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Telomere length in Thai Buddhist monks and Thai males aged 40 years and above

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ABSTRACT

Introduction: The daily lifestyles of Thai Buddhist monks and Thai males differ due to Buddhist practices, which potentially affect telomere length. Telomeres are DNA compounds located at the ends of chromosomes that shorten with each cell division. This study investigated the difference in telomere length between Thai Buddhist monks and Thai males aged ≥40 years. **Method:** This was a cross-sectional study involving 100 Thai Buddhist monks aged ≥40 years who had been ordained for more than five years and 100 Thai males aged ≥40 years. General information and health information were assessed by questionnaire. Nutritional status was determined by body composition and blood chemistry parameters. Telomere length was measured by Monochrome Multiplex Real-Time Quantitative PCR and expressed as T/S ratio. **Result:** Mean telomere length of Thai Buddhist monks was longer than that of Thai males (1.08±0.18 vs. 1.02±0.17; $p<0.050$). In both groups, the mean telomere length in subjects aged ≥60 years was shorter than that in subjects aged 40-59 years ($p<0.010$). Alcohol consumption, which affected Thai males ($p<0.050$), but meditation tend to slow down the shortening of telomeres ($r=0.167$; $p<0.050$) in both groups. **Conclusion:** Age was the parameter that affected telomere length the most. Furthermore, various factors in the Buddhist monk group, such as a peaceful lifestyle, meditation, non-alcohol consumption, and fewer underlying diseases, could explain for the longer telomere lengths in this group.

Keywords: age groups, meditation, nutritional status, telomere length, Thai Buddhist monks

INTRODUCTION

Telomeres are deoxyribonucleic acid (DNA) molecules located at the end of the chromosome. They have a nucleoprotein structure, which has a repetitive nucleotide sequence. The nucleotide sequence in mammals is TTAGGG (Lu *et al*., 2013). Telomeres are bound with telomere-specific proteins, known as telosomes or shelterin (de Lange, 2005), which protect chromosome tips from end-to-end fusion and chromosome degradation, thus increasing DNA stability. Telomeres are

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also known as chromosome caps because they resemble a plastic or metal casing at the tip of the shoelace that protects the laces from fraying. Telomere research has progressed because scientists have considered that telomeres may predict chronological ageing and slow down senescence.

Telomerase enzymes elongate telomeres and add telomeric hexanucleotide repeat sequences to the ends of chromosomes. However, telomerase is not normally active in most somatic cells, so telomeres naturally shorten with age, shrinking with each cell division until they undergo apoptosis (cell death) (Ramirez *et al.*, 2003). However, age-related diseases, such as hypertension (Bhupatiraju *et al.*, 2012) and diabetes (Olivieri *et al.*, 2009), as well as lifestyle factors, including obesity (Zannolli *et al.*, 2008) and smoking (Valdes *et al.*, 2005), can cause oxidative stress or DNA damage that accelerates telomere attrition. On the contrary, numerous studies have indicated that meditation is beneficial for one's health and affects telomere length (Alda *et al.*, 2016; Hoge *et al.*, 2013).

Thai Buddhist monks are males who have been ordained and must adhere to strict behavioural and practical rules. Their daily routine consists of alms rounds, Dharma study, prayer, and meditation. Additionally, they also adopt some special dietary habits (Ariyesako, 1998), such as eating meals before noon, after which they are not allowed to eat anything until the next morning, and not drinking alcoholic beverages. Hence, the lifestyle differences between Thai Buddhist monks and the general people, especially Thai males, are noticeable. The purpose of this study was to investigate the difference in telomere length between Thai Buddhist monks and Thai males aged ≥40 years, and to study the relationship between telomere length, nutritional status, and lifestyle factors. This study will provide new information on the effects of meditation and knowledge about the practices of Buddhist monks that may slow down telomere shortening.

MATERIALS AND METHODS

This was a cross-sectional study that was conducted with Thai Buddhist monks and Thai males; it was the first study of telomere length in Thai Buddhist monks. The inclusion criterion was age ≥40 years. Subjects who were Thai Buddhist monks had to have been ordained for more than five years. The group of Thai males was recruited from various clubs and communities close to the temples where data were collected. Thai males and Thai Buddhist monks with normal body weight and obesity were classified by percent body fat (Gallagher *et al.*, 2000). The total number of subjects was 100 Thai males and 100 Thai Buddhist monks. Those with liver disease, kidney disease, thyroid disease, or anaemia according to a screening blood examination were excluded.

