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Genetic Variations of SARS-COV-2: Distribution of Variants and Mutations in the N Gene

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ABSTRACT

The SARS-CoV-2 nucleocapsid (N) gene is the most frequently mutated gene after the spike gene. This research aims to analyze the distribution of SARS-CoV-2 and nucleotide variations of the N gene in the SARS-CoV-2 variant found in West Sumatra.

We analyzed 268 genome sequences of SARS-COV-2 at the start of the pandemic from April 2020 to March 2022 and compared them with NC45512. The range consists of three waves: the first, second, and third waves, all of which are dominated by the B.1.466.2 lineage.

Distribution lineage found 20 lineages, with the majority being B.1.466.2 (31.72%), a local variant. The delta variant is 19.20% divided into two lineages, AY.23 and AY24, and the Omicron variant is 14.18%. In this study, we found unique mutations in the N gene where the B.1.466.2 variant can be seen with the T205I amino acid change and Delta with the D64G, R203M, and G215C amino acid changes; omicron variant with amino acid changes P13L, DEL 31/33, R203K, and G204R.

Conclusion: We conclude that there are nucleotide variations in the West Sumatra SARS-CoV-2 N gene.

Keywords: SARS CoV-2, nucleocapsid gene, mutation variant B.1.466.2, delta, omicron

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome - Coronavirus 2 (SARS-CoV2). The first SARS-CoV2 was discovered in the Chinese city of Wuhan. [1, 2] SARS-CoV-2 is the seventh virus that currently infects humans. This virus is a Betacoronavirus with an encapsulated single-stranded ribonucleic acid (RNA) genome with a genome length of about 30 Kb. The first two-thirds of the genome is an overlapping ORF1a/b region, which encodes two polyproteins (pp1a and pp1ab) located at the 5' terminus. The 3' terminal end of SARS-CoV-2 consists of four structural protein-coding genes, which include spike (S), membrane (M), envelope (E), and nucleocapsid (N). Everything from host cell invasion to the formation of virus particles, various viral processes depend on these proteins. [3-9]

SARS-CoV-2, like other RNA viruses, is constantly adapting to its host. Since its emergence, it has continued to mutate to produce several variants of SARS-CoV-2 with different characteristics compared to its ancestral strain. Therefore, researchers conducted whole genome sequencing

(WGS) from West Sumatra to determine the distribution of SARS-CoV-2 and the genetic variation of the N gene. They also looked at the mutation pattern of the N gene from the dominant viral variant found in West Sumatra. Several studies have reported genetic variations in viruses resulting from several mutations: missense, synonymous, insertion, deletion, and non-coding mutations. In a previous study, Wang et al.[10] found 13 mutational variations in SARS-CoV-2 ORF1ab, S, ORF3a, ORF8, and the N region. In other studies, found nucleotide variations (204 mutations) between the SARS- CoV -2 isolated and sequenced from Egyptian COVID-19 patients. Of the 204 mutations, 131 were in ORF1ab, 30 in the S region, 23 in the N region, 1 in the E region, and 2 in the M region. [11]

Genetic variation analysis was necessary to broaden knowledge about new viruses, determine the specific geographic distribution of new variants, and define clinical and political strategies for dealing with outbreaks.[12] Biological characterization of these viral mutations can provide valuable insights for assessing viral drug resistance, immunity, and related mechanisms of pathogenesis. In addition, studies of viral mutations can guide the design of new vaccines, antiviral drugs, and diagnostic tests. Among the four structural proteins, the N protein is encoded by the Nucleocapsid gene (N gene) at the nucleotide position 28274 – 29533 (1260 bp). The N protein is considered a multifunctional RNA-binding protein involved in several aspects of the viral life cycle, such as the formation of the ribonucleoprotein helical structure during genome packaging, regulation of RNA synthesis during replication, transcription, and modulating host cellular metabolism.[8] Based on the above background, the authors are interested in examining the nucleotide variation of Gen N and its correlation with the SARS-CoV2 variant from an RNA isolate which is a collection of the Laboratory of Diagnostic and Research of Infectious Diseases, Faculty of Medicine, Andalas University, Padang. The existence of data on molecular variations originating from West Sumatra will make the data on molecular variations of SARS CoV2 more complete in Indonesia and is expected to be used as a basis for vaccine development.

