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Correlation between N-Acetyltransferase 2 (NAT2) Polymorphism Genotype with Plasma Isoniazid (INH) Concentration in MDR TB Patients Receiving Short Regimen in West Sumatera

Mega Senja, Masrul Basyar, Yessy Susanty Sabri, Afriani

Department of Pulmonology and Respiratory Medicine Faculty of Medicine, Universitas Andalas, Padang

Abstrak

Background: *Isoniazid (INH) is one of the most potent TB drug. High dose INH is used in short regimen MDR TB drugs. The genetic polymorphism of NAT2 affects the acetylation status. Awareness of the patients' acetylator status is important to determine the risk of toxicity, treatment failure and drug resistance. The aim of this study was to demonstrate NAT2 genotype association with INH plasma concentration after 2 hours of oral INH therapy.*

Methods: *This was a cross sectional study of MDR TB patients who received short term combination therapy at RSUP Dr. M.Djamil Padang, Achmad Muchtar Hospital Bukittinggiand West Sumatra Pulmonary Hospital from September 2019 to February 2020. Patients were examined for NAT2 genotype and plasma INH concentration. The results of the plasma INH concentrations obtained were evaluated based on the NAT2 acetylator phenotype group.*

Results: *The majority of the subjects weremen (62.5%), aged 40-64 years (50%), had the most common comorbid of diabetes mellitus (31.25%), were normoweight (75%) and had negative HIV status (93.8%). A total of 7 alleles consisting of 7 SNPs and 7 variations of the NAT2 genotype were found in MDR TB patients who received short-term therapy. The NAT2*12A alleles (56.25%) was the most common allele and was a fast acetylator. Based on the bimodal distribution, the median concentration of INH in the fast and slow acetylator were 1.25 µg/ml and 5.24 µg/ml, respectively. The median values of INH concentration based on the trimodal distribution for fast, intermediate, and slow acetylators were 1.25 µg/ml, 2.17 µg/ml and 5.24 µg/ml.*

Conclusion: *There were no correlations between the type of NAT2 acetylator phenotype and plasma INH concentrations. (**J Respirol Indones 2022; 42(1): 26-33)***

Keywords: *NAT2, INH, polymorphism, MDR TB*

Hubungan Polimorfisme Genotip N-Acetyltransferase 2 (NAT2) dengan Konsentrasi Isoniazid (INH) Plasma pada Pasien TB yang Mendapat Paduan Terapi Jangka Pendek di Sumatera Barat

Abstrak

Latar belakang*: Isoniazid (INH) adalah salah satu obat TB yang paling ampuh. INH dosis tinggi digunakan pada paduan terapi TB MDR jangka pendek. Polimorfisme genetik NAT2 berpengaruh terhadap status asetilasi. Pengetahuan mengenai status asetilator pasien penting untuk menilai risiko toksisitas, kegagalan pengobatan dan resistensi obat. Penelitian ini bertujuan mengetahui hubungan polimorfisme genotip NAT2 dengan konsentrasi plasma INH 2 jam setelah pemberian INH oral.*

Metode: *Penelitian potong lintang pada pasien TB MDR yang mendapat paduan terapi jangka pendek di RSUP Dr. M.Djamil Padang, RS Achmad Muchtar Bukittinggi dan RS Paru Sumatera Barat dari September 2019 hingga Februari 2020. Pasien diperiksa genotip NAT2 dan konsentrasi INH plasma. Hasil konsentrasi INH plasma yang didapatkan dievaluasi berdasarkan kelompok fenotip asetilator NAT2.*

Hasil:*Sebagian besar subjek penelitian adalah laki-laki (62,5%) berusia 40-64 tahun (50%), komorbid terbanyak ditemukan diabetes mellitus (31,25%), berstatus* normoweight*(75%) dan status HIV negatif (93,8%). Sebanyak 7 alel yang terdiri dari 7 SNP dan 7 variasi genotipe NAT2 ditemukan pada pasien TB MDR yang mendapat terapi jangka pendek. Alel NAT2*12A (56,25%) merupakan alel yang paling sering ditemukan dan merupakan asetilator cepat. Berdasarkan distribusi bimodal nilai tengah konsentrasi INH pada asetilator cepat dan lambat adalah 1,25µg/ml dan 5,24µg/m. Nilai tengah konsentrasi INH berdasarkan distribusi trimodal untuk asetilator cepat, menengah dan lambat secara berurutan adalah 1,25 µg/ml, 2,17µg/ml dan 5,24µg/ml.*

