

Conference Paper

Differences Between Rainy and Dry Seasons in Levels of Cholinesterase Activities and Psychological Distress due to Organophosphate Exposure among Farmworkers

Suratman

Faculty of Health Sciences, Universitas Jendral Soedirman, Purwokerto, Indonesia

Abstract

This study compared mean of erythrocyte acetylcholinesterase (EChE) and plasma cholinesterase (PChE) activity levels, and levels of psychological distress due to organophosphate (OP) exposure between rainy and dry seasons among farmworkers. This was a longitudinal study conducted in rainy and dry seasons on 30 farmworkers at a village in Brebes Regency, Indonesia. These EChE and PChE levels were measured from 10 μ L fingerprick blood samples using the Test-mate ChE field kit. Meanwhile, psychological distress levels were measured by a 10-item questionnaire developed by Kessler et al. (2002). Mean EChE activity levels in rainy season (29.45 \pm 3.68 U/g Hb) were higher than in dry season (26.33 \pm 3.69 U/g Hb) (p <0.05). In contrast, mean PChE activity levels in dry season (1.62 \pm 0.50 U/mL) were slightly higher than in rainy season (1.61 \pm 0.39 U/mL). Most of the farmworkers felt some symptoms of psychological distress both in rainy and dry season measurements. In addition, majority of them were categorized suffering from mental disorders in both measurements. However, the difference of EChE and PChE activity levels between rainy and dry seasons could be related to the time elapsed since last exposure. A proportion of the research participants suffered from mental disorders in the dry season was higher than that of in the rainy season. The difference of psychological distress levels between both seasons might be related to other external factors like high temperature, high humidity, or economic factors and not to the last OP application.

Keywords: Erythrocyte acetylcholinesterase; farmworkers; organophosphate; plasma cholinesterase; psychological distress.

1. INTRODUCTION

Organophosphate (OP) compounds particularly chlorpyrifos, diazinon, and malathion are the most commonly used in agriculture around the world ([10, 12, 26], WHO, 2009).

Corresponding Author:

Suratman

suratman_skm@yahoo.co.id

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In Indonesia, 3005 different pesticides were registered in 2014 [5]. Chlorpyrifos was the common active constituent of OP used by farmworkers in Indonesia [1, 16]. Previous studies demonstrated that long-term exposure to OP compounds caused adverse health effects like neurobehavioral and neurodevelopmental effects [17, 20, 25], lung cancer [2], and autoimmune disorders [24].

OP's toxicity occurs due to the inhibition of the neural target enzyme, acetylcholinesterase (AChE). Erythrocyte acetylcholinesterase (EAChE) and plasma cholinesterase (PChE) activity levels in blood samples are used as biomarkers of OP-related cholinesterase inhibition and are used to monitor farmworkers at risk of OP exposure [19, 21]. A study by Jintana et al. (2009) conducted on 90 Thai ethnic individuals occupationally exposed to OP and 30 controls showed that EAChE and butyrylcholinesterase (BuChE) activity levels statistically significantly decreased in the high-OP exposure period compared to the low-OP exposure period.

Pesticides also had been linked with depression experienced by farmworkers [9, 27, 28]. A study by Weisskopf et al. (2013) showed that as many as 83 of 567 farmworkers or 14.6% aged 37-78 years old were hospitalized for depression due to pesticide exposure.

The aim of this study was to compare mean of EAChE and PChE activity levels, and levels of psychological distress due to OP exposure between rainy and dry seasons among farmworkers.

2. METHODS

This was a longitudinal study conducted at a village in Brebes Regency, Indonesia in rainy and dry seasons in 2014. Inclusion criteria of population were: 1) male; and 2) had to be employed in farm work within the past 3 months. Thirty research participants who met these inclusion criteria were randomly selected from 52 farmworkers. Ethics approval was obtained from the Commission on Health Research Ethics, Faculty of Public Health, Diponegoro University, Semarang, Indonesia, with approval number: 183/EC/FKM/2013.