MATERIALS & METHODS

All samples were collected from patients from several areas in West Sumatra who were confirmed to have COVID-19 during the early period of the pandemic from April 2020 to March 2022. In this study, the SARS-CoV-2 RNA continued for WGS was a sample with a CT≤25 value. library preparations were constructed according to the Illumina RNA prep with enrichment (L) tagmentation kit protocol. DNA synthesis was carried out on isolated RNA samples and by the reverse transcriptase enzyme with a random hexamer primer. The viral genome was amplified using two primer pools in separate reactions. PCR amplification products were processed for adapter fragmentation and ligation using IDT for Illumina Nextera UD Indexes. The enrichment and clean-up steps were carried out according to the manufacturer's protocol. All samples finished cleaning up in the pool are in one tube. Pooled samples were quantified using the Qubit dsDNA High Sensitivity assay kit on a Qubit fluorometer (Invitrogen Inc, Carlsbad, CA, USA), and fragment sizes were analyzed in a Biorad CFX96 Touch Real-Time PCR Detection System. The pooled library will be prepared for the sequencing stage (Figure 1).

Variants or lineages of 268 SARS-CoV-2 genomes were identified using the PANGOLIN COVID-19 Lineage website. Data analysis was performed to determine the type of mutation and nucleotide variation of the N gene using the QIAGEN CLC Genomic Workbench app software Qiagen. The sequencing results of each sample were combined and aligned with the reference sequence NC_045512. The results of the nucleotide variation analysis of the SARS CoV-2 N gene are shown in tables

and figures. Locations and types of gene variations (substitutions, insertions, or deletions) and changes in amino acids are analyzed and presented in the table.

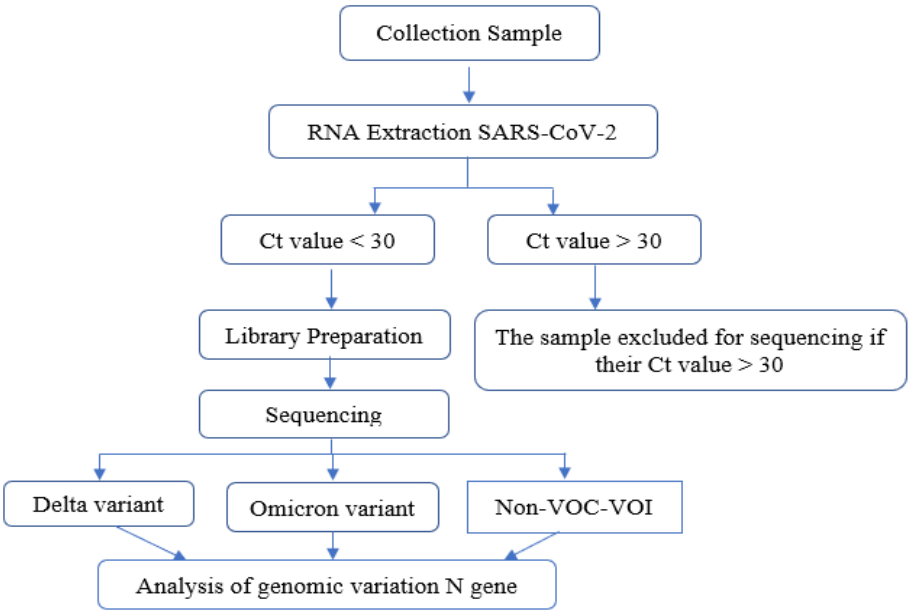

Figure 1. Schematic of preparation for analysis of Gen N nucleotide variation

RESULT

Distribution variant SARS-CoV-2

From the start of the pandemic to March 2022, 268 samples were further analyzed for WGS. These data were classified as Variants of Concern: omicron, delta, and non-VOI-VOC variants. The types and distribution of variants of the study sequences are shown in (Table 1). Samples were mainly collected at the start of the pandemic from April 2020 to March 2022. This range represents the peak of the first, second, and third waves of the Sars-CoV-2 outbreak (Figure 2.)