Kesimpulan: *Tidak ada hubungan antara jenis fenotip asetilator NAT2 dengan konsentrasi INH plasma. (**J Respirol Indones 2022; 42(1): 26-33)***

Kata kunci: *NAT2, INH, Polimorfisme, TB MDR*

Correspondence: Mega Senja
Email: dr.megasenja@gmail.com

INTRODUCTION

Indonesia is a country with the third-highest burden of Tuberculosis (TB) globally after India and China. One of the challenges faced today is the increasing number of drug-resistant TB cases. Multi Drug-Resistant Tuberculosis (MDR TB) is defined as TB that is resistant to at least two main anti-tuberculosis drugs, namely isoniazid (INH) and rifampin with or without other first-line drugs. According to the 2018 WHO Global Report, it was estimated that there were 558,000 cases of MDR TB worldwide. Indonesia itself ranks 10th in the incidence of MDR TB worldwide.[1]

In 2016 WHO began to recommend using a short-term regimen for the management of MDR-TB under certain conditions.[2] One of the drugs used in the short-term regimen is high-dose INH. Awareness of the acetylation status can help to decide which dose of INH to be used because treatment efficacy and INH toxicity are associated with increased metabolism (acetylation) in specific individuals, as determined by mutations in the NAT2 gene.[3]

INH has been an important component used for TB treatment since the early 1950s until now. The advantages of INH are effective against TB, affordable price, easy to digest, and well absorbed withthe maximum plasma concentration occurs 2 hours after administration. INH in the body is metabolized by the arylamine NAT2 enzyme, whose activity is influenced by genetic variations (polymorphisms).[4] This variation is responsible for plasma drug levels and drug half-life.[3]

Based on its ability to metabolize INH, the phenotype of the NAT2 gene can be categorized into distributions: bimodal and trimodal distributions. The bimodal distribution consists of fast acetylator and slow acetylator, while the trimodal distribution consists of fast acetylator, intermediate acetylator, and slow acetylator. The global distribution of NAT2 indicates that 15–40% of the world's population has a slow acetylator phenotype.[5] In Indonesia alone; a study conducted by Yuliwulandari, et al. on 212 people (Javanese and Sundanese ethnicity) found that the frequency of fast acetylator phenotype was 13.6%, intermediate acetylator of 50.8%, and slow acetylatorof 35.6%. The frequency of NAT2 genotype can vary between different ethnicities.[6]

A study conducted by Singh, et al. on 201 TB patients obtained a significant difference (P<0.001) between the variation of NAT2 genotype and plasma INH concentration. In this study, the fast acetylator group (13%) had a mean 2-hour plasma INH concentration of 2.4 µg/ml, the intermediate acetylator (32%) had a mean value of 4.1 µg/ml, while slow acetylator (55%) had 5.6 µg/ml.[7] Another study by Zabost, et al. on 130 patients found plasma INH concentrations for 3 hours in the fast (5.4%), intermediate (30%), and slow (64.4%) acetylator groups. were 1.2±0.6 µg/ml, 2.2±1.3 µg/ml and 4.4±1.5 µg/ml, respectively.[8]

Another study by Kumar, et al. in India regarding the genetic variation of NAT2 with 2-hours plasma INH concentration revealed that the median values in the three acetylator groups were significantly different, namely in the fast acetylator group (58%) had a median INH value of 10.2 µg/ml, the slow acetylator group (35%) had 8.1 µg/ml, while the intermediate acetylator group (23%) had 4.1 µg/ml.[9]

Study on NAT2 gene polymorphisms of TB patients in Indonesia, especially MDR TB, has not been widely carried out. The MDR TB combination treatment currently uses a short-term regimen, one of which is using high-dose INH drugs. Therefore, researchers are interested in investigating the polymorphism of the NAT2 gene and assessing INH levels based on their genotype in each MDR TB patient who received a standard short-term regimen at the MDR TB referral hospital in West Sumatra.