EAChE and PChE activity levels were measured according to the manufacturers instruction of the Test-mate ChE Cholinesterase Test System® [8]. Meanwhile, psychological distress levels were measured by a 10-item questionnaire developed by Kessler et al. (2002) based on questions about anxiety and depressive symptoms experienced by a person in the most recent 4 week period. These questions had five response options, namely 'none of the time', 'a little of the time', 'some of the time',

'most of the time', and 'all of the time' ranging from 1 to 5. A range of scores was 10-50 divided into four categories as follows: a) well if score was under 20; b) a mild mental disorder if score was 20-24; c) a moderate mental disorder if score was 25-29; d) a severe mental disorder if score was 30 and over [3, 14]. The questionnaire was translated into Indonesian language.

Continuous data were tested for normal distribution using the Shapiro-Wilk test [7, 23]. If a normal distribution was found, data were analyzed using paired t test and Pearson Product Moment test, otherwise data were analyzed using Wilcoxon test and Spearman's Rank Correlation test. Level of statistical significance was set at $\alpha = 0.05$.

EChE and PChE activity levels are also presented in clinical categories ranging from normal to severe inhibition. Clinical categories of inhibition of EChE based on percentage of normal EChE activity (taken as 31.4 U/g Hb) [8, 22] and grading of inhibition of PChE based on percentage of normal PChE activity taken as 2.55 U/mL [8, 22].

3. RESULTS

3.1. OP Application

Farmworkers were asked to estimate the last time they applied OP (defined as mixing, loading, and spraying). Most farmworkers applied OP to their crops in the preceding 1-6 days (83%) in rainy season, compared with most of them applying OP in the last 2-4 months (40%) in dry season (Figure 1). The active constituent of OP generally used by research participants was chlorpyrifos, about 50%.

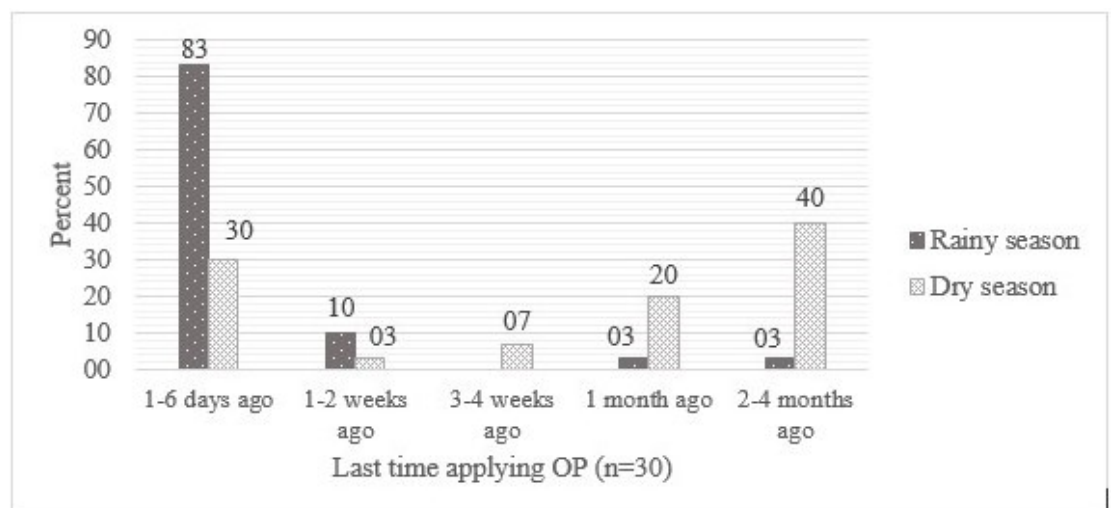


Figure 1: The last time applying OP by farmworkers in rainy and dry season measurements.

3.2. EAcHE Activity Levels

Generally, EAcHE activity levels slightly declined in dry seasons (Figure 2). However, EAcHE activity levels in some farmworkers decreased dramatically. There were statistically significant differences in EAcHE activity levels between rainy (29.45 ± 3.68 U/g Hb) and dry (26.33 ± 3.69 U/g Hb) among farmworkers ($p < 0.05$). In addition, the mean EAcHE activity levels among them were lower than the reference population value (31.4 U/g Hb) using this kit, $p < 0.05$.

In rainy season, mean EAcHE activity levels tended to be higher among farmworkers who applied OP more than a month ago (Figure 3). There was no clear pattern of EAcHE activity levels among farmworkers who applied OP in various latest application time in dry season. The results of Spearman's Rank test indicated that there was no statistically significant correlation between the last time applying OP and EAcHE activity levels in both rainy and dry seasons ($p > 0.05$).