Table 1. Distribution of SARS-CoV-2 variants from 268 sequences

Type of Variants	Lineage	n (268)	Collection Month	%
Non-VOC-VOI Variant	B.1.466.2	85	December 2020-July 2021	31.72
	B.1.36.19	27	July 2020- March 2021	10.07
	B.1.1.398	25	July 2020- February 2021	9.32
	B.1.468	17	September 2021- December 2021	6.34
	B.1	8	October- December 2020	2.99
	B.1.459	4	August-December 2020	1.49
	B.1.1	3	November-December 2020	1.12
	B.1.1.216	22	December 2020 and January 2021	0.75
	B.1.470	1	October 2020	0.37
	B.1.456	1	December 2020	0.37
	B.6	4	May-July 2020	1.49
	B	1	October 2020	0.37
Delta (VOC)	AY.23	38	June-July 2021	19.40
	AY.24	13	June-July 2021	
Type of Variants	Lineage	n (268)	Collection Month	%
Omicron (VOC)	BA.1	8	February-March 2022	14.18
	BA.1.13.1	14	February-March 2022	
	BA.1.15	2	February-March 2022	
	BA.2	8	February-March 2022	
	BA.2.3	5	February-March 2022	
	BA.2.32	1	February-March 2022	

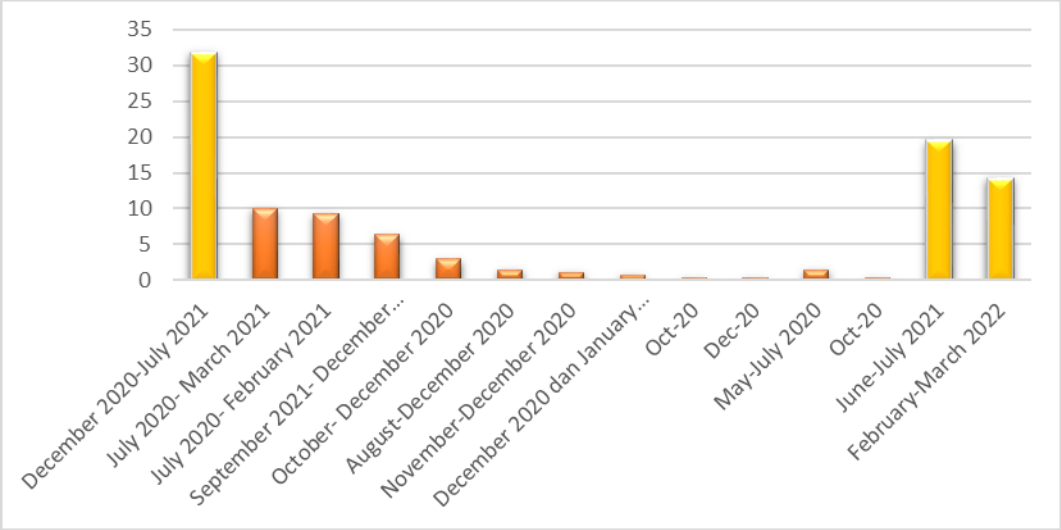

Figure 2. The number of variant cases peaked during December 2020-July 2021 (First wave) just before the Delta variant outbreak. The second wave for the June-July 2021 period is the Delta outbreak period and the third wave is the Omicron variant outbreak for the February - March 2022 period in the table highlighted in yellow.

Mutation N gene SARS-CoV-2

Analysis of nucleotide variations in the N gene of the SARS-CoV-2 virus shows that there are nucleotide variations in the N gene region because 89 point mutation substitutions (80 substitutions and 9 deletions) have been found in 1260 nucleotides (Table 2). Of the 80 point mutations, the most common base change was C/T, followed by G/T base change (Figure 3 and Table 3). A total of 80 substitution mutations further analyzed found 60 (75%) nonsynonymous mutations, and 20 (25%) synonymous mutations. There were also 30 transversion and 50 transition mutations (Table 3). SARS-CoV-2 continuously adapted to infect humans and produced variants with unique genomic profiles. The result of the alignment of the SARS-CoV-2 variant sequence with the reference sequence found a unique mutation. Based on Pango data, lineage-unique variant mutations are defined as non-synonymous substitutions or deletions that occur with a prevalence of more than 75% of sequence variants. The unique mutation pattern of the SARS-CoV-2 variant is shown in the table (Table 5).

