T/S ratio, we performed multiple regression analyses with the T/S ratio as dependent variable and age, sex, and MA-associated data as explanatory variables. However, not one of the latter variables was a significant predictor of the T/S ratio. **Table 2** shows that the T/S ratio was positively associated with past traumatic events (regression #1) and life-threatening accidents (regression #2). These effects remained significant after adjusting (forced entry) for MA-associated features (regression #3).

In order to examine whether the T/S ratio may predict MIP, we performed binary logistic regression analysis with MIP as dependent variable and no MIP as reference group with the T/S ratio and the MA-associated features as explanatory variables. **Table 3**, regression #1 shows that

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the T/S ratio was not significant in predicting MIP. The latter was significantly predicted by alcohol dependence, antisocial personality, and the MA-severity index and even after allowing for these effects the T/S ratio was not significant in this regression.

Finally, we have examined whether the T/S ratio is associated with withdrawal symptoms and, therefore, we have carried out binary logistic regression analysis with withdrawal symptoms as dependent variable and MA-associated features and antisocial personality as explanatory variables (see **Table 4**). The T/S ratio did not significantly predict withdrawal symptoms. Nevertheless, withdrawal was significantly associated with alcohol and nicotine dependence, antisocial personality disorder, and the MA-severity index (regression #1). Withdrawal either with depressed mood, agitation or psychomotor retardation (regressions #2-#4) was associated with nicotine dependence and MA-severity index, but the T/S ratio was not significant in these regressions.

Discussion

The first major finding of this study is that there was no significant difference in T/S ratio between patients with and without MIP. To the best of our knowledge, our study is the first to examine the relationship between T/S ratio and psychosis induced by MA. There were some early reports which showed shortening telomeres in schizophrenia [19, 20] but more recent studies found evidence of longer telomeres in this condition when compared to controls [48, 49]. Maurya et al. (2018) [48] studied the T/S ratio in 173 patients with schizophrenia as compared with healthy controls and they found that schizophrenia patients had a significantly higher T/S ratio than the control group. Moreover, non-remitted schizophrenia patients also showed a higher T/S ratio as compared to remitted individuals. Zhang et al (2018) [49] investigated 1241 patients with

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schizophrenia compared to healthy controls and reported the similarly longer telomere length in patients with schizophrenia relative to control group. They also found higher T/S ratio in elderly and long-term hospitalized patients compared to younger outpatients with short-term hospitalization. These results reflect the inconsistent association between telomere length and psychosis and the mechanism of longer telomeres in this group remains unclear.

MIP is regarded as a model of psychotic symptoms in schizophrenia [39]. One of mechanisms underpinning both MIP and schizophrenia is a dysfunction in LINE-1 partial methylation and this pattern is even more expressed in paranoid schizophrenia than in MIP [8]. Our a priori hypothesis was to find an altered T/S ratio in MIP because LINE-1 methylation, which may cause telomere shortening, is associated with MIP [39]. Changes in LINE1 methylation predicts changes in the T/S ratio in several diseases including diabetes mellitus [10], biliary atresia [9] and dyskeratosis congenita [50]. Furthermore, LINE-1 plays a role in genomic stability and is affected by severe oxidative stress and increased levels of IL-6 [40, 41], IL-8 [41] and TNF-α, which are associated with MA abuse [40, 42] and shortening telomeres [43]. Nevertheless, our negative result can be explained by several factors. Firstly, MIP is a temporary stage of paranoia which is induced by drugs, and therefore, it may not always be an adequate model for chronic schizophrenia. Secondly, in spite of the findings that oxidative stress may shorten telomeres, a cumulative inflammatory load appears to be indispensable. O'Donovan et al (2011) [43] investigated systemic inflammatory biomarkers and telomere length in 1,962 high-functioning elderly and found that only the combination of increased level of IL-6 and TNF-α, TNF-α and c-Reactive Protein (CRP) and IL-6, TNF-α and CRP are related with shortening telomere lengths as compared to those with high levels of IL-6, TNF-α or CRP alone. Thirdly, the age of the patients included in our study may be another factor explaining the negative results.

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Generally, telomere length is shortened by ageing [13, 14, 51] and inflammatory or degenerative medical conditions [16-18] while the average age of our sample was 26 years and patients were excluded for medical diseases.