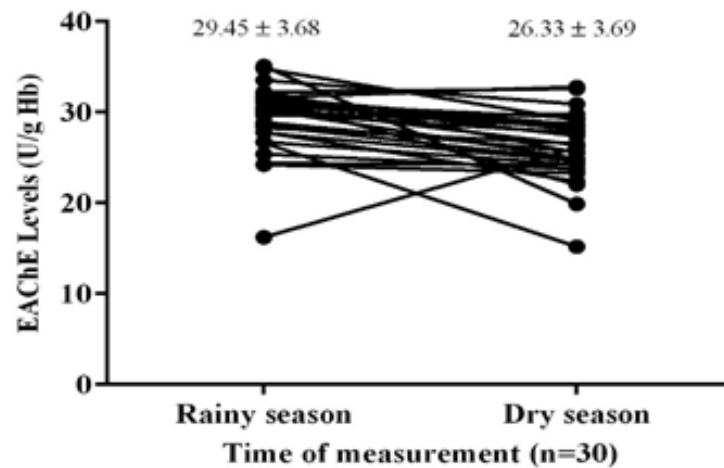


Figure 2: Activity levels of EAcHE in individually matched fingerprick blood samples in rainy and dry seasons among farmworkers ($p < 0.05$). (Note: The data labels used in Figure 2 were mean \pm SD. Abbreviation: SD, standard deviation).

3.3. PChE Activity Levels

Generally, mean PChE activity levels (1.61 ± 0.39 U/mL and 1.62 ± 0.50 U/mL respectively) were slightly higher in dry season than those taken in rainy season (Figure 4). However, PChE activity levels in some research participants decreased sharply. Overall, the results of paired t tests showed that there were no statistically significant differences in PChE activity levels between rainy and dry seasons ($p > 0.05$). In addition,

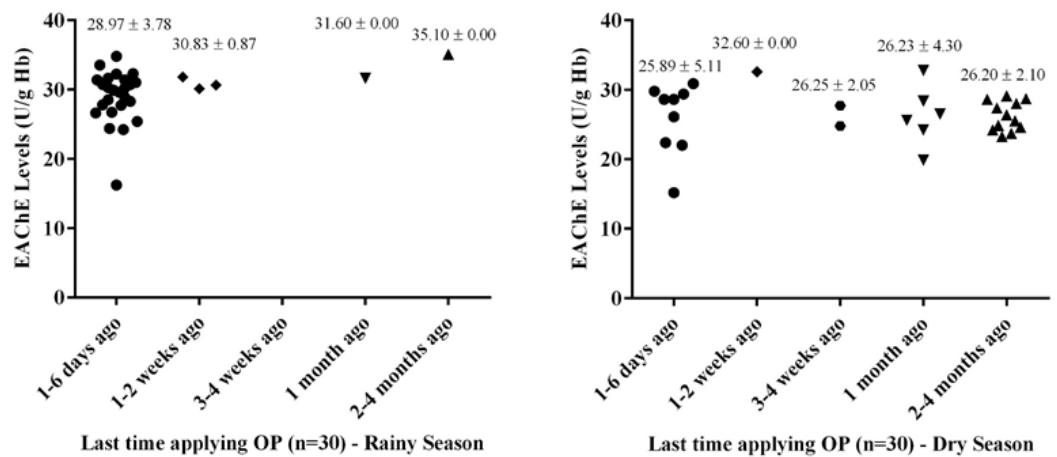


Figure 3: Last time applying OP against E.AChE activity levels in rainy and dry seasons among farmworkers. (Note: The data labels used in Figure 3 were mean ± SD. Abbreviation: SD, standard deviation).

the mean PChE activity levels among farmworkers were lower than the reference population value (2.55 U/mL) ($p < 0.05$).

In rainy season, mean PChE activity levels varied among farmworkers with various latest OP application time (Figure 5). For example, a farmworker who applied OP more than a month ago had the highest mean levels of PChE activity levels. Similarly, there was no specific pattern of PChE activity levels among farmworkers who applied OP in various latest application time in dry season. The results of Spearman’s Rank test indicated that there was no statistically significant correlation between the last time applying OP and PChE activity levels in both rainy and dry seasons ($p > 0.05$).