Table 2. Types of mutations in the SARS-CoV-2 N gene

Type of mutation	Number of Points of Gene N SARS-CoV-2 Mutations	Dominant mutation		
		Mutation	Position	n=268 (%)
Deletion	9	AGAACGCAG	28363-28371	38 (14.17%)
Substitution	80	C/T	28887	85 (31.71%)
		G/T	28461	52 (19.4%)

Table 3. Types of SARS-CoV-2 N gene substitution mutations

Mutation	n=80	%
Synonymous	20	25
Non-synonymous	60	75
Transition	50	37.5
Tranversion	30	62.5

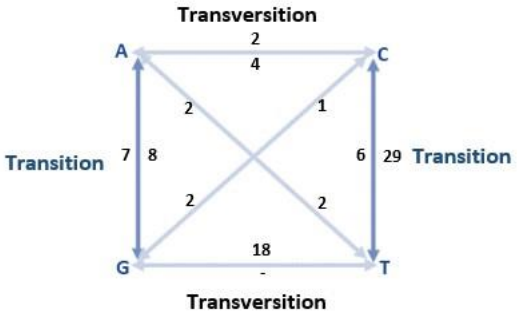

Figure 3. Schematic change of base. Arrows show how the base changes. The numbers next to the arrows indicate the amount of base change based on the number of samples. Dark blue arrows indicate transversion mutations and light blue transitions.

Table 5. The mutation pattern of the N gene in the SARS-CoV-2 variant > 75%

Posisi Mutasi Berdasarkan Urutan RefSeq NC_45512													
Variant	**n**	**28311**	**28363**	**28363-28371**	**28887**	**28461**	**28854**	**28881**		**28882**	**28883**	**28916**	**29402**
		C/T	**A/T**	**Del GAACGCAGT**	**C/T**	**A/G**	**C/T**	**G/T**	**G/A**	**G/A**	**G/C**	**G/T**	**G/T**
Delta	52					**(100 %)**		**(100 %)**				**(76.9 %)**	**(100 %)**
Omicron	**38**	**(100 %)**	**(100 %)**	**(100%)**					**(100 %)**	**(100 %)**	**38(100 %)**		
B.1.466.2	85				**(100 %)**								
B.1.36.19	27						**(100 %)**						
B.1.1.398	25								**(100 %)**	**(100 %)**	**(100%)**		
Change in amino acids		**P13L**	**SILENT**		**T205I**	**D63G**	**S194L**	**R203M**	**R203K**	**R203K**	**G204R**	**G215C**	**D377Y**

DISCUSSION

Distribution variant SARS-CoV-2

In this study, the distribution of the SARS-CoV-2 variant from May 2020 to March 2022 can be seen in Table 1 and Figure 2, showing that Lineage B.1.466.2 (31.72%) Delta (19.4%), Omicron (14.18%) lineage B.1.36.19 (10.67%), lineage B.1.1 .398 (9.32%), lineage B.1.468 (6.34%), and other minor variants lineage B.1 (2.99%), lineage B.1.459 and B.6 (1.49%), lineage B.1.1 (1.12%), lineage B.1.1.216 (0.75%) and lineage B.1.470 and B.1.456 and B (0.37% each). Lineage B1.466.2 was the most common before the delta variant (19.4%) broke out, the B.1.466.2 variant was the most dominant in Indonesia. This variant is more locally distributed in Indonesia compared to other Southeast Asian countries with a lower percentage. By the study results, Fibriani et al. reported that the B.1.466.2 variant of SARS-CoV-2 was found in West Java more than other variants (January to April 2021). [14] Meanwhile, Gunadi et al. also reported that the B.1.466.2 variant found in Central Java and Yogyakarta (May 2020 to June 2021) ranks second among non-Delta variants.[15]

In the early days of the pandemic, Indonesia lacked genomic surveillance until B.1.466.2 was discovered, so the trajectory of its population over time and how it is transmitted between islands in Indonesia is unclear. Then Zhu et al. analyzed whole genome sequencing data from lineage B.1.466.2 from Indonesia. As of 28 August 2021, the GISAID database recorded a total of 1953 complete genomes of the SARS-