Interestingly, we found a significant positive correlation with small effect size between T/S ratio with history of traumatic events and life-threatening accidents after controlling for MA-associated variables. Previous studies on stress and psychological trauma showed shortening telomeres especially in individuals with childhood trauma [28, 29, 52]. In an animal model, Beery et al. (2012) [53] found that the activity of telomerase, the enzyme that lengthens telomeres, was significantly increased (by 54%) in stressed rats than in controls. Similarly, in a prospective experimental study in humans, Epel et al. (2010) [54] reported increased telomerase activity with greater cortisol level in response to stressors. Jimenez et al. (2018) [55] found a positive correlation between T/S ratio and sexual abuse scores in depressive individuals in Latin America. Additionally, Boks et al (2015) [56] investigated soldiers in combat zone and found positive correlations between post-traumatic stress disorder (PTSD) symptoms and lengthening of telomeres. Further longitudinal studies with different stressors are required to test this hypothesis.

Another finding of our study is that different assessments of the severity of MA abuse and other drugs of dependence are not associated with T/S ratio. These findings extend those of a previous report by Yang et al. (2013) [44] who compared leukocyte telomere length in 415 drug abusers and 499 normal controls. They reported shortening telomeres only in individuals who use depressant drugs but not in individuals who use stimulants, including 168 MA users. A recent systematic review and meta-analysis by Navarro-Mateu et al. (2020) [57] showed that

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individuals with substance use disorder appear to have a shorter telomere length than controls, but most studies examined alcohol users.

The current study also showed some interesting results on the features of MIP. Firstly, MIP was predicted by alcohol dependence, MA dependency severity, and antisocial personality. There are studies indicating that polydrug use increases risk of substance-induced psychosis [58, 59]. Specifically, using alcohol combined with MA can produce more stimulating effects than using either drug alone [60] and frequent alcohol use was found to increase the risk of psychosis in MA users [61]. Other studies showed that MIP is associated with the severity of MA use [59, 61-63] and antisocial personality [63], whilst the latter can be associated with long-term use of MA which may increase risk of psychosis [64].

Secondly, we found that MA withdrawal symptoms are associated with alcohol and nicotine dependence, antisocial personality and MA severity. A study by McGregor et al. (2005) [65] found that duration of MA use and the number of DSM-IV dependence criteria (which reflects MA severity in our study) were predictors of MA withdrawal severity. Antisocial personality may be related with withdrawal symptoms because it increases the risk of long-term MA use [64]. Alcohol or nicotine use are very common in Thai individuals with heavy MA use increasing the risk of withdrawal symptoms when they stop using MA [66, 67].

Limitations

To date, our study is the first to investigate the effect of MIP and severity of MA use, as well as its associated features, on human leukocyte telomeres. Nevertheless, there are some limitations of our study that need discussion. Firstly, this is a cross-sectional study and, therefore, it is always possible that the T/S ratio would change at a later point in time. Secondly, our study

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included only inpatient users and, therefore, the results cannot be generalized to outpatient MA users or users in the community who are likely to have a less severe MA use.

Conclusions.

MIP and MA-use severity do not affect telomere length of leukocytes, but telomere length is affected by previous traumatic events and life-threatening accidents.

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Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

Author's contributions

All the contributing authors have participated in the preparation of the manuscript.

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Table 1. Socio-demographic and clinical features and *telomere to* single copy gene *(T/S) ratio* of patients with methamphetamine (MA) use and MA dependence and patients with MA-induced psychosis (MIP).

Variables	MA abuse n = 185	MA dependence n = 118	MIP n = 67	F/χ^2	df	P value
Age	26.0 (0.5)	26.6 (7.3)	25.9 (6.2)	0.36	2/367	0.699
Sex (male: female)	133/52	77/41	48/19	1.65	2	0.439
Education	8.0 (2.5)	7.9 (2.7)	7.9 (2.3)	0.05	2/367	0.952
T/S ratio*	1.92 (3.94)	1.67 (3.29)	1.51 (2.80)			
Never married/ widowed-divorced -separated/ married	120/20/45	86/16/16	50/9/9	8.03	4	0.091
Employment status (Y/N)	84/101[B, C]	35/83[A]	19/48[A]	10.43	2	0.005
Antisocial personality (N/Y)	177/8[B, C]	103/15[A, C]	39/28[A, B]	58.27	2	<0.001
Tolerance (N/Y)	174/11[B, C]	54/64[A, C]	15/52[A, B]	142.54	2	<0.001
Withdrawal (N/Y)	170/15[B, C]	39/79[A, C]	4/63[A, B]	191.29	2	<0.001
Age of onset use (years)	18.9 (4.6)	18.6 (5.9)	17.4 (4.3)	2.05	2/367	0.131
Duration of use (years)	6.8 (5.2)	7.4 (5.2)	8.1 (5.0)	1.71	2/365	0.182
Episode of heaviest use in life	1.2 (0.4) [B, C]	1.7 (0.5) [A]	1.7 (0.5) [A]	51.40	2/364	<0.001
Number of pills/days	1.1 (0.3) [B, C]	1.5 (0.5) [A]	1.5 (0.5) [A]	38.95	2/367	<0.001
Day/months of meth use	10.9 (10.1) [B, C]	21.5 (8.8) [A]	22.8 (8.9) [A]	63.69	2/367	<0.001
Severity of dependence	1.6 (1.1) [B, C]	5.1 (1.4) [A, C]	6.2 (1.0) [A, B]	494.79	2/367	<0.001
Severity of withdrawal	1.7 (1.8) [B, C]	4.1 (1.8) [A, C]	5.0 (1.8) [A, B]	112.02	2/367	<0.001
Withdrawal depression (N/Y)	180/5 [B, C]	94/24 [A, C]	41/26 [A, B]	54.76	2	<0.001
Withdrawal agitation (N/Y)	175/10 [B, C]	90/28 [A, C]	41/26 [A, B]	43.36	2	<0.001
Withdrawal psychomotor retardation (N/Y)	137/48 [B, C]	31/87 [A]	13/54 [A]	94.35	2	<0.001