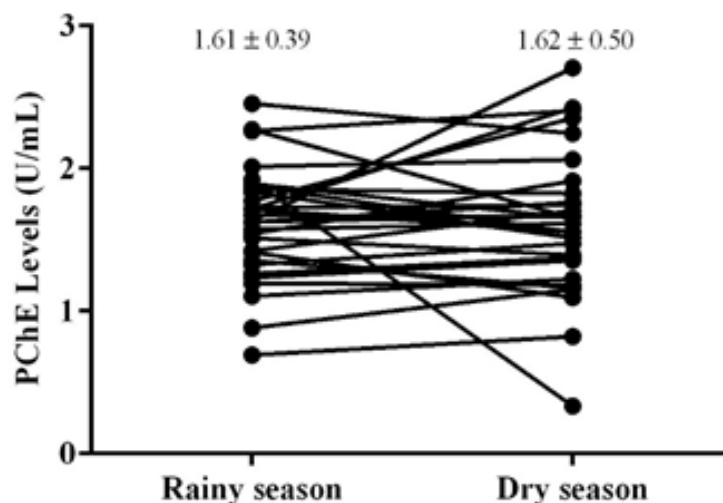


Figure 4: Activity levels of PChE in individually matched fingerprick blood samples in rainy and dry seasons among farmworkers ($p > 0.05$). (Note: The data labels used in Figure 4 were mean ± SD. Abbreviation: SD, standard deviation).

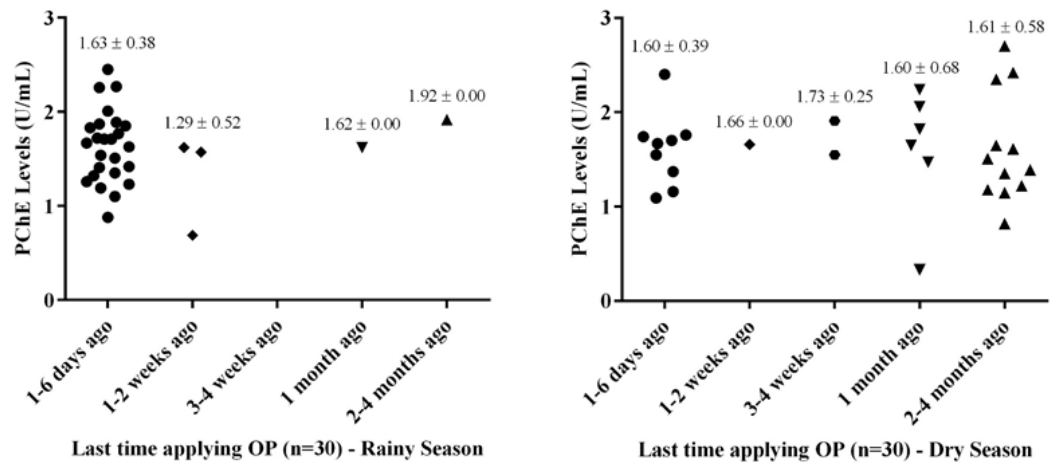


Figure 5: Last time applying OP against PChE activity levels in rainy and dry seasons among farmworkers. (Note: The data labels used in Figure 5 were mean ± SD. Abbreviation: SD, standard deviation).

3.4. Clinical Categories of EChE and PChE Activity Levels

Nearly all farmworkers had normal levels of EChE (Table 1). At the same time, most of the research participants suffered from mild inhibition of PChE.

TABLE 1: Clinical categories based on percentage of normal EChE and PChE activity levels among farmworkers in rainy and dry seasons.

Variable	Period Time Measurement	
	Rainy Season n (%)	Dry Season n (%)
EChE		
Normal	29 (96.7%)	25 (83.3%)
Mild inhibition	1 (3.3%)	5 (16.7%)
Moderate inhibition	-	-
Severe inhibition	-	-
PChE		
Normal	5 (16.7%)	7 (23.4%)
Mild inhibition	24 (80.0%)	22 (73.3%)
Moderate inhibition	1 (3.3%)	1 (3.3%)
Severe inhibition	-	-

3.5. Psychological Distress

In rainy season, most of the farmworkers felt tired out for no good reason (73%); nervous (57%); and that everything was an effort (73%) (Table 2). In contrast, in dry

season, a majority of them felt tired out for no good reason (100%); nervous (77%); so nervous that nothing could calm you down (80%); hopeless (67%); restless or fidgety (97%); so restless you could not sit still (83%); depressed (67%); that everything was an effort (97%); and so sad that nothing could cheer you up (57%).