CoV-2 B.1.466.2 strain from 24 countries. Of these, it was found that 84.9% were from Indonesia, followed by Malaysia (4.6%), Singapore (4.3%), Australia (1.4%), Japan (1.4%), and Papua New Guinea (0.9%). The first sample was isolated from Banten Province (part of Java), Indonesia on 6 August 2020. The geographic distribution of the 1302 samples found 493 samples from Sumatra and 406 from Java. The B.1.4662 variant reached its peak in March 2021 until the end of May 2021. Unlike the May 2021 Delta variant, it experienced a sharp increase so it became the newest dominant variant in Indonesia at that time.[16]

The period from December 2020 to July 2021 is a transitional period from the local variant outbreak to the delta variant. The delta variant was found during this period, but it was still dominated by the B.1.466.2 variant the original Indonesian variant. By the results research of Zhu et al. analyzed the B.1.466.2 variant which contributed 31.7% when the variant was endemic in Indonesia (second after the delta variant).[16] . In the period before the Delta variant appeared, the B.1.466.2 variant was the most dominant in Indonesia, then followed by the B.1.470 variant. The B.1.470 variant was first detected in Indonesia on April 9, 2020, then spread to the closest neighboring countries, such as Malaysia, South Korea, and Japan. Other unique variants found in Indonesia that were detected in the pre-Delta period were B.1.1.398 at 5.7% and B.1.468 at less than 5%. The Indonesian variant was no longer found significantly during the Delta outbreak, except for B.1.466.2. [17-19]

From Gisaid data, the original Delta variant (B.1.617.2) was first detected in India in October 2020, while in Indonesia it was first detected in January 2021 with a proportion below 5% lower than sub-lineage AY.23 and AY.24 of the total genome that has been in sequencing. In the case of the VoC Delta (sub-lineages AY.23 and AY.24), the predominance found in Indonesia caused the second wave of the pandemic. At that time the local variant B.1.466.2 was still found with decreasing numbers. [19] From our study, the delta variant found sublineage AY. 23 and AY.24 while lineage B.1.617.2 was not found. Lineage AY.23 is found more frequently than AY.24 (AY.23 n=38; AY.24 n=24). Lineages AY.23 and AY.24 were first sequenced on 29 July 2020, a sample from the city of Jambi. West Sumatra was first discovered on June 7, 2021. [20] Interestingly, in August 2021, a neighboring country, Malaysia, also reported that the Delta lineage AY.23 variant was dominant until October 2021, being replaced by lineage AY.59 and AY.79. As east Malaysia borders the Indonesian Borneo region on Borneo Island, active migration across the border may have facilitated the transmission of lineage AY.23 to east Malaysia. [21]

From Table 1 the distribution of omicron variants in West Sumatra is 14.18% with several sub-lineages namely BA.1, BA.1.13.1, BA.1.15, BA.2, and BA.2.3, BA.2.32. BA.1.13.1 is also called Indonesian lineage because it can be found in Indonesia. Based on sequencing data for 17 November 2020 lineage BA.1.13.1, 3691 sequences were sequenced, of which 3554 sequences were from Indonesia (96%). In Indonesia, it was discovered for the first time on December 16, 2021, and finally on September 22, 2022, while in West Sumatra it was first discovered on January 20, 2022, and finally on August 1, 2022. [20]

Analysis of Nucleotide Variation in the N Gene of the SARS-CoV-2 Virus

Since its emergence in 2019, SARS-CoV-2 infection has continued to spread rapidly throughout the world. Genetic sequencing studies have found many mutations, especially single nucleotide polymorphisms (SNPs) and insertions/deletions (indels), which are mostly neutral or can cause interference. However, a few mutations can increase resistance and help the virus adapt. Substitution or deletion can change the polarity of the peptide, affecting the structure and function of proteins involved in pathogenicity, infectivity, transmissibility, and antigenicity.[22]

Mutations are changes in the nucleotide sequence that cause genetic variation in a species. Mutations can occur due to replication errors but they can also be caused by external factors such as ultraviolet (UV) light or mutagenic chemicals. Generally, reading errors resulting from this mutation can be corrected by DNA polymerase enzymes. However, if this error escapes the repair mechanism, this will result in a point mutation which is of the substitution type. The types of mutations found include substitution, insertion, deletion, and duplication.[18]