METHODS

This was a cross-sectional study of all MDR TB patients who received short-term combination therapy at RSUP Dr. M.Djamil Padang, Achmad Muchtar Hospital Bukittinggi, and West Sumatra Pulmonary Hospital from August 2019 to February 2020. The inclusion criteria were MDR TB patients on short-term intensive phase treatment for at least

4 weeks, aged >17 years, and willing to participate in the study by signing the consent form after receiving an explanation.

The analysis in this study consisted of univariate and bivariate analysis. Univariate analysis was carried out to determine the frequency distribution of MDR TB patients' characteristics and genotypic polymorphisms in MDR TB patients who received short-term regimens. In addition, an analysis was also carried out to see the mean and median plasma INH concentrations after 2 hours of administration. Bivariate analysis was performed using the Kruskal Wallis test for the trimodal group and the Mann-Whitney test for the bimodal group to examine the correlation between NAT2 genotypic polymorphisms and plasma INH concentrations after 2 hours.

RESULTS

This study analyzed data from a total of 16 patients. The characteristics of the patients were mostly male (62.50%), with the highest age group of 40–64 years old. Most of the patients (75%) were normoweight (BMI 18.5–24.9%). Subjects with comorbid of diabetes mellitus were 31.25%, however, only 1 subject with HIV (6.25%) was found. Rapid molecular testof most patients resulted in medium detectable MTB (Table 1).

Sequencing analysis could be performed on 16 samples. There were 7-point mutations (SNP). The combination of SNPs produced 7 different alleles which carried acetylator properties. The most common allele found was NAT2*12A (56.25%) which had a point mutation of 803 A>G (rs1208) and moved withfast acetylation properties. One sample had a mutation at point 341 T>C 776C>A 803 G>A. The mutation at point 776 C>A had not been published in the NAT2 database (accessible at http://nat.mbg.duth.gr), so the allele type has not been determined (Table 2).

In this study, the phenotype predictionwas divided based on the distribution of trimodal (fast acetylator, intermediate acetylator and slow acetylator) and bimodal (fast and slow acetylators). The acetylator phenotype is a combination of two alleles.

Based on trimodal phenotype predictions, the most common phenotypes found in this study were fast acetylators (62.5%) namely NAT2*4/*4, NAT2*12A/*12A; intermediate acetylators (12.50%) namely NAT2*12B/NAT2*12B, NAT2*6C/*12B; and slow acetylators (18.75%) namely NAT2*6C/*6C, NAT*5A/*5A, NAT2*7C/*7C. Meanwhile, 75% of fast acetylator and 18.75% of slow acetylator were found based on the bimodal distribution. There was 1 sample (6.25%) whose acetylator phenotype was unknown. Since it was unknown, the data was published in the NAT2 database (accessible at http://nat.mbg.duth.gr) so that the type of acetylator could not be determined (Table 3).

Table 1. Characteristics of MDR-TB patients receiving short-term regimen in West Sumatra.

Characteristic	Total (N)	Frequency (%)
Gender		
Male	10	62.5
Female	6	37.5
Age		
18–39 years	7	43.8
40–64 years	8	50.0
≥65 years	1	6.2
Body Mass Index		
Underweight (BMI<18.5)	4	25
Normoweight (18.5–24.9)	12	75
Comorbidity		
Diabetes mellitus	5	31.2
No comorbid	11	68.8
HIV Status		
Positive	1	6.2
Negative	15	93.8

All samples were examined for plasma INH levels 2 hours after drug administration. The median value of INH levels in the fast acetylator group was 1.25 µg/ml, the intermediate acetylator group of 2.17 µg/ml, and the slow acetylator groupof 5.24 µg/ml (Table 4). Meanwhile, based on a bimodal distribution, the median value of isoniazid levels in the fast acetylator group was 1.25 µg/ml, and in the slow acetylator group was 5.24 µg/ml (Table 4, Table 5).