General and health information

General and health information, including dietary behaviour and stress information, were collected with a questionnaire.

Body composition assessment

The parameters of body composition were height (cm), body weight (kg), body mass index (BMI, kg/m^2) and body fat (% of body weight), which were used to divide participants according to obesity and normal body weight (Gallagher *et al.*, 2000), fat mass (kg), fat-free mass (FFM, kg), muscle mass (kg), total body water (%), bone mass (kg), and visceral fat, which was used to classify normal fat and excess visceral fat levels (Tanita, 2000). These parameters were assessed using a Tanita BC-420 MA body composition analyser (Tanita Co. Ltd., Japan).

Biochemical assessment
After a ten-hour overnight fast, fifteen millilitres of blood was collected and analysed using an automated blood BS-400 Chemistry Analyzer (Mindray Bio-Medical Electronics Co., Ltd. Shenzhen, China) for haemoglobin, fasting plasma glucose (FPG), blood urea nitrogen (BUN), and creatinine.

Telomere length measurement
DNA was purified from the sample (buffy coat) by a QIAamp DNA Blood Mini Kit (Qiagen, Germany). Telomere length was determined using the monochrome multiplex quantitative PCR (MMq-PCR) method, which evolved from the singleplex quantitative PCR assay (Cawthon, 2009). This method uses less DNA and takes less time to detect telomere length than other methods. The result of this method was the relative average telomere length, which is defined as a ratio of the quantity of telomere DNA (T) normalised to the copy number of single-copy nuclear genes (S), expressed as T/S ratio calculated per sample by CT values (T/S ratio = 2 (CT), where CT = CT sample – CT reference curve).

Ethics approval and permission
This study, including the protocols and consent forms signed by the subjects, was approved by the Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (ID 10-61-59). Written informed consent was obtained from the subjects.

Statistical analysis
Statistical analysis was performed using SPSS statistics version 18.0. General information, health information, dietary behaviour data, stress information, nutritional status, and telomere length measurements were all expressed as mean (± standard deviation, *SD*) or percentage. The independent sample *t*-test was used to examine the difference in mean telomere length among different variables. Pearson's correlation coefficient and multiple linear regression were used to assess the relationship between telomere length and various variables. Statistical significance was defined as a *p*-value <0.05.

RESULTS
Table 1 shows that age was divided into two groups: 40-59 years and ≥60 years. The average ages of Thai males and Thai Buddhist monks were not significantly different in each age group. In both Thai males and Thai Buddhist monks, the average values for each body composition variable were not significantly different. The average fasting plasma glucose (FPG) level of Thai Buddhist monks was within the normal range (97.22±22.17 mg/dL), while Thai males had an average FPG level within the pre-diabetes range (101.62±18.21 mg/dL). However, there was no significant difference in FPG levels between the groups.

Thai male subjects had more underlying diseases (62.0%) than Thai Buddhist monks (52.0%). Hypertension, dyslipidaemia, and diabetes were the three most common underlying diseases in this study. A total of 19.0% of Thai males consumed alcoholic beverages, while Thai Buddhist monks are not allowed to consume alcoholic beverages for the duration of their ordained life. Non-smokers accounted for 75.0% and 58.0% of Thai males and Thai Buddhist monks, respectively. Most of the subjects engaged in physical activity for 3-5 days/week for 30-60 minutes/day. The percentages of subjects who added sugar to their beverages were 47.0% among Thai males and 39.0% among Thai Buddhist monks. A total of 93.0% of Thai