TABLE 2: Proportion of psychological distress answers for each question in rainy and dry seasons (n=30).

Questions	Rainy Season					Dry Season				
	None of the time n (%)	Little of the time n (%)	Some of the time n (%)	Most of the time n (%)	All of the time n (%)	None of the time n (%)	Little of the time n (%)	Some of the time n (%)	Most of the time n (%)	All of the time n (%)
Tired out for no good reason	8 (27%)	1 (3%)	2 (7%)	19 (63%)	-	-	3 (10%)	7 (23%)	20 (67%)	-
Nervous	13 (43%)	1 (3%)	3 (10%)	13 (43%)	-	7 (23%)	2 (7%)	17 (57%)	4 (13%)	-
So nervous that nothing could calm you down	15 (50%)	1 (3%)	1 (3%)	13 (43%)	-	6 (20%)	7 (23%)	10 (33%)	7 (23%)	-
Hopeless	19 (63%)	2 (7%)	1 (3%)	8 (27%)	-	10 (33%)	5 (17%)	7 (23%)	8 (27%)	-
Restless or fidgety	15 (50%)	2 (7%)	2 (7%)	9 (30%)	2 (7%)	1 (3%)	9 (30%)	16 (53%)	4 (13%)	-
So restless you could not sit still	16 (53%)	2 (7%)	3 (10%)	9 (30%)	-	5 (17%)	4 (13%)	17 (57%)	4 (13%)	-
Depressed	16 (53%)	1 (3%)	1 (3%)	11 (37%)	1 (3%)	10 (33%)	5 (17%)	12 (40%)	3 (10%)	-
That everything was an effort	8 (27%)	-	1 (3%)	11 (37%)	10 (33%)	-	1 (3%)	5 (17%)	13 (43%)	11 (37%)
So sad that nothing could cheer you up	18 (61%)	1 (3%)	1 (3%)	10 (33%)	-	13 (43%)	6 (20%)	7 (23%)	4 (13%)	-
Worthless	22 (73%)	3 (10%)	2 (7%)	3 (10%)	-	27 (90%)	-	1 (3%)	2 (7%)	-

Furthermore in rainy season, 20%, 10%, and 33% the research participants suffered from mild, moderate, and severe mental disorders, respectively (Table 3). These proportions of moderate and severe mental disorders increased in dry season to 23% and 40%, respectively. Meanwhile, a proportion of the farmworkers who were well in rainy season (37%) dramatically decreased to 17% in dry season.

TABLE 3: Categories of psychological distress levels among farmworkers in rainy and dry seasons (n=30).

Categories of psychological distress	Rainy Season		Dry Season	
	n	%	n	%
Well	11	36.7	5	16.7
A mild mental disorder	6	20.0	6	20.0
A moderate mental disorder	3	10.0	7	23.3
A severe mental disorder	10	33.3	12	40.0

4. DISCUSSION

This study found that mean EAcH activity levels in dry season were statistically different from rainy season among farmworkers (Figure 2). Generally, mean EAcH activity levels in dry season were lower than that in rainy season. Mean PChE activity levels did not significantly differ between rainy and dry seasons (Figure 4). The mean PChE levels in dry season were slightly higher than that in rainy season. Both mean EAcH (Figure 3) and PChE (Figure 5) activity levels tended to increase among farmworkers who applied OP more than a month ago.

PChE inhibition is a biomarker of exposure to OP, whereas inhibition of EAcH indicates a biomarker of toxicity and more indicative of the severity of poisoning [18]. PChE inhibition has been shown to not have relationship with EAcH inhibition [18].