Table 2. Distribution and frequency of NAT2 allele polymorphisms in MDR TB patients receiving short-term regimen in West Sumatra

Combines SNP (Haplotype)							NAT2 Allele	Prediction Phenotype	Allele Total (n)	Allele Frequency (%)
282 C>T (rs1041983)	341 T>C (rs1801280)	481 C>T (rs1799929)	590 G>A (rs1799930)	803 A>G (rs1208)	857 G>A (rs1799931)	776 C>A (rs1304162037) **				
C	T	C	G	A	G	C	NAT2*4	Fast Acetylator	2	6.25
C	C*	T*	G	A	G	C	NAT2*5A	Slow Acetylator	2	6.25
T*	T	C	A*	G*	G	C	NAT2*6C	Slow Acetylator	3	9.375
T*	T	C	G	G*	A*	C	NAT2*7C	Slow Acetylator	2	6.25
C	T	C	G	G*	A	C	NAT2*12A	Fast Acetylator	18	56.25
T*	T	C	G	G*	G	C	NAT2*12B	Fast Acetylator	3	9.375
C	C	C	G	A	G	A	Others	Unknown	2	6.25

Note: * Known and published SNPs; ** Unpublished SNP

Table 3. Distribution of NAT2 genotypes and prediction of acetylator phenotype

No	Genotype	Total	%	Phenotype Prediction			
				Trimodal distribution	%	Bimodal Distribution	%
1	NAT2*4/*4	1	6.25	Rapid	62.50	Rapid	75
2	NAT2*12A/*12A	9	56.25	Rapid		Rapid	
3	NAT2*12B/*12B	1	6.25	Intermediate	12.50	Rapid	
4	NAT*6C/12B	1	6.25	Intermediate		Rapid	
5	NAT*6C/*6C	1	6.25	Slow	18.75	Slow	18.75
6	NAT2*5A/*5A	1	6.25	Slow		Slow	
7	NAT2*7C/*7C	1	6.25	Slow		Slow	
8	Unknown	1	6.25	-		-	

Table 4. INH concentration after 2 hours of administration based on trimodal distribution

Variable	Fast Acetylator (n=10)	Intermediate Acetylator (n=2)	Slow Acetylator (n=3)
INH concentration (µg/ml)	1.25 (0–8.50)	2.17 (0–4.33)	5.24 (0.25–14.16)

Table 5. INH concentrations after 2 hours of oral administration in MDR TB patients based on bimodal group phenotype

Variable	Fast Acetylator (n=12)	Slow Acetylator (n=3)
INH concentration	1.25 (0–8.50)	5.24 (0.25–14.16)

Table 6. Correlation of INHlevels in the bimodal and trimodal group

Variable	Median	Min-Max	*P*
INH level	1.56	0–14.16	0.448
Bimodal	1.00	1–2	
INH level	1.56	0–14.16	0.598
Trimodal	1.00	1–3	

This study correlated the NAT2 genotypic polymorphism with INH concentration after 2 hours of oral administration in MDR TB patients based on the acetylator phenotype (bimodal and trimodal). The results showed that there were no correlations between INH levels in the trimodal group (*P*=0.598) as seen in Table 6.

DISCUSSION

Most of MDR TB patients who received short-term intensive phase therapy in this study were male (62.50%), with the highest age group being 40-64 years (50%), comorbid of diabetes mellitus (31.25%), and had normal BMI (75%). Several studies have obtained results that were not much different from this study. Pradipta, et al. reported that most of MDR TB patients who received short-term therapy at the Persahabatan Hospital (61.5%) were male with the age range being 41-50 years, had comorbid of diabetes mellitus (30.8%) but was dominated by subjects with underweight BMI status (32.7%).[10]

The NAT2 genotype polymorphism affects the acetylation status. The correlation between the acetylation phenotype and the NAT2 genotype has been reported in several studies.[11,12] In this study,

we obtained 7 different SNPs and there were 7 variations of SNP/polymorphism of the NAT2 genotype based on the results of the coding region sequencing, namely rs1041983, rs1801280, rs1799929, rs1799930, rs1208, rs1799931, and rs1304162037. Point mutations cause polymorphisms of the NAT2 gene (SNPs) in nucleotides, and their combinations produce different alleles.[13]

The allele variations in this study, namely NAT2*4, NAT2*5A, NAT2*6C, NAT2*7C, NAT2*12A, and NAT2*12B, were adjusted according to the guidelines of The Arylamine N-acetyltransferase Gene Nomenclature Committee accessed at http://nat.mbg.duth.gr/Human%20NAT2%20alleles_2013.htm. The alleles NAT2*4, NAT2*12A, NAT2*12B carry fast acetylator properties, while NAT2*5A, NAT2*6C, and NAT2*7C are carriers for slow acetylator properties.[13]