Table 1. Characteristics of subjects

Characteristics	*Thai males (n=100)*		*Thai Buddhist monks (n=100)*	
Age (years), mean±*SD*				
40–59 years	50.0±5.9	(*n*=50)	49.5±5.7	(*n*=62)
≥ 60 years	69.5±6.8[a]	(*n*=50)	68.5±6.8[a]	(*n*=38)
Body composition, mean±*SD*				
Weight (kg)	70.2±12.2		69.4±13.1	
BMI (kg/m^2)	25.0±4.1		25.5±4.1	
Body fat (%bw)	22.3±6.2		22.9±5.9	
Fat mass (kg)	16.2±7.1		16.6±7.3	
Visceral fat	13.2±4.6		13.2±4.3	
FPG (mg/dL), mean±*SD*	101.6±18.2		97.2±22.2	
Underlying diseases, *n* (%)				
Yes	62 (62.0)		52 (52.0)	
No	38 (38.0)		48 (48.0)	
Alcohol consumption, *n* (%)				
Drinker	19 (19.0)		-	
Non – drinker	81 (81.0)		100 (100.0)	
Smoking status, *n* (%)				
Smoker	9 (9.0)		20 (20.0)	
Ex – smoker	16 (16.0)		22 (22.0)	
Non – smoker	75 (75.0)		58 (58.0)	
Physical activity[†], *n* (%)				
Yes	74 (74.0)		82 (82.0)	
No	26 (26.0)		18 (18.0)	
Adding sugar to beverages, *n* (%)				
Yes	47 (47.0)		39 (39.0)	
No	53 (53.0)		61 (61.0)	
Meditation				
Yes	47 (47.0)		93 (93.0)	
No	53 (53.0)		7 (7.0)	

[a]Significant difference from 40–59 years, $p<0.001$
[†]Physical activity for Thai Buddhist monks: walk for alms, clean the temple yard
BMI: body mass index, FPG: fasting plasma glucose

Buddhist monks meditated, and they spent 10-30 minutes/day meditating. However, half of Thai male subjects (53.0%) did not practise meditation.

The mean relative telomere length (TL) of Thai Buddhist monks was significantly higher than that of Thai males, at 1.08±0.18 and 1.02±0.17, respectively ($p<0.05$). In both Thai males and Thai Buddhist monks, the mean TL of subjects aged 40-59 years was significantly longer than that of subjects aged ≥60 years, as shown in Table 2.

Table 3 shows the mean TL of Thai males and Thai Buddhist monks classified by body composition parameters. Thai Buddhist monks showed a higher mean TL than Thai males across all BMI categories, especially significant in obese group (I). Thai Buddhist monks showed longer TL than Thai males in all body fat

Table 2. Mean (±SD) and range of relative telomere length classified by subject group and age group

Age (years)	*T/S ratio (range)*			
	Thai males	*n*	*Thai Buddhist monks*	*n*
40-59	1.08±0.14 (0.74–1.48)	50	1.13±0.17 (0.84–1.56)	62
≥60	0.97±0.17[a] (0.70–1.48)	50	1.00±0.16[a] (0.67–1.47)	38
Total	1.02±0.17 (0.70–1.48)	100	1.08±0.18[b] (0.67–1.56)	100

[a]Significant difference from aged 40–59 years, $p<0.010$
[b]Significant difference from Thai males, $p<0.050$

percentage criteria and fat mass index groups. At both visceral fat groups, the mean TL of Thai Buddhist monks was longer than that of Thai male subjects, significantly in the groups with excess visceral fat levels. However, there was no difference in TL when comparing normal weight subjects and obese subjects.

In subjects who did not have underlying diseases, Thai Buddhist monks had a significantly higher mean TL than Thai males and Thai Buddhist

Table 3. Mean±*SD* relative telomere length classified by subject group and body composition parameters