More than 80% of the research participants applied OP within one to six days prior to the first measurement (Figure 1). This contrasts with most of the research participants in the dry season measurement (40%) estimating that they had applied OP 2-4 months ago. From a practical point of view, possible differences between the groups might be related to the time elapsed since last exposure. Most of the research participants applied OP 1-6 days before at rainy season, and only 30% at dry season. However, Figure 3 and Figure 5 showed that mean EAcH and PChE activities among farmworkers who applied OP 1-6 days prior to data collection in dry season (25.89 ± 5.11 U/g Hb and 1.60 ± 0.39 U/mL respectively) were lower than that in rainy season (28.97 ± 3.78 U/g Hb and 1.63 ± 0.38 U/mL respectively). The Test Mate is useful for confirmation of a decrease in enzyme activity is suspected poisoning but it is not ideal for measuring more subtle changes [8, 22]. Afriyanto (2008) reported that generally farmworkers in Indonesia sprayed OP using backpack sprayer. In addition, most of farmworkers did not wear Personal Protective Equipment (PPE). The use of PPE plays an important role in the inhibition of EAcH and PChE [12].

Interestingly, EAcHE and PChE activity levels in one farmworker dropped dramatically even though he reported applying OP at least a month prior to the second measurement (Figure 2 and Figure 4). This might indicate unwitting OP exposure experienced by the farmworker. The risk of OP exposure is increased among agricultural workers resulting from unwittingly taken home OP on clothing, shoes and other items (Ackerman & Cizmas, 2014). Immediately after exposure, PChE activity is more inhibited than EAcHE [18]. The half-life of PChE recovery after OP exposure was about 12 days and complete recovery has been reported to occur after about 50 days [19]. This contrasts with complete recovery of EAcHE (attaining unexposed activity) after about 82 days, shorter than the normal life-span of erythrocyte (about 120 days) [19]. Recovery from mild inhibition has been shown to be about 1-3 days whereas recovery from moderate inhibition is 1-2 weeks [29].

In this study, nearly all farmworkers had EAcHE activity within the 'normal' range, when compared with the clinical guidelines for OP toxicity (Table 1). This contrasts with most research participants in rainy and dry season measurements suffered from mild inhibition of PChE activity levels (80% and 73% respectively) and about 3% of farmworkers suffering from moderate inhibition of PChE activity. These results are not consistent with a study conducted by Jintana et al. (2009) that indicated inhibition of PChE activity occurred in high-exposure period (3.73 ± 0.19 U/mL) compared to low-exposure period (4.92 ± 0.19 U/mL). Even though PChE inhibition is a biomarker of exposure to OP, this parameter correlates very poorly with clinical signs or with EAcHE inhibition [6].

EAcHE measurement is a better predictor for effects compared with PChE measurement because EAcHE found on erythrocyte membranes is similar to that found in neuronal tissue [13, 19, 21].

A proportion of the farmworkers suffering from some symptoms of psychological distress increased in dry season compared to those suffering from these symptoms in rainy season (Table 2). In addition, a proportion of the farmworkers categorized suffering from moderate and severe mental disorders in rainy season dramatically increased in dry season (Table 3). The finding of this study was different from the result of a study conducted by Wesseling et al. (2010) that found past poisoning with OP was associated with an overall excess of symptoms of psychological distress and a study conducted by [15] that reported the highest number of neurological symptoms including depressed and anxious occurred among OP applicators during the application season. An increase of psychological distress proportions suffered by the research participants during dry season whereas the use of OP was very low might not be

caused by OP exposure. Other external factors like high temperature, high humidity, economic factors, etc. might contribute to their psychological aspects.

5. CONCLUSION

Mean EAcHE activity levels in the dry season were statistically different from that in the rainy season. Mean PChE activity levels slightly increased from 1.61 ± 0.39 U/mL in the rainy season to 1.62 ± 0.50 U/mL in dry season. However, mean PChE activities did not significantly differ between the rainy and dry seasons. It is not clear whether the observed increase in PChE activity levels was a result of no OP application or a result of a longer period between prior exposure and testing. Most of the research participants had normal EAcHE levels, otherwise most of them suffered from mild inhibition of PChE.

The inhibition of cholinesterase (ChE) activity levels after applying OP compounds indicates exposure among farmworkers. OP application conducted by the research participants 1-6 days before collection and analysis of blood samples might increase possibility of ChE inhibition.

A proportion of the research participants suffered from mental disorders in the dry season was higher than that of in the rainy season. However, the difference of psychological distress levels between rainy and dry seasons could be related to other external factors like high temperature, high humidity, or economic factors and not to the last OP application.

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