In this study, the NAT2*12A allele was the dominant allele (56.25%), followed by NAT2*5A (12.50%) and NAT2*6C (9.375%), while the other allele frequencies were <10%. The NAT2*4 allele is the reference allele or called the wild-type allele because no mutation was found.[14] In this study, the frequency was 6.225%. This finding was different from the study of Yuliwulandari, et al. who reported the frequency of the NAT2*4 allele was 36.9% and was the dominant allele. In contrast, the NAT2*12A allele was lower than the current study, which was <2% (0.8%).[15] Susilowati, et al. reported that the dominant allele was NAT2*6A (38%), while the NAT2*12A allele in that study was only 4%.[16] Study from Patin, et al. reported the frequency of NAT2*5B allele was 23.3%, NAT2*12A of 22.6%, and NAT2*6A of 18.6%.[17]

In previous studies in China, Japan, Indonesia, and Thailand, the NAT2*4 and NAT2*6 alleles were the most common.[15] In this study, new allele variations were found and were not reported in the NAT2 nomenclature database. The allele was mutated at 341 T>C, 776 C>A (rs1304162037), and 823 T>A (rs14856670). Based on the NAT2 gene data accessed at http://asia.ensembl.org, it was found that the point mutation 776 C>A (rs1304162037) was only released in April 2020, and there has been no publication so that the type of allele for this mutation could not be determined.[18]

The frequency of allele variations found in various studies was caused by variations in the NAT2 phenotype in different ethnic groups. The variations in some of the studies above may be due to differences in the ethnicity of the research subjects, such as this study that was conducted in West Sumatra, dominated by the Minang ethnic, study from Yuliwandari was conducted on the Javanese and Sundanese, study from Susilowati on the Indonesian Malay ethnicity and the study of Patin, et al. in the African population.[15–17] Variation can be caused by natural mutations, demographic influences, the influence of food consumed, and the influence of lifestyle as reported by Sabbagh et al. A study of the NAT2 gene variation in six Central Asian populations revealed clear differences between populations with different lifestyles and eating habits, which in this study were found to be slow acetylators in sedentary farmers (55–63%) compared to herders.[19]

Based on the bimodal distribution group, the frequency of the acetylator phenotype in this study was 75% fast acetylator and 18.75% slow acetylator while based on the trimodal distribution, the frequency of the acetylator phenotype in this study was 62.50% fast acetylator, intermediate acetylator 12.50% and 18.75% slow acetylator. The details of the combination of fast acetylator alleles (phenotypes) in this study are NAT*12A/NAT2*12A, NAT2*4/NAT2*4, NAT2*12B/NAT2*12B, NAT2*6C/NAT2*12B) and acetylators (NAT2*6C/NAT2*6C, NAT2*5A/NAT2*5A, NAT2*7C/NAT2*7C). In general, the dominant phenotype observed in this study was fast acetylator.

The prevalence of the NAT2 phenotype differs geographically, such as standard slow acetylator status in Egypt (83%) and in the United States (67%) but rare in northern Asia (12% in China). Study from Singh in Indian population notified that the dominant phenotype was slow acetylator (55%). Susilowati, et al. reported that in the Malay tribe the dominant phenotype was fast acetylator (62%).[15,16] Pramono,

et al. in the East Nusa Tenggara population also expressed that fast acetylator was the dominant phenotype.[20] A meta-analysis conducted by Paspinodya, et al. stated that fast acetylators were more likely to experience microbiological conversion failure and relapse than slow acetylators. The study also concluded that individual doses for TB might be more effective than standard doses in the DOTS program. This study could not prove this hypothesis because it did not correlate with treatment outcome.[21]

Mutations in the NAT2 gene are responsible for most of the acetylator phenotype affecting plasma concentrations of isoniazid and its metabolites.[12,22] The 1973 WHO report stated the importance of determining the patient's acetylation phenotype in INH administration because NAT transforms the drug in the liver.[8] Variations in the NAT2 gene among different populations might influence INH metabolism, disposition, and side effects. The division of INH acetylation into fast, intermediate, and slow acetylators, shows different plasma drug concentrations after administration of the same dose. The rate of INH elimination also differs between fast and slow acetylators.[6] Patients with the NAT2*4/NAT2*4 genotype (fast acetylator phenotype) exhibited lower INH concentrations than other NAT2 genotypes. Patients with a slow acetylator phenotype have two to seven-fold higher INH concentrations at 3 and 6 hours after drug administration than those with fast acetylator phenotype.[8]