Parameters	*T/S ratio*			
	n (%)	*Thai males*	*n (%)*	*Thai Buddhist monks*
BMI (kg/m^2)				
Normal	30 (30.0)	1.02±0.17	28 (28.0)	1.07±0.15
Overweight	19 (19.0)	1.03±0.18	22 (22.0)	1.05±0.16
Obese I	39 (39.0)	1.02±0.17	34 (34.0)	1.12±0.20[a]
Obese II	12 (12.0)	1.05±0.14	16 (16.0)	1.07±0.19
Body fat (%bw)				
Normal	53 (53.0)	1.01±0.16	53 (53.0)	1.06±0.16
Obese I	38 (38.0)	1.04±0.19	31 (31.0)	1.11±0.17
Obese II	9 (9.0)	1.02±0.12	16 (16.0)	1.08±0.23
Fat mass (kg)				
≤50th percentiles	49 (49.0)	1.02±0.16	52 (52.0)	1.06±0.15
>50th - ≤ 75th percentiles	25 (25.0)	1.03±0.19	25 (25.0)	1.12±0.21
>75th percentiles	26 (26.0)	1.03±0.17	23 (23.0)	1.09±0.20
Visceral fat level				
Normal level	37 (37.0)	1.05±0.17	39 (39.0)	1.07±0.13
Excess visceral fat level	63 (63.0)	1.01±0.17	61 (61.0)	1.09±0.20[a]

[a]Significant difference from Thai males, $p<0.050$
BMI: body mass index

Table 4. Mean±*SD* relative telomere length classified by subject group, lifestyle and health parameters

Parameters	*T/S ratio*			
	n (%)	*Thai males*	*n (%)*	*Thai Buddhist monks*
Underlying diseases				
Yes	62 (62.0)	1.03±0.19	52 (52.0)	1.03±0.16
No	38 (38.0)	1.02±0.12	48 (48.0)	1.14±0.17[a*b]
Smoking status				
Smoker	9 (9.0)	1.10±0.27	20 (20.0)	1.07±0.20
Ex – smoker	16 (16.0)	1.00±0.16	22 (22.0)	1.02±0.16[c]
Non – smoker	75 (75.0)	1.02±0.15	58 (58.0)	1.11±0.17[a**]
Adding sugar to beverages				
Yes	47 (47.0)	1.01±0.18	39 (39.0)	1.11±0.19[a***]
No	53 (53.0)	1.04±0.16	61 (61.0)	1.07±0.17
Meditation				
Yes	47 (47.0)	1.04±0.16	93 (93.0)	1.09±0.18
No	53 (53.0)	1.01±0.16	7 (7.0)	0.97±0.15
FPG				
Normal	55 (60.4)	1.06±0.17	72 (79.1)	1.09±0.18
IFG	36 (39.6)	0.98±0.14[d]	19 (20.9)	1.07±0.15[a***]

[a]Significant difference from Thai males; *$p<0.001$, **$p<0.005$,***$p<0.050$
[b]Significant difference from yes group; $p<0.005$
[c]Significant difference from non-smoker; $p<0.050$
[d]Significant difference from normal; $p<0.050$
FPG: fasting plasma glucose; IFG: impaired fasting glucose

monks who had underlying diseases. In the Thai Buddhist monk group, the average TL of subjects who were ex-smokers was significantly shorter than those who were non-smokers. Among the subjects who added sugar to their beverages, the mean TL of Thai Buddhist monks was significantly longer than that of Thai males. Furthermore, the average TL of subjects who had normal FPG was longer than subjects who had impaired fasting glucose (IFG), particularly significant in Thai male subjects. In the IFG group, Thai Buddhist monks showed significantly longer TL than Thai males. In both Thai Buddhist monks and Thai males, the average TL of those who meditated was longer than those who did not meditate. Furthermore, as shown in Table 4, Thai Buddhist monks had longer TL than Thai males in the meditation group.

Additionally, Table 5 shows that age and underlying diseases had significant negative associations with TL (r=-0.407; $p<0.001$ and r=-0.163; $p<0.050$, respectively), but meditation was found to have a significant positive association with TL (r=0.167; $p<0.050$) in all subjects. Age (β= -0.407, $p<0.001$) and alcohol consumption (β= -0.163, $p<0.050$) were predictor variables that influenced telomere length when age, BMI, body fat, fat mass index, visceral fat, FPG, underlying diseases, alcohol consumption, daily smoking, adding sugar in beverages, and meditation were entered as independent variables in a multiple linear regression model for telomere length.