The N gene in SARS-Cov-2 has many mutations, especially those found at amino acid positions 203-205 (Table 5). These three mutations occurred in the R203K+G204R region (multiple mutations) which were present in the alpha, gamma, and omicron variants, as well as the T205I amino acid mutation which was found in the beta variant, and the R203M mutation which was present in the kappa and delta variants.[23] In addition to other studies also found three mutations in the N gene, namely Del28877-28894 which caused the deletion of six AAs, the substitution of GGG for AAC at 28881-28883 changed two AAs, and a frameshift mutation caused by the deletion of 28877-28878. found in variant P.1 (P1.1). Mutations in the N gene region are associated with higher viral loads in COVID-19 patients found in Saudi Arabia. The results of a mutational association study between the SARS-CoV-2 SNP and patient mortality identified three consecutive SNPs (G28881A, G28882A, G28883C) underlying the R203K/G204R mutation. [24-25]

As a result, it can be seen that substitutions are the most common type of mutation found in the N gene, namely 80 substitutions, 9 deletions, and insertions not found in the N gene. In a previous study, Zekri et al. found that substitution is the most common type of substitution mutation compared to deletion.[13] Of the 80 point mutations, the most frequent base changes were C/T, followed by G/T base changes (Figure 1 and Table 2). This is also in line with the results of a previous study which showed that the base that changed the most was C/T followed by a change in base G/T.[26-27]

A total of 52 samples of the delta variant were found, divided into 2 lineages, namely AY. 23 and vv. 24, both are mostly Indonesian lineage (more are found in Indonesia). Lineage AY.23 and AY.24 have several unique mutations located in the NTD, LKR, and CTD domains sequentially in mutations A28461G (D63G), G28881T (R203M), and G29402T (D377Y) and in the AY.23 sub-variant, a unique mutation was found in the LKR region G28916T (G215C). Similar results were also found in global sequences of the variant delta. [30-31]

In previous studies, Rahman et al.[32] explained that synonymous mutations cause helical or sheet changes and compositional turns in the secondary structure of proteins. A significant impact will be seen in the structure of the NTD and CTD domains which change the secondary structure and function of the domain. Meanwhile, transversion substitution will affect the tertiary structure of the entire domain.

In our study, the R203M mutation was found in 52 variant delta sequences (100%) and G215C was found in 40 variant delta sequences where it was more commonly found in lineage AY.23. The results of previous research explained explained that mutations in amino acid 203 affect the polymerization phase, separation/phosphorylation of N protein has an impact on ribonucleocapsid (RNP) assembly, thus increasing viral immunity and reducing the effectiveness of the SARS CoV-2 vaccine, in other words, the R203M mutation causes its escape. delta variant of the antibody.[33] Inserting the R203M mutation from the delta variant into SARS-CoV2 - wild type also increased viral replication. In addition, Lin et al., (2021) also found a synergistic effect of mutations with S and N proteins in increasing viral immunity.

The results of the analysis found 18 non-VOC – VOI variants (B.1.466.2, B.1.36.19, B.1.1.398, B.1.468, B.1, B.1.459, B.6, B.1.1, B. 1.1.216, B.1.456 and B.1.470). Variant B.1.466.2 which was found the most was 85 samples out of 268 (31.72%). B.1.466.2 has a unique mutation in the N gene, namely a substitution at the C28887T nucleotide position which causes a change in the amino acid threonine to isoleucine (T205I) which is found in all sequences of B.1.466.2 (100%). The same thing was found by Massi et al.[13] from a genomic analysis of the Makasar sequences, a C28887T substitution was found in all sequences (100%) lineage B.1.466.2. Bourossa et al.[34] stated that T205I is a common mutation of the N gene in the global consensus sequence, with a prevalence of 42.9%, the mutation is located in the 419 aa protein area with no available structure, with T205I in the serine arginine-rich region.

CONCLUSION

The lineage B.1.466.2 (first wave) Delta (during the 2nd wave) and Omicron VOCs (during the 3rd wave) were very predominant among sequences SARS-CoV2 in our research. Changes in the N gene in the samples in the study were found at 89 treatment points with 80 substitutions and 9 deletions. The most mutation was found at position C28887T which is a mutation found in 100% of the B.1.466.2 variant

Declaration by Authors

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