Assessment of plasma INH concentrations is carried out by the chromatographic method, which is faster than other methods.[23] This method is useful for scientific purposes and for keeping track of TB patients undergoing treatment by monitoring changes in drug concentrations. In this study, the median plasma concentration of the drug was obtained after 2 hours of administration, based on the type of acetylator. The median plasma INH concentration for two hours after administration of the drug in rapid acetylators was 1.25 µg/ml (range 0–8.50 µg/ml), lower than that of intermediate acetylators (2.16 µg/ml, range 0–4.33 µg/ml) and slow acetylators (5.24 µg/ml, range 0.25–14.16 µg/ml). The concentrations in this study were lower than that of Kumar, et al., namely 4.1 µg/ml in fast acetylator, 8.1 µg/ml in intermediate acetylator, and 10.2 µg/ml in slow acetylator.[9] Another study by Singh, et al. found the value of INH concentration after 2 hours in fast acetylator was 2.4 µg/ml, while in slow acetylator was 5.6 µg/ml; these results were not much different from the results in this study.[7] The acetylator phenotype was almost the same, namelythe plasma INH concentration in the fast acetylator group was lower than that in the intermediate and slow acetylator groups.

This study analyzed the correlation between plasma isoniazid levels and the distribution of acetylator phenotypes. In both the trimodal and bimodal distributions, no correlations were found. This was in contrast to Kumar, et al., who obtained a significant difference in plasma INH concentrations between the three acetylators.[9] This might be due to the smaller number of samples in this study.

Several studies to date have reported the effect of NAT2 genotype and plasma INH concentration on the clinical condition of patients.[23,24] Adverse effects such as INH-induced hepatotoxicity have been reported from various studies. Based on this, the difference in INH concentration variations based on the NAT2 genotype is essential to know. Slow acetylators with higher INH concentrations are more susceptible to INH-induced hepatotoxicity. A meta-analysis by Wang, et al., 17 of 14 studies consisting of 474 cases and 1446 controls, showed a significant association between NAT2 slow acetylators and the risk of TB drug-induced liver toxicity.[22]

In contrast to slow acetylators, fast acetylators are associated with an increase in the acetylating capacity of the NAT2 enzyme, thereby lowering drug levels and showing poor drug efficacy in some reports. This leads to possibly slower bacteriological conversion rate, resulting in treatment failure, higher risk of recurrence and drug resistance.[21] Many factors affect the efficacy of INH. In addition to plasma INH concentration, the effectiveness of INH is also influenced by the MIC of mycobacterium

tuberculosis in individuals. In patients with low INH concentrations, the drug efficacy in these patients was not necessarily low.[25] In this study and most studies in several regions in Indonesia, the dominant acetylator was fast acetylator. Cases of relapsed TB and MDR TB in Indonesia were quite high based on 2014 WHO data of as many as 7,840 points, and in West Sumatra, the figures were relatively high. However, this study could not conclude the study hypothesis because it did not include data on the outcome of MDR TB patients.

The results of this study supported the importance of knowing the variation of the patient's acetylator phenotype because it was one of the factors which played a role in plasma INH concentrations. Based on this, the INH dosing regimen could be tailored to the individual to increase efficiency and reduce INH side effects and the risk of recurrence and resistance. In addition, this study supported the monitoring of treatment adherence by assessing plasma INH concentrations. In this study, low plasma INH concentration was found, even though the patient was taking medication before the sample was taken. Researchers could only confirm that the patient took the drug on that day. The low INH concentration in these patients could not be ascertained whether it was influenced purely by the acetylator phenotype or other factors such as non-adherence and other host factors.

This study had limitations in the relatively small number of samples, namely from 26 subjects whose venous blood was taken; only 16 patients could be sequenced.

CONCLUSION

The NAT2 genotype polymorphism influenced the acetylation status. The concentration of isoniazid was different in the three groups of fast, intermediate, and slow acetylators. This study found no correlations between the NAT2 acetylator phenotype and plasma INH concentrations among the trimodal and bimodal distributions.

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