Table 5. Pearson's correlation coefficient of relative telomere length with studied parameters

Parameters	*T/S ratio*	
	r	*p value*
Age (years)	-0.407	<0.001
Underlying diseases	-0.163	<0.050
Smoking	0.002	NS
Adding sugar to beverage	0.007	NS
Meditation	0.167	<0.050
BMI (kg/m^2)	0.046	NS
Body fat (%bw)	0.026	NS
Fat mass (kg)	0.054	NS
Visceral fat level	-0.089	NS
FPG	-0.090	NS

BMI: body mass index, FPG: fasting plasma glucose; NS: Not significant

DISCUSSION

In this study, a difference in telomere length between Thai Buddhist monks and Thai males was observed. When assessed according to BMI, especially obesity status (I), excess visceral fat, adding sugar to beverages, impaired fasting glucose (IFG) level, and meditation, Thai Buddhist monks had longer telomere length than Thai males, which could be attributed to their different lifestyles. Thai Buddhist monks leave their families and get ordained in Buddhism, where they study Dharma and follow the rules and norms of Buddhist monks. Some of their principles include no sexual intercourse, no gossiping, restriction of physical activity, which allows Buddhist monks to only walk for alms every day in the morning, perform walking meditation, and clean the temple yard as their daily movements (Agence France-Presse, 2018), and eating before noon, after which only beverages without pulp are allowed (Ariyesako, 1998). They also pray and meditate daily. These characteristics are different between Thai Buddhist monks and Thai males. As a result of these behaviours, Thai Buddhist monks may have longer telomere lengths than Thai males. When we compared telomere length in females from a previous study to telomere length in males from this study, we discovered that females had longer telomeres than males, probably due to oestrogen causing telomerase to add telomere repeats to the end of chromosomes (Nawrot *et al.*, 2004).

Telomere shortening occurs with each somatic cell replication until they reach the *Heyflick* limit (Hayflick, 1998), at which stage they finish cell replication and enter the apoptosis (cell death) process. In our study, age had an adverse correlation with telomere length, with subjects aged 40-59 years having telomere lengths longer than those aged ≥60 years. Moreover, in a previous study (Nantanawut, 2021), which investigated the difference in telomere length between individuals with normal weight and obesity in various age groups in Thai subjects, age was negatively associated with relative telomere length in both normal weight and obese groups, and other studies have shown that telomere length shortens with age (Charoenying *et al.*, 2020; Srettabunjong *et al.*, 2014). The length of telomeres is used to calculate the biological age of the body, which is the age determined by the functioning of the body as a result of natural deterioration of the human process.

Furthermore, oxidative stress has been reported to increase in older age individuals, most likely as a result of an uncontrolled generation of free radicals induced by mitochondrial senescence and a decrease in antioxidant defences (Andriollo-Sanchez *et al.*, 2005). Oxidative stress is caused by an imbalance between antioxidants and reactive oxygen species (ROS); it occurs naturally in metabolic processes in the body or as a result of unhealthy behaviours, which can breakdown the single-stranded DNA of telomeres. Additionally, telomeres are high in guanines, which make DNA more easily oxidised and vulnerable to damage, and telomere DNA is incapable of repairing single-strand breaks, causing telomere length to shorten faster than it should (Singh *et al.*, 2019). Our research found a negative correlation between telomere length and underlying diseases, despite the weak relationship in our analysis. However, such diseases as hypertension, diabetes, and cardiovascular disease have been related to telomere shortening (Bhupatiraju, 2012.; Olivieri *et al.*, 2009.; Xu *et al.*, 2020). In addition, the average age of subjects with underlying diseases was higher than the average age of subjects without underlying diseases. As a result of telomere shortening, ageing may be one of the factors contributing to cellular senescence or age-related diseases, which induces oxidative stress and inflammation (Houben *et al.*, 2008).

In this study, Thai Buddhist monks who were non-smokers had telomere lengths longer than those who were ex-smokers, which is consistent with the results of Valdes *et al.* (2005), who reported that non-smokers had longer telomere lengths than ex-smokers. Smoking is a health risk factor that increases oxidative stress and some inflammatory markers were found to increase among ex-smokers 10-20 years after quitting (Yanbaeva *et al.*, 2007). As a result, telomere shortening may be accelerated.

Thai Buddhist monks had significantly longer telomeres than Thai males among subjects who added sugar to their beverages. While the amount of sugar added to beverages was not correlated with telomere length in this study, it was observed that the age of those who added sugar in both subject groups were significantly different, with Thai males having a significantly higher average age than Thai Buddhist monks. As a result, age may have a greater impact on telomere length in Thai males than in Thai Buddhist monks in the context of this parameter.