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Withdrawal craving (N/Y)	175/10 [B, C]	87/31 [A]	39/28 [A]	49.56	2	<0.001
Alcohol dependence (N/Y)	168/17 [C]	100/18 [C]	40/27 [A, B]	34.41	2	<0.001
Nicotine dependence (N/Y)	137/48 [A, B]	52/66 [C]	21/46 [C]	47.92	2	<0.001
Total severity index (z score)	-0.755 (0.590) [B, C]	0.615 (0.695) [A, C]	1.028 (0.642) [B, C]	273.12	2/364	<0.001
Traumatic events (N/Y)	146/39	99/19	46/21	5.93	2	0.052

All data are shown as mean (SD). *Processed in Ln transformation

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Table 2: Results of multiple regression analysis with the *telomere to* single copy gene (*T*/*S*) *ratio* as dependent variable

No	Explanatory variables	β	t	p	F model	df	p	R^2
#1	Model				5.04	1/363	0.025	0.014
	Traumatic event	0.117	2.45	0.025				
#2	Model				8.79	1/363	0.003	0.024
	Life threatening accident	0.154	2.97	0.003				
#3	Model				1.63	6/360	0.138	0.026
	Traumatic event	0.122	2.27	0.024				
	Sex	0.050	0.87	0.384				
	Age	-0.013	-0.23	0.818				
	Alcohol dependence	-0.048	-0.86	0.393				
	Nicotine dependence	-0.057	-1.03	0.306				
	MA severity index	-0.038	-0.69	0.487				

MA: methamphetamine

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Table 3: Results of logistic binary regression analysis with methamphetamine (MA)-induced psychosis (MIP) as dependent variable

#binary regression	Explanatory variables	β	SE	Wald	df	p	OR	95% CI
#1	T/S ratio	0.038	0.126	0.09	1	0.761	1.04	0.81-1.33
#2	Alcohol dependence	0.849	0.341	6.20	1	0.013	2.34	1.20-4.56
	Antisocial PD	1.412	0.357	15.62	1	<0.001	4.10	2.04-8.26
	MA severity index	0.847	0.156	29.36	1	<0.001	2.33	1.72-3.17

OR: Odds ratio, 95% CI: 95% confidence intervals

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Table 4: Results of logistic binary regression analyses with methamphetamine (MA)-associated withdrawal symptoms as dependent variables

Dependent variable	**Explanatory variables**	**β**	**SE**	**W**	**df**	**p**	**OR**	**95% CI**
#1. Withdrawal	Alcohol dependence	1.009	0.348	8.41	1	0.004	2.74	1.39-5.43
	Nicotine dependence	0.940	0.256	13.47	1	<0.001	2.56	1.55-4.23
	Antisocial PD	0.914	0.384	5.65	1	0.017	2.49	1.17-5.30
	MA severity index	0.909	0.133	46.76	1	<0.001	2.48	1.91-3.22
#2. Withdrawal, depressed mood	Nicotine dependence	1.520	0.357	18.12	1	<0.001	4.57	2.27-9.21
	MA severity index	0.641	0.167	14.80	1	<0.001	1.90	1.37-2.63
#3. Withdrawal, psychomotor retardation	Nicotine dependence	0.582	0.234	6.21	1	0.013	1.79	1.13-2.83
	MA severity index	0.749	0.121	37.97	1	<0.001	2.17	1.67-2.68
#4. Withdrawal, agitation	Nicotine dependence	0.950	0.309	9.46	1	<0.001	2.59	1.41-4.74
	MA severity index	0.739	0.157	22.22	1	<0.001	2.09	1.54-2.85