The study discovered that the mean telomere length in Thai males and Thai Buddhist monks with impaired fasting glucose (IFG) levels was lower than that in individuals with normal fasting plasma glucose (FPG) levels in both subject groups, which is consistent with a research that found that elderly patients with type 2 diabetes and myocardial infarction had shorter leukocyte telomere length (Olivieri *et al.*, 2009). Similarly, from the research of Sampson *et al.* (2006), who studied type 2 diabetes participants compared to control subjects, revealed that the diabetic group had a shorter mean monocyte telomere length than the control group, as well as a trend towards increased oxidative stress, which leads to telomere shortening.

We observed that alcohol consumption was one of the variables that affected telomere length in this study, similar to other studies that found that shorter telomeres were associated with alcohol consumption in older age (Dixit *et al.*, 2019) and that decreasing telomere length was associated with increased drink units (drinking >4 drink units) (Pavenello *et al.*, 2011). Excessive drinking (five glasses in one sitting or fifteen drinks per week) produces reactive oxygen species (ROS) and increases protein, lipid, and DNA peroxidation, leading to DNA damage (Wu & Caderbaum, 2003). Alcohol consumption was only found in Thai male subjects, implying that abstaining from intoxicants that cloud the mind, such as alcohol or narcotics, was one of the precepts that Thai Buddhist monks must follow (Lin, 2017).

On the contrary, meditation can slow telomere shortening. This study found a slight positive relationship between meditation with telomere length, as over 90% of Thai Buddhist monks meditated. However, among Thai males, only approximately half practised meditation. This could be another reason why telomeres of Thai Buddhist monks were longer than those in Thai males in our study, which is in line with a study assessing telomere lengths in people from the Soto Zen Spanish Buddhist community who have practised meditation (Alda *et al.*, 2016). The study found that Zen meditators who practised meditation for more than 10 years and spent at least 60 minutes/ day meditating had significantly longer telomeres than the control group (Alda *et al.*, 2016), as did a study of loving-kindness meditators, which found that the meditation group had significantly longer relative telomere length than the control group (Hoge *et al.*, 2013). Furthermore, Thai Buddhist monks were able to control their emotions more than Thai males, and they also had relatively less anxiety than Thai males. According to Buddhist doctrines that teach how to deal with anger and stress consciously, identifying the cause of the problem and resolving it effectively with wisdom and mindfulness are all related to meditation. Meditation, which focuses on the present moment, such as diaphragmatic breathing (slow and deep breathing) or loving-kindness meditation (focusing on developing a positive intention), can positively affect telomere length by reducing oxidative stress and increasing oxygen transportation to cells (Matarelli *et al.*, 2011).

CONCLUSION

Thai Buddhist monks showed longer telomere length than Thai males when the same parameters were assessed. Age was the parameter that affected telomere length the most. It was also found that underlying diseases, alcohol consumption, and meditation were related to telomere length. As a result, it is important to provide information on the advantages of meditation for self-relieving stress or problems that emerge in daily life, and the risks of drinking alcohol, which is one of the detrimental health behaviours found in the general public, except among Buddhist monks. The findings could be useful for the general public with application in health care for the prevention of telomere shortening and used as a guideline for future research. To observe telomere changes in the future, longitudinal study with larger sample sizes should be conducted. It would be interesting to study rural Buddhist monks (the Thai Forest tradition group) who place a strong focus on mental training during meditation in order to gain knowledge, they ventured out into the forest to be far away from any outside disruptions, which results in distinct daily routines than Buddhist monks who concentrate on understanding the Buddhist principles in order to share the knowledge gained with the general public, most of them stay in temples located in the city or village.

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Authors' contributions

PW, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; PL, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript, reviewed the manuscript; AT, assisted data analysis and interpretation, reviewed the manuscript; WP, assisted in data collection and reviewed the manuscript; SB, assisted in data collection and reviewed the manuscript; NP, assisted in data collection and reviewed the manuscript.

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Conflict of interest

No conflict of interest.

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